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Role of p53 in cell metabolism, ferroptosis, and stemness

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Role of p53 in cell metabolism, ferroptosis, and stemness

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Editorial: Role of p53 in cell metabolism, ferroptosis, and stemness

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Editorial on the Research Topic
Role of p53 in cell metabolism, ferroptosis, and stemness

p53 is among the most critical tumor suppressor genes and is the most extensively studied gene in tumor biology within the past 40 years (Dolgin, 2017). Due to its multiple functions in tumor, p53 has been recognized as a promising target to treat cancer (Duffy et al., 2022). In order to leverage the crucial roles of p53 in tumor therapy, it is necessary to illuminate the exact mechanisms for p53 function and how p53 is regulated (Liu et al., 2019). Conventional activities of p53, such as inducing cell cycle arrest, senescence, and apoptosis have been accepted as the major checkpoints in stress responses and tumor suppression for a long period. However, findings in recent years argue against the necessity of these classical activities for p53-mediated tumor suppression (Mello and Attardi, 2018). Accumulating evidence implicates the importance of other mechanisms, including regulating cancer cell metabolism, ferroptosis, and cell stemness to support p53's anti-cancer role (Liu and Gu, 2022a; Liu and Gu, 2022b). Besides its crucial role in tumor biology, p53 has been demonstrated to function in various other physiological and pathological processes, such as obesity, aging, and immune response. Additionally, the role of mutant p53 and other p53 family members (p63 and p73) are new focuses in research (Mantovani et al., 2019; Osterburg and Dotsch, 2022).

Here we have organized this Research Topic titled "Role of p53 in cell metabolism, ferroptosis, and stemness" and wish to collect manuscripts about the emerging roles of p53 in mediating cell metabolism, ferroptosis, and stemness, which link p53 with diverse diseases, particularly tumors (Figure 1). We have now accepted 11 high quality manuscripts in total. These papers, including research articles and reviews, cover a wide range of Research Topic about p53. We would like to briefly summarize them as follows.

MicroRNA is a type of short non-coding RNA with diverse functions by targeting different genes (Liu et al., 2016; Liu et al., 2017a; Liu et al., 2017b; Liu et al., 2018; Ma et al., 2019). p53 is a master regulator of cell metabolism and is capable of regulating microRNA expression (Hermeking, 2012; Liu and Gu, 2022a). However, the role of the p53-microRNA axis in thermogenesis is largely unknown. Brown adipose tissue (BAT) is the major tissue in mouse to exert non-shivering thermogenesis. Reinisch et al. found that under acute fasting, p53 in BAT was activated to induce the expression of miR-92a-1-5p. They also demonstrated

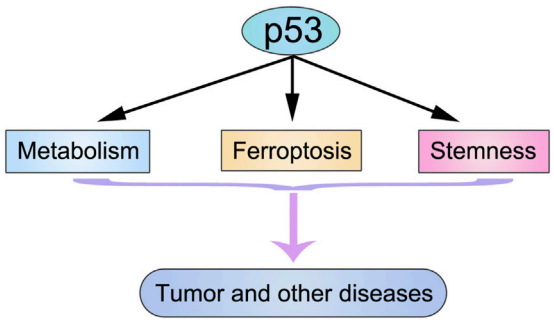

FIGURE 1
Modulating cell metabolism, ferroptosis, and stemness are three novel functions of p53.

a fructose transporter Slc2a5 to be a repressive target of miR-92a-1-5p. The downregulation of Slc2a5 reduced the import of fructose, glycolysis, and finally thermogenesis. This study revealed a novel p53/miR-92a-1-5p/Slc2a5 axis in fasting-regulated thermogenesis.

Lei et al. in their comprehensive review focused on the role of p53 in the brain. They firstly introduced a basic background of p53 and its regulation. They then discussed how p53 regulates various diseases in the brain, including glioma, cerebral stroke, and neurodegenerative diseases.

Ferroptosis is a newly discovered cell death type, which is caused by the iron dependent peroxidation of cell membranes (Stockwell, 2022). p53 has been proved to be a critical regulator of ferroptosis (Liu and Gu, 2022b). Zhou et al. wrote a short review about ferroptosis and lymphoma, highlighting that targeting the p53-mediated ferroptotic pathway may be a promising method to treat lymphoma. pan and Wang contributed a review about p53 and ferroptosis in osteosarcoma. In this review, they classified ferroptosis into typical and atypical types. In both types of ferroptosis, p53 has a vital role. Ferroptosis also contributes to diseases other than cancer, such as in the regulation of diabetes. In the review by Du et al., authors depicted the major mechanism of ferroptosis and its effect in diabetes. Notably, they pointed out the crucial role of p53-regulated ferroptosis in the pathology of diabetes. In an interesting study from Hu et al., authors investigated the function of a bioactive peptide G1dP3 in MH7A cells. They showed that G1dP3 significantly suppressed the viability of MH7A cells by inducing ferroptosis. Mechanistically, they demonstrated that G1dP3 could activate p53 expression, resulting in the suppression of the SLC7A11/GPX4 axis.

p53 functions as a checkpoint molecule to suppress tumorigenesis. To overcome this, p53 is often mutated or deleted in tumor cells. Mutant p53 may have different functions compared to wild type p53 (Mantovani et al., 2019). Liu et al. presented an informative review about how p53 mutation or deletion remolds the tumor immune microenvironment. There are at least three ways that mutant p53 or loss of p53 contributes to an immunosuppressive status: 1) induce immunosuppressive cytokines and downregulate proinflammatory factors; 2) augment the expression of immunosuppressive ligands; and 3) facilitate immunosuppressive cells differentiation. Correspondingly, targeting mutant p53 would be a reasonable choice to restore the tumor immune microenvironment. Corazzari and Collavin wrote an attractive review to summarize the ferroptosis-regulatory activity of both wild type and mutant p53. They proposed that the regulated ferroptosis could be a stress response to diverse stimuli in and out of the tumor cells. K120 is a critical site for p53 function. Monti et al. analyzed the transactivation activity of the p53 K120R mutant. The K120R mutation abrogates the acetylation at this site, preventing p53 from promoting apoptosis. However, this mutant could still retain some activity to modulate cellular metabolism. Mutant p53 also influences the efficacy of tumor treatment. Li et al. reported a pulmonary sarcomatoid carcinoma case with p53 T125K mutation. Fortunately, treatment of Tislelizumab in combination with Anlotinib enabled this patient to go into complete remission. We hope that this therapeutic regimen may benefit other similar patients.

Other members of the p53 family, such as p63 and p73, also have fundamental roles in tumor (Osterburg and Dotsch, 2022). Signal transducer and activator of transcription 3 (STAT3) is a vital transcription factor in stem cells. The review by Wei et al. discussed the crosstalk between p63 and STAT3 in the regulation of cancer stemness.

To summarize, this Research Topic of papers present novel information about some unconventional functions of p53 (and p63). Elucidating the mechanisms of p53 that underlie its versatile roles will not only benefit the basic research about p53 and tumor biology, but also open promising doors for developing therapeutics targeting p53 and related pathways for the treatment of cancer and other diseases. We hope this Research Topic can bring new knowledge and ideas to those who are interested in the function of p53.

Author contributions

YL, JL, and YaZ wrote the manuscript. JY, SC, and JiZ reviewed and revised the manuscript. The authors read and approved the final manuscript. The requirements for authorship have been met. Each author believes that the manuscript represents honest work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Dolgin, E. (2017). The most popular genes in the human genome. *Nature* 551 (7681), 427–431. doi:10.1038/d41586-017-07291-9

Duffy, M. J., Synnott, N. C., O'Grady, S., and Crown, J. (2022). Targeting p53 for the treatment of cancer. *Semin Cancer Biol.* 79, 58–67. doi:10.1016/j.semcancer.2020.07.005

Hermeking, H. (2012). MicroRNAs in the p53 network: Micromanagement of tumour suppression. *Nat. Rev. Cancer* 12 (9), 613–626. doi:10.1038/nrc3318

Liu, Y. Q., Liu, R., Yang, F., Cheng, R. J., Chen, X. R., Cui, S. F., et al. (2017a). miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. *Mol. Cancer* 16, 53. doi:10.1186/s12943-017-0625-8

Liu, M. H., Yin, K., Guo, X., Feng, H. J., Yuan, M., Liu, Y. Q., et al. (2017b). Diphthamide biosynthesis 1 is a novel oncogene in colorectal cancer cells and is regulated by MiR-218-5p. *Cell. Physiol. Biochem.* 44 (2), 505–514. doi:10.1159/000485087

Liu, Y., and Gu, W. (2022a). The complexity of p53-mediated metabolic regulation in tumor suppression. *Semin Cancer Biol.* 85, 4–32. doi:10.1016/j.semcancer.2021.03.010

Liu, Y. Q., and Gu, W. (2022b). p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell. death Differ.* 29 (5), 895–910. doi:10.1038/s41418-022-00943-y

Liu, Y. Q., Chen, X. R., Cheng, R. J., Yang, F., Yu, M. C., Wang, C., et al. (2018). The Jun/miR-22/HuR regulatory axis contributes to tumourigenesis in colorectal cancer. *Mol. Cancer* 17, 11. doi:10.1186/s12943-017-0751-3

Liu, Y. Q., Tavana, O., and Gu, W. (2019). p53 modifications: exquisite decorations of the powerful guardian. *J. Mol. Cell Biol.* 11 (7), 564–577. doi:10.1093/jmcb/mjz060

Liu, Y. Q., Guo, Y., Liang, H. W., Cheng, R. J., Yang, F., Hong, Y., et al. (2016). miR-181b functions as an oncomiR in colorectal cancer by targeting PDCD4. *Protein Cell.* 7 (10), 722–734. doi:10.1007/s13238-016-0313-2

Ma, L. J., Chen, X. R., Li, C., Cheng, R. J., Gao, Z., Meng, X. B., et al. (2019). miR-129-5p and-3p co-target WWP1 to suppress gastric cancer proliferation and migration. *J. Cell. Biochem.* 120 (5), 7527–7538. doi:10.1002/jcb.28027

Mantovani, F., Collavin, L., and Del Sal, G. (2019). Mutant p53 as a guardian of the cancer cell. *Cell. death Differ.* 26 (2), 199–212. doi:10.1038/s41418-018-0246-9

Mello, S. S., and Attardi, L. D. (2018). Deciphering p53 signaling in tumor suppression. *Curr. Opin. Cell. Biol.* 51, 65–72. doi:10.1016/j.ceb.2017.11.005

Osterburg, C., and Dotsch, V. (2022). Structural diversity of p63 and p73 isoforms. *Cell. death Differ.* 29 (5), 921–937. doi:10.1038/s41418-022-00975-4

Stockwell, B. R. (2022). Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. *Cell.* 185 (14), 2401–2421. doi:10.1016/j.cell.2022.06.003

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p53 Regulates a miRNA-Fructose Transporter Axis in Brown Adipose Tissue Under Fasting

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Active thermogenic adipocytes avidly consume energy substrates like fatty acids and glucose to maintain body temperature upon cold exposure. Despite strong evidence for the involvement of brown adipose tissue (BAT) in controlling systemic energy homeostasis upon nutrient excess, it is unclear how the activity of brown adipocytes is regulated in times of nutrient scarcity. Therefore, this study aimed to scrutinize factors that modulate BAT activity to balance thermogenic and energetic needs upon simultaneous fasting and cold stress. For an unbiased view, we performed transcriptomic and miRNA sequencing analyses of BAT from acutely fasted (24 h) mice under mild cold exposure. Combining these data with in-depth bioinformatic analyses and *in vitro* gain-of-function experiments, we define a previously undescribed axis of p53 inducing miR-92a-1-5p transcription that is highly upregulated by fasting in thermogenic adipocytes. p53, a fasting-responsive transcription factor, was previously shown to control genes involved in the thermogenic program and miR-92a-1-5p was found to negatively correlate with human BAT activity. Here, we identify fructose transporter *Slc2a5* as one direct downstream target of this axis and show that fructose can be taken up by and metabolized in brown adipocytes. In sum, this study delineates a fasting-induced pathway involving p53 that transactivates miR-92a-1-5p, which in turn decreases *Slc2a5* expression, and suggests fructose as an energy substrate in thermogenic adipocytes.

Keywords: p53, metabolism, fasting, brown adipose tissue, miRNA, fructose

INTRODUCTION

Cold-activated brown adipocytes are specialized cells that dissipate chemical energy to drive non-shivering thermogenesis (NST), thereby acting as a "metabolic sink" by taking up large amounts of glucose (Klepac et al., 2019) and fatty acids (Townsend and Tseng, 2014) from the circulation. The thermogenic functions of brown adipose tissue (BAT) are largely mediated by uncoupling the proton gradient from the electron transport chain *via* uncoupling protein 1 (UCP1) to generate heat instead of ATP (Nedergaard et al., 1977). NST is mainly driven by cold-induced activation of the sympathetic nervous system, which activates a downstream cascade that culminates in the activation of UCP1 (Harms and Seale, 2013). Recent advances in the field have delineated a set of peptides, signaling molecules, and hormones that influence the activity of brown adipocytes independently of or in addition to an increased sympathetic drive (Li et al., 2019). Interest in the search for BAT activators was spurred by the description of the central role of BAT in controlling whole-body energy homeostasis and metabolic health (Bartelt and Heeren, 2014).

The first evidence, coupling energy expenditure in BAT with nutrient intake, came from studies suggesting that BAT is activated upon caloric excess to counteract the effects of chronic overeating (Rothwell and Stock, 1997). Therefore, studies on thermogenic adipocytes have focused on finding ways to exploit the energy-dissipating properties of BAT as a pharmacological target to treat metabolic disease (Harms and Seale, 2013). However, reports on the functions and activities of BAT in nutrient shortage are currently limited (Reinisch et al., 2020).

From the viewpoint of a thermogenic cell, fasting and cold stress seem to be two processes with opposing biochemical demands. Whereas thermogenesis depends on effective utilization of energy substrates, upon nutrient deprivation, energy substrates (especially glucose) need to be conserved to be directed to the brain. Additionally, several fasting-induced metabolic pathways and signaling molecules, such as β-adrenergic signaling, fatty acids, ketone bodies, and fibroblast growth factor 21 (Fgf21), are well-known inducers of BAT activity (Reinisch et al., 2020). Therefore, counterregulatory mechanisms that balance preservative processes under fasting with the catabolic drive under cold exposure must exist in thermogenic adipocytes.

This study aimed to define BAT activity upon nutrient deprivation with simultaneous mild cold exposure and to elucidate molecular mechanisms that balance thermogenic and energetic demands in BAT of mice. By using unbiased omics approaches, we examined transcriptome and microRNA (miRNA) signatures in BAT upon fasting in mildly cold-stressed mice. In combination with functional *in vitro* experiments, we identified a fasting-selective pathway in BAT involving transcription factor p53, the miRNA miR-92a-1-5p, and the fructose transporter *Slc2a5*, implying a regulated, nutrient-dependent uptake of fructose as an energy substrate in brown adipocytes.

RESULTS

Acute Fasting Mediates Major Alterations in BAT of Mildly Cold-Stressed Mice

The elaborate metabolic response to nutrient deprivation is achieved through a fine-tuned transcriptional program, which is tissue-specific and dependent on the duration and frequency of the fasting stimulus (de Cabo and Mattson, 2019). Due to the lack of information about the transcriptional signature of BAT upon fasting, we performed mRNA transcriptome analysis of male C57BL/6J mice fasted for 24 h or *ad libitum* chow diet-fed controls and maintained under mild cold stress (22°C, singly housed in grid bottom cages). It is clearly established that room temperature is below thermoneutrality in mice (~30°C), which is compensated by a substantial increase in the thermogenic properties of BAT (Ganeshan and Chawla, 2017). Under these conditions, 1,307 genes were upregulated and 1,306 genes were downregulated in BAT of fasted vs. control fed mice (**Figure 1A**), illustrating a striking remodeling of the BAT transcriptomic landscape upon fasting. Performing gene set enrichment analysis revealed a shift towards downregulation of genes in pathways closely associated with the thermogenic program: Oxidative phosphorylation, adipogenesis, and angiogenesis were among the top-downregulated metabolic pathways under acute fasting (**Figure 1B**). Decreased oxidative phosphorylation is consistent with reduced fasting mRNA levels of Pgc1α (**Figure 1C**), the master regulator of mitochondrial biogenesis (Wu et al., 1999). In line with these results, the expression of BAT-selective genes was significantly downregulated after 24 h of fasting (**Figure 1C**). Four hours of refeeding reversed the fasting-mediated decrease in the expression of most thermogenic genes. To shed light on the immediate-early starvation response, BAT samples were harvested from mice maintained at room temperature after 1, 3, 6, 12, or 24 h of food withdrawal. To control for circadian rhythmicity of BAT activity (Gerhart-Hines et al., 2013), control samples were harvested at the same time points from mice fed *ad libitum* with chow diet. Our data showed that *Ucp1* mRNA expression followed a different rhythmicity in the fed compared to the fasted group (**Figure 1D**). *Ucp1* expression levels increased after the first 12 h of fasting, but were significantly decreased after 24 h of fasting compared to *ad libitum* controls. Similar to transcript levels, we found that UCP1 protein levels were also significantly increased after 6 and 12 h of nutrient deprivation and decreased to control levels after 24 h of fasting (**Figure 1E**). Furthermore, brown adipocyte cell size was decreased (**Figures 1F,G**), UCP1 immunostaining was diminished (**Figure 1F**), and BAT weight was reduced (**Figure 1H**) in 24 h fasted mice compared to fed control mice. Systemically, the respiratory exchange ratio (RER), as an indicator of whole-body fuel utilization, was significantly reduced during fasting (**Figures 1I,J**), coinciding with a marked decrease in energy expenditure (**Figures 1K,L**). In situations of severe cold and/or prolonged fasting, mice can enter torpor, which is a hibernation-like state characterized by strongly reduced locomotion, a slowdown in metabolism, and a body temperature below 32°C (Geiser et al., 2014). However, we did

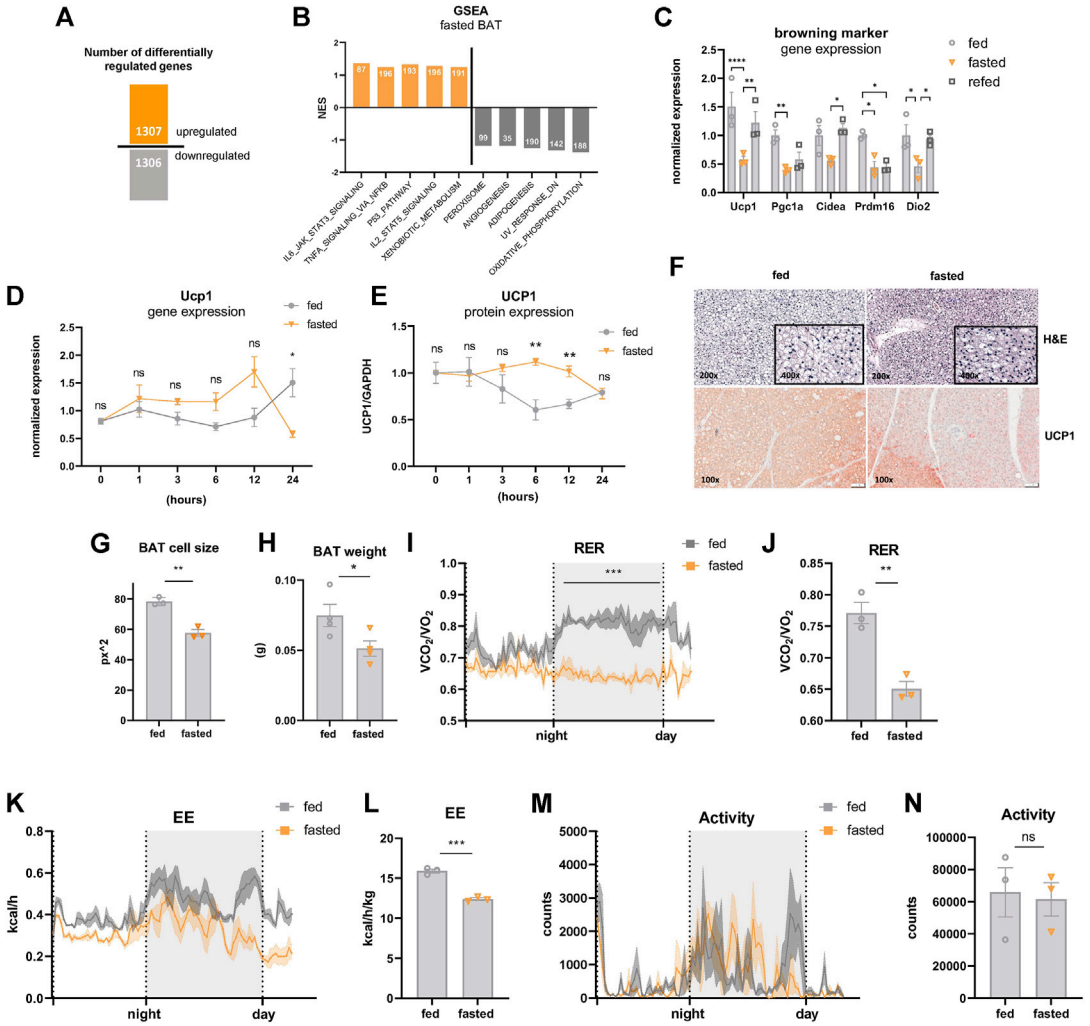

FIGURE 1 | Acute fasting-mediated alterations in BAT of mildly cold-stressed mice. **(A)** Number of up- and downregulated (1.5 x, FDR5) genes in BAT of 24 h fasted mice. **(B)** GSEA Hallmark analysis results with top five pathways with highest or lowest normalized enrichment scores (NES). Numbers of mapped genes are shown in the bars. **(C)** BAT mRNA expression of genes encoding for browning markers of ctrl, 24 h fasted, and 4 h refed mice. **(D)** *Ucp1* mRNA and **(E)** UCP1 protein expression (from WES digital western blotting) in BAT harvested after 1, 3, 6, 12, and 24 h of fasting with samples from circadian-matched *ad libitum* fed controls. GAPDH served as loading control. **(F)** Representative images (magnification, ×100) of BAT of ctrl and 24 h fasted mice stained with hematoxylin and eosin **(E–H)** and immunohistochemistry with anti-UCP1 antibody. **(G)** Cell size of was analyzed by using ImageJ and indicated as square pixels (px^2). **(H)** BAT weight in grams (g) of ctrl and 24 h fasted mice. **(I,J)** Respiratory exchange ratio (RER), **(K,L)** energy expenditure (EE), and **(M,N)** motility of ctrl and 24 h fasted mice as validated by indirect calorimetry in metabolic cages. Data are presented as mean values ± SEM. Significant differences in the mRNA expression of fed, fasted, and refed mice were analyzed by 2-way ANOVA and Tukey's multiple comparisons test, for BAT weight, RER and energy expenditure unpaired t-test was performed. Differences not indicated with asterisks or indicated with ns are not statistically significant ($p > 0.05$). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

not detect any signs of torpor, as indicated by unchanged activity of the mice upon 24 h of fasting in mild cold (**Figures 1M, N**). These data suggest that BAT activity of mice under mild cold stress gradually increases in the first hours after nutrient withdrawal, but is dampened upon 24 h of fasting.

p53 Signaling Is Activated by Acute Fasting in Brown Adipocytes

The transcriptional landscape upon fasting is tightly regulated by a network of distinct nutrient-responsive transcription factors (Schupp et al., 2013; Goldstein et al., 2017). p53 was recently added to the list of fasting-related transcription factors due to its implications in the maintenance of energy homeostasis in the liver of fasted mice (Goldstein and Hager, 2015; Prokesch et al., 2017; Gonzalez-Rellan et al., 2021). In agreement with these reports, we found that p53 signaling was among the top-five upregulated pathways under fasting conditions in BAT of mildly cold-stressed mice (**Figures 1B** and, **2A, B**). We confirmed the upregulation of the p53 target genes *Cdkn1a* and *Ddit4* in fasted BAT by qPCR (**Figure 2C**). The fasting-mediated increase in the expression of p53 target genes was abrogated by 4 h of refeeding (**Figure 2C**), indicating that the p53 activation is dependent on nutritional state. To test if the fasting-triggered increase in p53 signaling is a brown

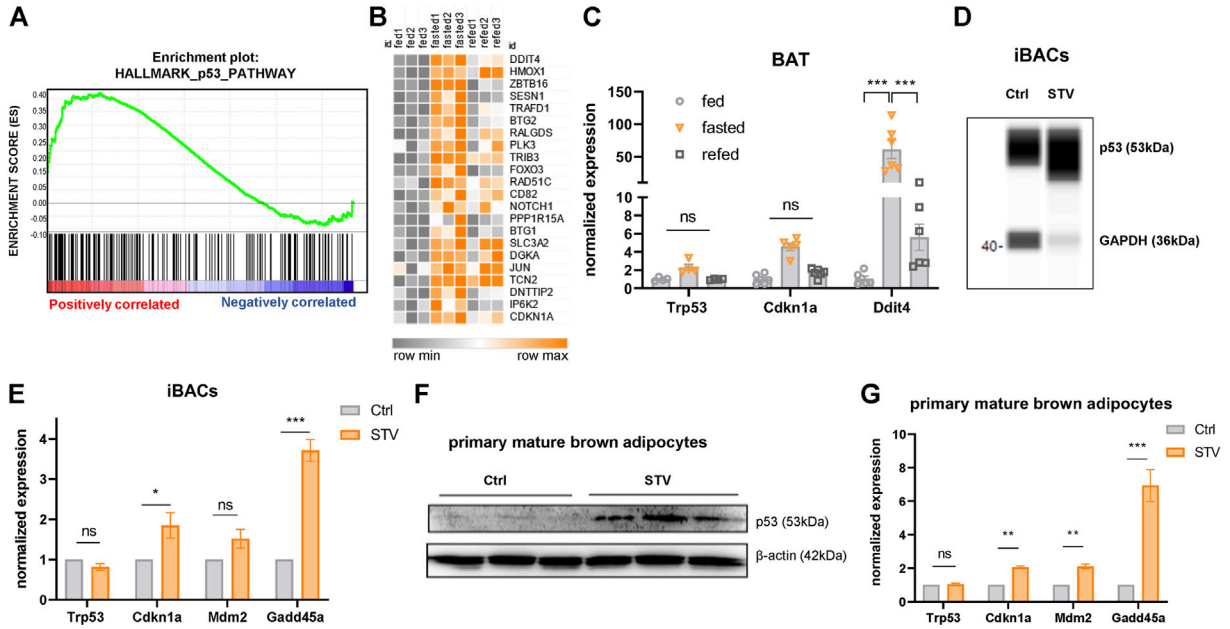

FIGURE 2 | p53 signaling is activated by acute fasting in brown adipocytes. **(A)** Gene set enrichment blot of 193 genes annotated as p53 pathway (NES = 1.33, nominal *p*-value = 0.00) in BAT of 24 h fasted versus fed mice. **(B)** Heatmap showing p53 target genes differentially regulated in BAT of control fed, 24 h fasted and 4 h refed mice. **(C)** mRNA expression of p53 target genes in BAT of ctrl, 24 h fasted, and refed mice as validated by qPCR. **(D)** WES digital western blot of p53 protein of differentiated iBACs cultured in growth medium (Ctrl) or starvation medium (STV) for 24 h. GAPDH served as a loading control. **(E)** mRNA expression of p53 target genes in differentiated iBACs in Ctrl or STV conditions. **(F)** Western blot of p53 in primary mature brown adipocytes in Ctrl or STV conditions. *β*-actin served as loading control. **(G)** mRNA expression of p53 target genes in primary mature brown adipocytes in Ctrl or STV conditions. Mean values ± SEM are shown, significant differences in the mRNA expression of fed, fasted, and refed mice were analyzed by 2-way ANOVA and Tukey's multiple comparisons test. For *in vitro* data, unpaired t-test was performed. Differences not indicated with asterisks or indicated with ns are not statistically significant ($p > 0.05$). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

adipocyte-autonomous mechanism, we used two *in vitro* models: immortalized brown adipocytes (iBACs) and primary preadipocytes isolated and differentiated from the stromal vascular fraction of BAT. In line with the *in vivo* data, p53 protein expression and the transcript levels of p53 target genes were induced in mature iBACs (**Figures 2D,E**) and primary brown adipocytes (**Figures 2F,G**) after 24 h in starvation medium (STV). These data indicate that, also in brown adipocytes, p53 is a fasting-selective transcription factor that might be involved in the regulation of the switch from anabolic to catabolic processes upon nutrient deprivation.

miR-92a-1-5p, a Previously Described Negative Marker for BAT Activity, Is Upregulated in Fasted BAT

Our data showing p53 activation with concomitantly decreased BAT activity after 24 h of fasting is consistent with a recent study that demonstrated p53 as a negative regulator of the thermogenic program in brown adipocytes in mice (Zhao et al., 2020). Therefore, we further investigated the potential underlying regulatory mechanisms. Evidence about p53 acting as a direct transcriptional repressor is very scarce. Indeed, a meta-analysis has suggested that p53 acts solely as a transcriptional activator (Fischer, 2017), whereas most of the repressive effects observed upon p53 signaling activation may be indirect. We reasoned that these indirect effects might be a consequence of p53-mediated expression of transcriptional co-repressors or of miRNAs. To evaluate a possible contribution of miRNAs, we performed miRNA sequencing from the same BAT samples used for transcriptomics. miR-92a-1-5p, which has been previously described as a negative marker for BAT activity (Chen et al., 2016), emerged as the top upregulated miRNA in fasted BAT, whereas the expression of most other miRNAs was reduced (**Figure 3A**). qPCR verified the increased expression of miR-92a-1-5p in fasted BAT (**Figure 3B**) which returned to control levels after 4 h of refeeding, reflecting the nutrient-dependent signatures of p53 target genes (**Figures 2B,C**). More detailed analyses of fasting kinetics showed that mRNA expression levels of miR-92a-1-5p were immediately and increasingly induced by fasting, as validated in our time-course fasting experiment (**Figure 3C**). Finally, we confirmed the upregulation of miR-92a-1-5p in iBACs exposed to the starvation medium for 24 h (**Figure 3D**), indicating a brown adipocyte-autonomous mechanism of miR-92a-1-5p upregulation. Taken together, we delineated the miRNA profile of fasted BAT and identified miR-92a-1-5p as the top fasting-induced miRNA.

Fasting-Induced miR-92a-1-5p Downregulates the Nutrient-Regulated Fructose Transporter *Slc2a5* in Brown Adipocytes

Combining our transcriptomic analysis with seed match predictions in the 3′ untranslated regions (UTR), we generated

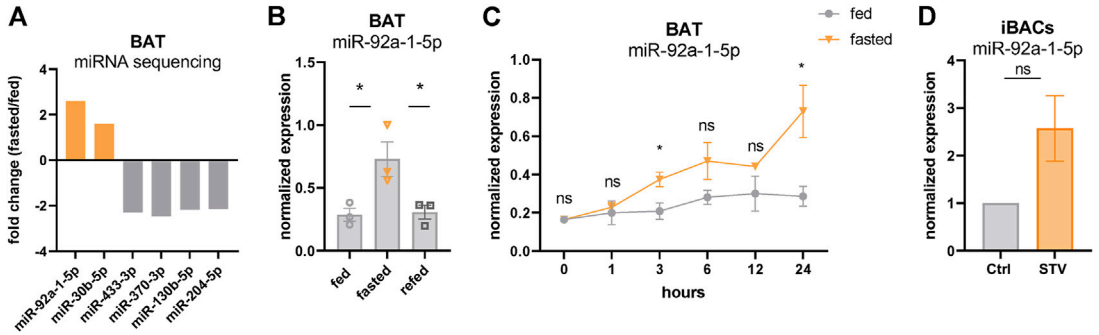

FIGURE 3 | miR-92a-1-5p is upregulated in fasted BAT. **(A)** Top up- and downregulated miRNAs in 24 h fasted BAT determined by miRNA sequencing ($n = 3$). **(B)** Expression of miR-92a-1-5p in BAT of ctrl, 24 h fasted, and refed mice validated by qPCR. **(C)** Time-series of miR-92a-1-5p expression in BAT harvested after 1, 3, 6, 12, and 24 h of fasting in mildly cold-stressed mice. As control, BAT was harvested from *ad libitum* fed mice at the respective circadian-matched time points ($n = 3$). **(D)** Expression of miR-92a-1-5p in differentiated iBACs cultivated in growth medium or starved for 24 h ($n = 3$). Mean values ± SEM are shown, significant differences in the mRNA expression of fed, fasted and refed mice were analyzed by 2-way ANOVA and Tukey's multiple comparisons test. For *in vitro* data, unpaired t-test was performed. Differences not indicated with asterisks or indicated with ns are not statistically significant ($p > 0.05$). *$p < 0.05$.

a list of potential miR-92a-1-5p target genes (**Figure 4A**), that are downregulated by fasting (**Figure 4B**) and associated with thermogenesis, lipid metabolism, or carbohydrate uptake. To functionally validate these predictions, we analyzed alterations in the expression of these candidate genes upon overexpression of miR-92a-1-5p mimic in iBACs (**Figure 4C**). We found no overt differences in the expression of most candidate genes tested, except for the markedly reduced expression of fructose transporter *Slc2a5* in iBACs (**Figure 4D**). In our transcriptome data, *Slc2a5* was the most diminished gene upon fasting in BAT (19-fold downregulation, **Figure 4E**). The putative interaction between miR-92a-1-5p and the predicted miRNA recognition element in the *Slc2a5* 3′UTR is shown in **Figure 4F**. Furthermore, 24 h exposure of iBACs to starvation medium, conditions in which p53 protein was stabilized (**Figure 2D**) and miR-92a-1-5p expression increased (**Figure 3D**), led to a drastic decrease in *Slc2a5* expression (**Figure 4G**). To validate the direct interaction between miR-92a-1-5p and *Slc2a5*, we cloned the 3′UTR of *Slc2a5* containing the predicted seed matches of miR-92a-1-5p into a luciferase reporter vector and performed co-transfection with miR-92a-1-5p mimic or a non-targeting control in HEK293 cells. Co-transfection of miR-92a-1-5p mimic trended to reduce the luciferase activity (**Figure 4H**), suggesting a direct interaction of miR-92a-1-5p with the 3′UTR of *Slc2a5*, which may lead to subsequent degradation of *Slc2a5* mRNA. To probe if increased nutrient supply modulates the expression of *Slc2a5 in vivo*, we challenged C57BL/6 mice with a high-glucose diet. Interestingly, high-glucose feeding led to a substantial increase in the expression of *Slc2a5* in BAT (**Figure 4I**), with a concomitant decline in the expression of p53 target genes (**Figure 4I**). These data suggest *Slc2a5*, a highly nutrient-responsive gene in BAT, as a direct target of fasting-induced miR-92a-1-5p. Moreover, we demonstrated that the expression of *Slc2a5* in BAT is induced by high-glucose feeding *in vivo*, corroborating a potential functional role for fructose metabolism in BAT.

Fructose Is Taken up and Metabolized by Brown Adipocytes

SLC2A5 (also called GLUT5) is a member of the GLUT family of facilitated sugar transporters that, unlike the other members, has an almost exclusive affinity for fructose (Mueckler and Thorens, 2013). SLC2A5 is mainly expressed at the apical surface of intestinal epithelial cells, but also to a lower extent in testis, kidneys, brain, skeletal muscle, and adipose tissues (Cura and Carruthers, 2012). Previous studies have shown that fructose can be metabolized in an *in vitro* model of mature white adipocytes (Varma et al., 2015a; 2015b). However, the functional role of fructose in thermogenic adipocytes is unknown. To investigate whether fructose can be taken up by brown adipocytes, we supplemented the standard growth medium of iBACs with fructose and analyzed the abundance of intracellular metabolites by nuclear magnetic resonance metabolomics. Strikingly, fructose was avidly taken up by brown adipocytes, concomitant with a significant decrease in the abundance of branched-chain amino acids [BCAAs; isoleucine, leucine, valine, which are substrates for NST (Yoneshiro et al., 2019)] and glycine (**Figure 5A**). In white adipocytes, it was suggested that fructose is not catabolized *via* fructolysis but feeds into glycolysis after being phosphorylated at position six by hexokinase (Legeza et al., 2017). To probe if fructose is indeed metabolized via glycolysis, we quantified the extracellular acidification rate (ECAR) of iBACs after acute exposure to fructose or glucose in a Seahorse glycolytic function assay. Acute fructose infusion led to increased proton generation (or acidification), suggestive of conversion of fructose to lactate (**Figures 5B,C**), albeit to a lower extent than after glucose infusion (**Figures 5B,D**). Fructose is a potent inducer of signaling through the carbohydrate response element-binding protein (ChREBP), which controls the expression of genes involved in lipogenesis, glycolysis, and fructolysis (Ortega-Prieto and Postic, 2019). Analysis of ChREBP target genes in iBACs exposed to fructose revealed a trend toward increased expression of *Mid1ip1, Khk-a,* and

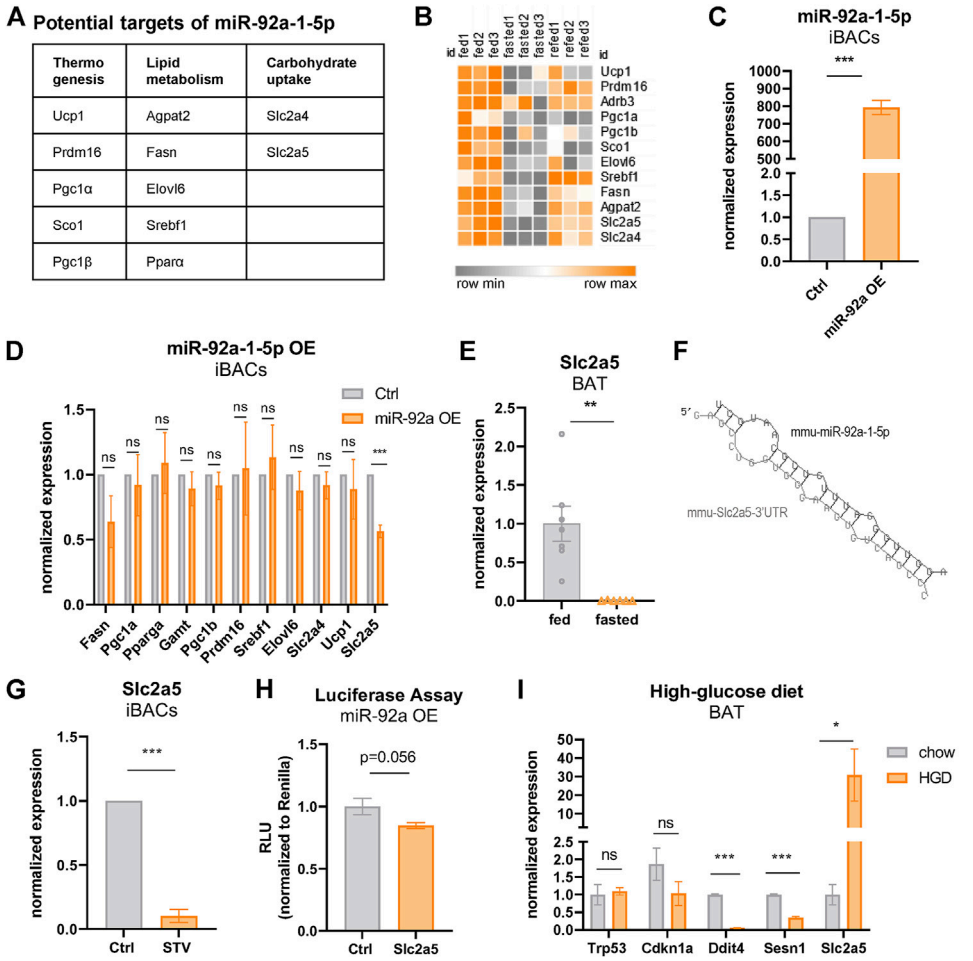

FIGURE 4 | Fasting-induced miR-92a-1-5p downregulates the fructose transporter *Slc2a5* in brown adipocytes. **(A)** Predicted target genes of miR-92a-1-5p downregulated by fasting in BAT. **(B)** Heatmap of predicted, fasting-downregulated miR-92a-1-5p targets in BAT of ctrl, 24 h fasted, and refed mice. **(C)** qPCR expression levels of miR-92a-1-5p in iBACs after transfection with miRNA-mimic-92a-1-5p or non-targeting control. **(D)** mRNA expression of predicted target genes of miR92a-1-5p in iBACs after transfection of miR-mimc-92a-1-5p or non-targeting control. **(E)** mRNA expression of *Slc2a5* in BAT of fed or 24 h fasted mice. **(F)** Putative miRNA seed match of miR-92a-1-5p within the 3'UTR of *Slc2a5*. **(G)** mRNA expression of *Slc2a5* in iBACs cultured in standard growth medium or starved for 24 h. **(H)** Luciferase assay was performed by co-transfecting a reporter vector containing the 3'UTR of *Slc2a5* harboring the predicted binding sites of miR-92a-1-5p, with miR-92a-1-5p mimic or non-targeting control in HEK293 cells. **(I)** mRNA expression of *Slc2a5* and p53 target genes in BAT of mice fed chow or high-glucose diet. Mean values ± SEM are shown, significant differences in the mRNA expression of fed and fasted mice were analyzed by performing an unpaired t-test. For *in vitro* data, unpaired t-test was performed. Differences not indicated with asterisks or indicated with ns are not statistically significant ($p > 0.05$). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

Chrebp-β, and a significant induction of *Elovl6* (**Figure 5E**), which is critically involved in fatty acid elongation. Taken together, we demonstrated that fructose 1) can be taken up by brown adipocytes, 2) is, at least in part, directed into the glycolytic pathway, and 3) leads to partial activation of the carbohydrate-responsive ChREBP pathway. However, the metabolic pathways and consequences of fructose utilization in brown adipocytes requires further research.

p53 Binds and Activates the miR-92a-1-5p Locus

Based on the function of p53 in regulating metabolism in response to various nutritional cues (Labuschagne et al., 2018) and on the co-regulation of miR-92a-1-5p and p53 targets in BAT (**Figures 2C**, **3B**), we hypothesized that miR-92a-1-5p might be a direct nutrient-responsive target of p53. In line with this notion, JASPAR binding site predictions yield a p53 binding site in the mouse miR-92-1 locus (**Figure 6A**, red shaded boxes). Intriguingly, this p53 binding site is fully conserved in the human MIR92A1 locus (**Figure 6A**, lowest panel). To further examine if miR-92a-1-5p is a direct target of p53, we modulated the expression of p53 in mature iBACs. Pharmacologic stabilization and activation of p53 with the small molecule nutlin (**Figure 6B**) resulted in a significantly increased expression of miR-92a-1-5p (**Figure 6C**) and a tendency, albeit not significant, to downregulation of *Slc2a5* (**Figure 6D**). In addition, chromatin immunoprecipitation

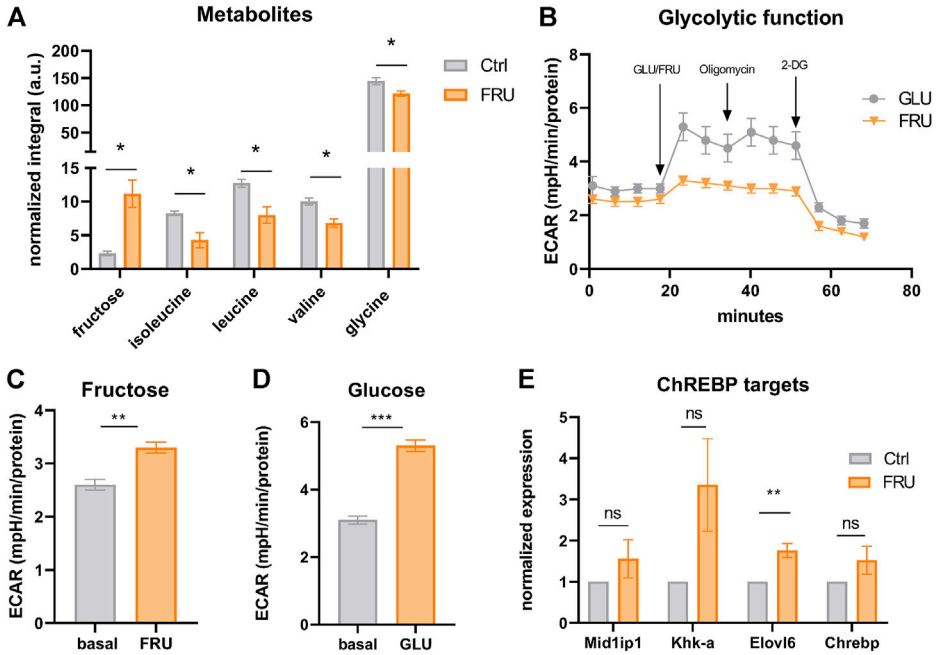

FIGURE 5 | Fructose is taken up and metabolized by brown adipocytes. **(A)** Abundance of intracellular fructose and the amino acids isoleucine, leucine, valine, and glycine in iBACs cultivated in growth medium or supplemented with 5 g/L fructose measured by nuclear magnetic resonance (NMR). **(B)** Continuous extracellular acidification rate (ECAR) in BACs after the injection of 5 g/L fructose or glucose. Oligomycin and 2-deoxyglucose (2-DG) were acutely injected after measuring basal ECAR. **(C,D)** Basal ECAR (analyzed after 20 min of measurement) and stimulated ECAR after acute infusion of 5 g/L fructose **(C)** or glucose **(D)**. **(E)** mRNA expression of ChREBP target genes in iBACs kept in growth medium or supplemented with 5 g/L fructose. Mean values ± SEM are shown, significant differences in the mRNA expression of intracellular metabolites and seahorse experiments were analyzed by performing an unpaired t-test. For mRNA expression of ChREBP targets, unpaired t-test was performed. Differences not indicated with asterisks or indicated with ns are not statistically significant ($p > 0.05$). ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

qPCR showed a significant enrichment of p53, over a negative control region and IgG antibody control, at the miR-92a-1-5p locus, confirming a direct binding of p53 to the miR-92 locus (**Figure 6E**). p53 binding in a known recognition element in the *Cdkn1a* (p21) locus served as a positive control (**Figure 6E**). In line with these findings, overexpression of full-length p53 (**Figure 6F**) increased the expression of miR-92a-1-5p (**Figure 6G**) with concomitant decrease of *Slc2a5* (**Figure 6H**). Taken together, p53 activation, followed by an increased miR-92a-1-5p abundance with concomitant downregulation of *Slc2a5* may represent a functional signaling cascade regulating fructose utilization in brown adipocytes with p53 as the nutrient-responsive upstream regulator (**Figure 7**).

DISCUSSION

In many peripheral tissues, nutrient deprivation elicits a tightly controlled switch from anabolic to energy-conserving mechanisms that provide energy substrates to the brain, which cannot adequately store energy or efficiently utilize fatty acids that are abundantly released during fasting (Secor and Carey, 2016). Little is known about the fasting response of BAT, especially during simultaneous cold exposure when demands for energy conservation theoretically counteract energy consumption for thermogenesis (Reinisch et al., 2020). We defined the transcriptomic signature of BAT from 24 h fasted mice exposed to mild cold stress at the mRNA and miRNA level. Our data suggest that within 24 h, maintenance of energy homeostasis is favored over thermogenesis. This was reflected in decreased expression of BAT-selective genes and genes involved in oxidative phosphorylation, diminished BAT weight, and reduced RER, as well as in disruption of diurnal mRNA and protein rhythmicity of Ucp1, that was recently shown to be under control of circadian and metabolic regulator Rev-erbα (Gerhart-Hines et al., 2013). Decreased oxidative phosphorylation was in line with a recent publication showing reduced mitochondrial content in BAT of mice that were intermittently fasted (Harney et al., 2021). In contrast, earlier studies showed no differences in norepinephrine turnover as a proxy for sympathetic drive, in the expression of thermogenic genes, or in BAT weight after 24 h of fasting in mildly cold stressed mice (Knehans and Romsos, 1983; Ding et al., 2016). These discrepant results may be attributed to different housing conditions of mice. The mice in our study were housed individually, without nesting material, and in grid-bottom cages to prevent coprophagy, eating of bedding material, and external thermo-homeostasis through nesting and shared body heat. Thus, the housing conditions in our study represent a more severe cold and fasting stress.

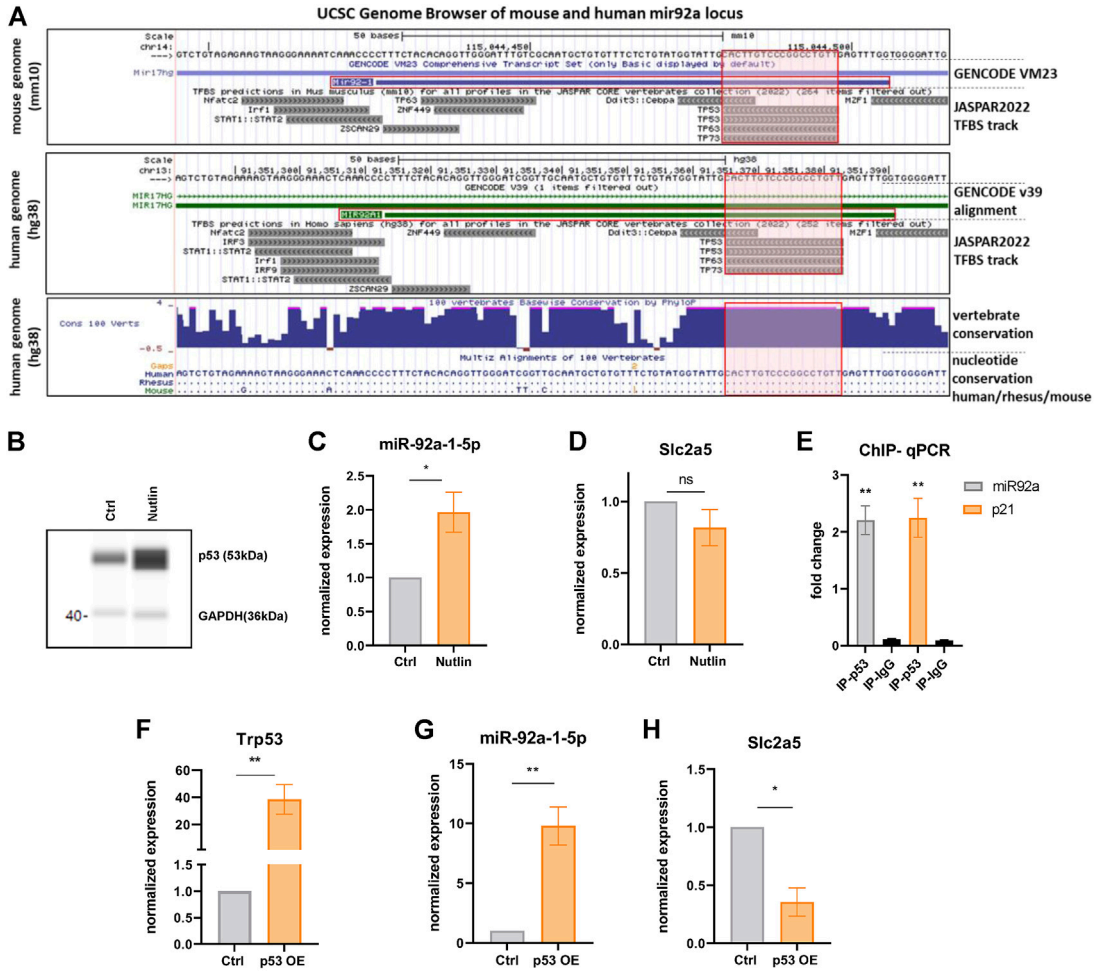

FIGURE 6 | p53 binds and activates the miR-92a-1-5p locus. **(A)** Excerpt from the UCSC genome browser tracks from mouse (mm10) and human (hg38) genomes in the vicinity of the mir92a locus (Mir92-1 for mouse chromosome 14, MIR92A1 for human chromosome 13). Respective JASPAR tracks illustrate p53 binding motives (red shaded boxes) that are fully conserved between human, rhesus monkey, and mice (lowest box; dots depict sequence identity). **(B)** WES digital western blot of p53 protein of iBACs after nutlin or vehicle treatment. GAPDH served as a loading control. **(C)** miR-92a-1-5p and **(D)** *Slc2a5* expression after nutlin or vehicle treatment of iBACs. **(E)** Fold-enrichment of a p53-binding site at the miR-92a-1-5p locus. ChIP-qPCR was used to amplify chromatin derived from immunoprecipitations with anti-p53 antibody or anti-IgG antibody. A known p53 binding site in the *Cdkn1a* locus served as positive control and primer pairs targeting distant loci without predicted p53 binding sites served as negative control. **(F)** mRNA expression of *Trp53* upon overexpression of full length wild-type p53 (or transfection with empty control vector) in iBACs. Expression of **(G)** miR-92a-1-5p and **(H)** *Slc2a5* upon overexpression of p53 or control in iBACs. Data are presented as mean values ± SEM. Significances were determined by unpaired Student's t-tests. Differences not indicated with asterisks or indicated with ns are not statistically significant ($p > 0.05$). *$p < 0.05$, and **$p < 0.01$.

Previously described fasting-induced core regulators of the thermogenic program involve metabolites like fatty acids and ketone bodies, signaling pathways like norepinephrine signaling, and hormones like glucagon, glucocorticoids, and FGF21 (Reinisch et al., 2020). However, whereas fasting-selective transcription factors have been well-described in the liver (Goldstein and Hager, 2015), the transcriptional regulation of the thermogenic program upon fasting in adipocytes has received little attention. The transcription factor p53 was originally described as main player in cancer development, as evidenced by the high prevalence of *TP53* mutations in several cancer types in humans (Baker et al., 1990a; Baker et al., 1990b; Olivier et al., 2002). However, besides its vital function as tumor suppressor, more recent studies have established p53 as an important regulator of metabolism and tissue homeostasis in non-cancer contexts (Lacroix et al., 2020). This was corroborated by findings of our group, which demonstrated that the p53 pathway is activated within 24 h of nutrient withdrawal in the major fasting-responsive mouse tissues [white adipose tissue, liver, and skeletal muscle (Schupp et al., 2013)]. Moreover, p53 has been delineated as a nutrient-responsive transcription factor that is pivotal for the physiological response to fasting in the liver (Prokesch et al., 2017; Gonzalez-Rellan et al., 2021). In these studies, p53 activity was regulated through stabilization of the p53 protein by upstream stress stimuli, rather than through upregulation of p53 mRNA. This is consistent with our

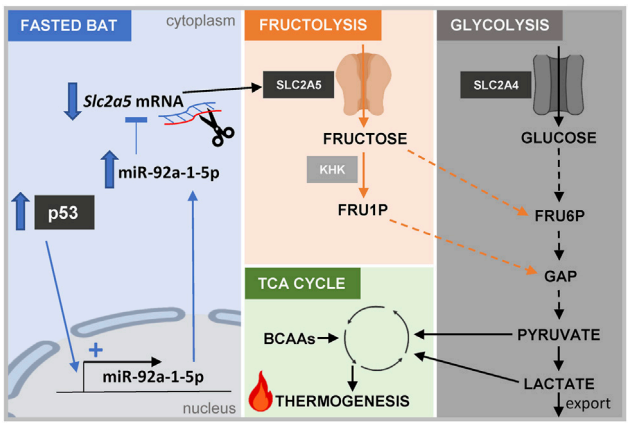

FIGURE 7 | Graphical abstract. Scheme depicting the proposed connection between the p53/miR-92a-1-5p/Slc2a5 axis, fructose and glucose metabolism, and thermogenesis in brown adipocytes. Abbreviations: KHK, keto-hexokinase; FRU1P, fructose-1-phosphate; FRU6P, fructose-6-phosphate; GAP, glyceraldehye-3-phosphate; BCAAs, branched-chain amino acids.

findings in BAT where p53 mRNA levels remained unchanged by fasting (**Figure 2C**), while p53 signaling was broadly activated (**Figures 2A–C**). Interestingly, earlier work in colon cancer cells showed p53 stabilization upon AMPK-mediated phosphorylation (Jones et al., 2005), a mechanism that could also play a role in brown adipocytes under fasting.

In adipocytes, there is increasing evidence for a function of p53 in the control of lipid metabolism, (brown) adipocyte differentiation, thermogenesis, and systemic energy homeostasis (Al-Massadi et al., 2016; Krstic et al., 2018). Whereas a previous report described p53 as a positive regulator of the thermogenic program (Al-Massadi et al., 2016), a more recent study demonstrated KMT5c-knock out-dependent p53 induction responsible for the diminished expression of genes in the thermogenic program (Zhao et al., 2020), highlighting the context-dependent mode of action of p53.

Thus, based on our data and previous reports, p53 seems to act as fasting-dependent negative regulator of the thermogenic program in BAT. However, since there is little evidence for p53 as a direct transcriptional repressor (Fischer, 2017), we hypothesized that the repressive actions of p53 in BAT might be regulated indirectly by miRNAs. While we cannot entirely rule out a direct impact of p53 on Slc2a5, our collective data including luciferase assay and miR-92a-1-5p overexpression, indicate that p53 acts through miR-92a-1-5p. In general, p53 regulates a plethora of different miRNAs in various contexts, either by inducing their transcription or by promoting their processing and maturation (Hermeking, 2012; Goeman et al., 2017). In addition, several BAT-derived miRNAs have been described to affect the thermogenic program (Goody and Pfeifer, 2019). Here, we elucidated miR-92a-1-5p as the top fasting-responsive miRNA in BAT. Intriguingly, Chen et al. (2016). showed that miR-92a is present in human and mouse BAT-derived exosomes and that the abundance of miR-92a in serum exosomes inversely correlates with human BAT activity. Seed match prediction analysis revealed a plethora of thermogenesis-, lipid-, and carbohydrate-associated genes as potential targets of miR-92a-1-5p. However, in *in vitro* experiments we could only confirm the fructose transporter *Slc2a5* as a direct target of miR-92a-1-5p. Notably, in our transcriptome analysis, *Slc2a5* was the most downregulated gene in fasted BAT.

Furthermore, our data provide evidence that iBACs can take up fructose avidly and metabolize it, suggesting a physiological significance of fructose uptake by SLC2A5 in brown adipocytes. Previous studies have already shown that the supplementation of fructose to the growth medium of white adipocyte precursor cells can increase their differentiation capacity and that fructose is a potent lipogenic substrate, triggering the formation of oleate and palmitate in human mature white adipocytes (Varma et al., 2015a; 2015b). Moreover, it was recently shown that despite the low abundance of fructose in the systemic circulation in physiological conditions, intravenously administered labeled fructose can be detected in BAT of mice (Jang et al., 2018) and that *Slc2a5* is strongly induced in BAT of mildly cold-stressed mice (Sanchez-Gurmaches et al., 2018). However, further studies need to examine the fate of fructose, the regulatory role of fructose and/or products of fructose catabolism, and the implications of ChREBP signaling and BCAA metabolism in BAT during times of nutrient restriction.

Of note, our findings are derived from male mice only and a recent report showed systemic differences (mostly male hyperinsulinemia) in the fasting/feeding response in male and female C57Bl/6J mice (Bazhan et al., 2019). In this study BAT weight and the expression of genes measured in BAT was not different between males and females (Bazhan et al., 2019). As the functional axis we report here seems to act cell autonomous, it is possible that it also operates in females, although this needs to be formally tested.

Taken together, our study delineated the transcriptome and miRnome responses of BAT from mice that were challenged with an acute 24 h fasting bout and mild cold stress. Targeted *in vitro* experiments validated a novel, fasting-selective pathway involving p53 signaling to regulate the fructose transporter *Slc2a5* by miR-92a-1-5p modulation. Furthermore, our results add to data suggesting a metabolic role of fructose as an energy substrate in brown adipocytes (**Figure 7**).

Contribution to the Field Statement: Active BAT is a highly energy-consuming tissue and is of critical importance for the regulation of whole-body energy homeostasis. Thermogenic active depots found in adult humans, frame BAT as an attractive pharmacological target for the treatment of metabolic diseases. However, the regulation of thermogenic properties of BAT under fasting conditions, especially under simultaneous mild cold stress, has not been explored until now. We elucidated the transcriptional and miRNA signature in BAT of mildly cold-stressed, acutely fasted mice and identified a highly nutrient-dependent pathway involving the transcription factor p53, miR-92a-1-5p, and the fructose transporter *Slc2a5*. We propose that repression of *Slc2a5*, by miR-92a-1-5p, downstream of p53, could represent a mechanism for limiting the thermogenic properties of BAT during fasting.

METHODS

Mouse Experiments

All animal studies were approved by the Austrian Ministry for Education, Science and Research (Vienna, Austria, BMWFW-66.010/0087-WF/V/3b/2017) and performed strictly according to its guidelines. Animals (C57BL/6J, 12 weeks of age) were housed in a temperature-controlled (22°C) environment with a 12:12 h light-dark cycle. For fasting experiments, food was withdrawn at 9:00 a.m. for 24 h. To prevent coprophagy, fasted and *ad libitum* chow diet-fed mice were single-housed on grid bottoms without nesting material during the intervention. For high-glucose feeding experiments, C57BL/6J mice were fed a high-glucose diet (Ssniff Spezialdiaeten GmbH, Soest, Germany, E15629-34) for 12 weeks (Huber et al., 2019). Mice were sacrificed by cervical dislocation, and harvested interscapular BAT depots were immediately frozen in liquid nitrogen.

Metabolic Cages

Metabolic assessment of mice was performed using an indirect calorimetry system (TSE PhenoMaster, TSE Systems, Bad Homburg, Germany). The animals were single-housed at room temperature, a regular light-dark (12:12 h) cycle, and with free access to food and water. Mice were acclimated to the metabolic cages for 48 h before metabolic recording. After 1 week of metabolic recording, the fasting experiment was performed by withdrawing the food at 9:00 a.m. for 24 h. O_2 consumption, CO_2 production, and locomotor activity (using infrared sensor frames) were measured every 15 min.

Cell Culture

iBACs

Immortalized brown preadipocytes [iBACs (Harms et al., 2014)] were a kind gift of Patrick Seale. iBACs were maintained in DMEM (Thermo Fisher Scientific, Waltham, MA, United States, 41966-029) containing 4.5 g/L glucose supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, HyClone TM, Thermo Fisher Scientific, Waltham, MA, United States, SV30160.03), 1% penicillin and streptomycin (Thermo Fisher Scientific, Waltham, MA, United States, 15140-122), and 20 mM HEPES (Thermo Fisher Scientific, Waltham, MA, United States, 1,560-080). Cells were cultivated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. iBACs were induced to undergo adipogenesis at a confluence of ~90% by the addition of 500 nM dexamethasone, 1 nM triiodothyronine, 0.5 mM 3-isobutyl-1-methylxanthine, 1 µg/ml insulin, and 125 mM indomethacin (all Sigma-Aldrich, St. Louis, MI, United States) to the growth medium. From day three on, the growth medium was supplemented with 1 µg/ml insulin and 1 nM triiodothyronine, and changed every other day. Fully differentiated iBACs (day 7–10) were used for the experiments.

Stromal Vascular Fraction Isolation and Differentiation Into brown Adipocytes

BAT depots were harvested, finely minced with scissors, and incubated in collagenase solution (4 mg/ml collagenase Type II (Thermo Fisher Scientific, Waltham, MA, United States, 17101015), 10 mM $CaCl_2$, 0.5% FFA-free BSA (Sigma-Aldrich, St. Louis, MI, United States, 126609) for ~10 min at 37°C. Digestion was stopped by adding 30 ml of growth medium and cells were filtered through a 100 µm sieve. SVF was pelleted by centrifugation at 600 *x g* for 15 min. After adding 1 ml of erythrocyte lysis buffer and incubating for 1 min, 30 ml of growth medium was added and filtered through a 70 µm sieve. The SVF was pelleted by centrifugation at 600 *x g* for 15 min und seeded in T75 flasks in growth medium (DMEM/F12 with glutamax (Thermo Fisher Scientific, Waltham, MA, United States, 10565018), supplemented with 10% FBS, 1% penicillin and streptomycin). To induce differentiation, the growth medium was supplemented with 1 µM dexamethasone, 100 nM triiodothyronine, 0.5 mM 3-isobutyl-1-methylxanthine, 1.5 µg/ml insulin, and 1 µM rosiglitazone (all Sigma-Aldrich, St. Louis, MI, United States). After 3 days, medium was changed to growth medium supplemented with 1. 5 µg/ml insulin and 100 nM triiodothyronine. Primary brown adipocytes were harvested 7–9 days after differentiation start.

Treatments

For starvation experiments, cells were washed with PBS (Thermo Fisher Scientific, Waltham MA, United States, 10010-015) and maintained in starvation medium [HBSS (Thermo Fisher Scientific, Waltham, MA, United States, 14175-053) supplemented with 10 mM HEPES (Thermo Fisher Scientific, Waltham, MA, United States, 1560-080)] for 24 h. To pharmacologically stabilize p53, iBACs were treated with 1 µM Idasanutlin (Selleck Chemicals, Houston, United States, RG-7388) for 24 h. For fructose experiments, iBACs were incubated for 24 h in growth medium supplemented with 5 g/L fructose (Sigma-Adrich, St. Louis, MI, United States, F3510).

Tissue Isolation

Tissues were homogenized in Qiazol (Qiagen, Hilden, Germany, 79306) using Magnalyser beads (PeqLab, Radnor, United States, 412-0201) at 6.500 rpm for 20 s and two runs with the TissueLyser (Qiagen, Hilden, Germany). Samples were cooled with short taps in N_2 between the runs and incubated for 5 min at room temperature. RNA was isolated with PeqGOLD total RNA kit (Peqlab, Radnor, United States, 12-6634) according to the manuals. Sample purification and concentration was quantified with NanoDrop® ND-1000 (Peqlab, Radnor, United States). For western blotting experiments, tissues were homogenized with Magnalyser beads in radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 0.1% SDS, 0.5% Na-deoxycholate, 1% NP-40, adjusted to pH 7.2 — 7.4) supplemented with PIC (complete Tablets EASYpack, Roche, Basel, Switzeland, 04693116001) and PhosStop (Roche, Basel, Switzeland, 04906837001), incubated on ice for 20 min, and centrifuged at 15.000 *x g* for 15 min. The protein concentration of cleared supernatants was analyzed with a bicinchoninic acid assay kit (BCA, Thermo Fisher Scientific, Waltham, MAs, United States).

qPCR Analysis

For qPCR analysis, isolated total RNA was reverse transcribed to cDNA by using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, United States, 4368814) and amplified using Blue SybrGreen qPCR mastermix (Biozym

TABLE 1 | Nucleotide sequences for qPCR, cloning, and ChIP primers.

	Fwd (5′–3′)	Rev (5′–3′)
Ucp1	GGATTGGCCTCTACGACTCA	TAAGCCGGCTGAGATCTTGT
Pgc1a	GGTCAAGATCAAGGTCCCCA	TCATAGCTGTCGTACCTGGG
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATTCGCTTGTG
Dio2	GTCCGCAAATGACCCCTTT	CCCACCCACTCTCTGACTTTC
Trp53	ACATGACGGAGGTCGTGAG	AATTTCCTTCCACCCGGATA
Cdkn1a	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
Mdm2	AGCAGCGAGTCCACAGAGAC	ATCCTGATCCAGGCAATCAC
Gadd45a	CCGAAAGGATGGACACGGTG	TTATCGGGGTCTACGTTGAGC
Ddit4	TCTTCGCTGACCGCGCTAGC	CGGCCGGAGTTCGAGACGAG
Slc2a5	GCTGCAGCCAAATTGCCCAATCG	CGGGGCCAGCTCCCCTAAGT
Fasn	CACCAATACAGATGGCAGCA	CCAGCTGGCTGATACAGAGA
Ppara	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGGTTCTT
Gamt	GCAGCCACATAAGGTTGTTCC	CTCTTCAGACAGCGGGTACG
Srebf1	AAGCCAATCACTGAAGGACCTGG	AAAGACAAGGGGCTACTCTGGGAG
Elovl6	CGTAGCGACTCCGAAGATCAGCC	AGCGTACAGCGCAGAAAACAGGA
Glut4	GGCATCAATGCTGTTTTCTAC	GCTGGAACCGCTTCCAGC
Sesn1	CGGACCAAGCAGGTTCATCC	TGATGTTATCCAGACGACCCAAA
Mid1ip1	GGT GAA CAA CAT GGA CCA GA	CGC TGA CCT CGT CTA TCT CC
Khk-a	TGGACTTACGATATGTCCTT	GCCTCGTTGATGATGACTGTAG
Chrebp-b	TCTGCA GATCGCGTGGAG	CTT GTCCCGGCATAGCAAC
TFIIb	GTCACATGTCCGAATCATCCA	TCAATAACTCGGTCCCCTACAA
	CLONING PRIMER	
Slc2a5 insert	TTGCTCGAGCACAGCCATCTT	TGGCGGCCGCCAGAATGTGCTT
	ChIP	
miR-92a-1-5p	TTGGGATTTGTCGCAATGCTG	TCTGGTCACAATCCCCACCA
p21	CTGTTGCCTCTCGGAGACC	CCTGAAGGCCAGAAAGCTAGT
Neg. Ctrl	TGAGCACAGGAGAAAAGGCAA	GCCTACCAAGACAAATGAGCAG

Scientific, Olendorf, Germany, 331416XL). All primer sequences are listed in **Table 1**. TFIIb was used as reference gene.

Western Blot Analysis

Immunoblotting was performed as described elsewhere (Prokesch et al., 2016). WES digital western blot (Bio-techne, Proteinsimple, Minneapolis, and Minnesota) was performed according to the manufacturer's guidelines, using 10 µg of protein.

Antibodies used: p53 (D2H9O, Cell Signalling, Danvers, MA, United States, 32532), GAPDH (Cell Signalling, Danvers, MA, United States, 2118S), β-actin (Abcam, Cambridge, United Kingdom, ab6276).

miRNA Isolation

miRNA isolation from iBACs and BAT was performed with the Qiagen miRNeasy Kit (Qiagen, Hilden, Germany, 217004) according to the manufacturer's guidelines. In short, about 100 mg of BAT was dissected in 700 µl Qiazol (Qiagen, Hilden, Germany, 79306) as described above. 140 µl of chloroform was added to the samples, shaked vigorously and incubated for 2–3 min at room temperature. After spinning the samples at 12.200 rpm for 15 min at 4°C, the supernatant was mixed with 1.5 volume of 100% ethanol. A maximum of 500 µl was loaded to the MiniElute tubes and the protocol was processed as outlined by the manufacturer's instructions and total RNA was eluted in 20 µl EB-buffer (Qiagen, Hilden, Germany). All samples were quality checked on a BioAnalyzer BA2100 station (Agilent, Foster City, CA, United States).

miRNA-Reverse Transcription and miRNA-qPCR

Reverse transcription (miRCURY LNA RT Kit, Qiagen, Hilden, Germany, 339340) and qPCR (miRCURY LNA SYBR Green PCR Kit, Qiagen, Hilden, Germany, 339345) of miRNAs was performed according to the manufacturer's guidelines.

Transcriptome Analysis and miRNA Sequencing

For whole Transcriptome analysis 200 ng of total RNA were used with the GeneChip™ Human Transcriptome Assay 2.0 kit (Thermo Fisher Scientific, Waltham, MA, United States) according to manufacturer's instructions. Arrays were washed after hybridization on a GeneChip™ Fluidics Station 450 and scanned on a GeneChip™ Scanner 3,000 7G. Raw microarray data have been submitted to Gene Expression Omnibus (GEO accession number GSE199963).

For miRNA library preparation 100 ng of total RNA were used with the NEBNext® Small RNA Library Prep Set for Illumina® (New England Biolabs, Ipswich, MA, United States, E73305) according to manufacturer's instructions. Quality of libraries was checked on an Agilent BioAnalyzer BA2100 station, pooled and sequenced in an Illumina HiSeq lane (Illumina, Eindhoven, Netherlands). FastQ raw data are publicly available in the European Nucleotide Archive (ENA) with the accession number PRJEB51729 (https://www.ebi.ac.uk/ena, last access March 2022).

Transcriptome and miRNA raw data were normalized and analyzed using Partek® Genomics Suite® Software 6.6 (Partek Incorporated, St. Louis, MI, United States) according to standard settings. Array data were normalized using robust multi-chip average normalization (RMA).

Gene Set Enrichment Analysis (GSEA) was performed with the GSEA app (Broad Institute) on a list ranked according to expression changes between BAT of fasted and fed mice. Hallmark analysis was conducted according to default settings and top five enriched and de-enriched hallmark pathways were identified and displayed (Subramanian et al., 2005).

Putative targets of mmu-miR-92a-1-5p (miRBase accession number MIMAT0017066) were obtained from miRWalk 2.0 (Dweep et al., 2011; Sticht et al., 2018), integrating the output of several miRNA-target prediction algorithms. The query for potential mmu-miR-92a-1-5p MREs was limited to 3′UTRs of mRNAs. The interaction of mmu-miR-92a-1-5p and *Slc2a5* was predicted by rna22 (Miranda et al., 2006) and RNAhybrid (Rehmsmeier et al., 2004).

Glycolysis Stress Test

iBACs were detached at day 5 of differentiation with 0.5 mg/ml collagenase P (Sigma-Aldrich, St. Louis, MI, United States, 11213857001) and 2.5% Trypsin (Thermo Fisher Scientific, Waltham, MA, United States, 15400054) in PBS and seeded at a density of 5 x 10⁴ cells per well in a seahorse 96-well plate (Agilent, Santa Clara, CA, United States. After 24 h, a glycolysis stress test was performed, according to manufacturer's instructions (Agilent, Santa Clara, CA, United States). The extracellular acidification rate (ECAR) was measured using XF96 Extracellular Flux Analyzer (Agilent, Santa Clara, CA, United States) before and after acute injection of 5 g/L fructose or glucose (Sigma-Aldrich, St. Louis, MI, United States, 50-99-7), 1 μM oligomycin-A or 50 mM 2-deoxy-glucose.

Nuclear Magnetic Resonance Metabolomics

NMR metabolomics was performed as previously published (Alkan et al., 2018). In short, iBACs were treated with growth medium supplemented with 5 g/L Fructose for 24 h iBACs were washed extensively with PBS and harvested for NMR metabolomics as described. Metabolites were extracted using methanol, NMR spectra were recorded and processed in Matlab 2014a to obtain aligned and normalized datasets.

Luciferase Assay

For Luciferase assay, the 3′UTR of *Slc2a5* harbouring predicted seed matches for miR-92a-1-5p was cloned in a PsiCheck2 vector (Promega, Madison, WI, United States, C8021). HEK293 cells were transfected with 1 μM mmu-miR-92-1-5p-mimic (Horizon discoveries, Waterbeach, United Kingdom, MIMAT0017066) or non-targeting control (Horizon, CN-001000-01–05) and 0.2 μg PsiCheck2 vector using lipofectamine 3,000 (Thermo Fisher Scientific, Waltham, MA, United States, as transfection reagent. The medium was changed after 24 h of transfection and luciferase assay was performed on day 2 after transfection. The luciferase assay was performed according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, United States, 16185).

ChIP-qPCR

ChIP-qPCR was performed according to an established protocol (Prokesch et al., 2016). In short, fully differentiated iBACs were crosslinked with 1% formaldehyde (Thermo Fisher Scientific, Waltham, MA, United States) for 15 min at RT. Crosslinking was stopped by adding 125 mM glycin to the medium for 5 min. Afterwards, chromatin was sonicated in Bioruptor® Pico Microtubes (Diogenode, Denville, United States, C30010016) for 10 cycles (30 s on/30 s off) by using the Diogenode Bioruptor (Diogenode, Denville, United States, B01020001) and fragment size was analyzed by running the DNA/Chromatin fragments on an agarose gel. Sonicated samples were washed twice according to the protocol. IP was performed using precleared Protein G DynaBeads magnetic beads (Thermo Fisher Scientific, Waltham, MA, United States, 10003D). 1.25 μg of the following antibodies was used: a-p53 (D2H9O, Cell Signalling, Danvers, MA, United States, 32532), a-IgG (Santa Cruz, Santa Cruz, United States, sc-2027). Immunoprecipitated chromatin and input chromatin were reverse cross-linked and column purified. DNA was subjected to SYBR green qPCR. Primers designed at loci without p53 binding sites served as negative control. All primer sequences are listed in **Table 1**.

Histology

Immunohistochemical staining of formalin-fixed, paraffin-embedded BAT depots was performed after antigen retrieval (93°C, 15 min at pH 6) and peroxidase blocking (Agilent, Foster City, CA, United States, S202386-2) using the UltraVision LP detection system (Thermo Fisher Scientific, Waltham, MA, United States, 12643997) according to the manual with UCP1-antibody (1.25 μg/ml; Abcam, MA, United Kingdom, 10983). AEC (3-amino-9-ethyl carbazole) chromogen (Thermo Fisher Scientific, Waltham, MA, United States, 001122) was used for color detection. Counterstaining with hematoxylin was done on all slides. Hematoxylin and eosin stainings were quantified by using ImageJ Brown adipocyte area was indicated as square pixels.

Statistical Analysis

If not stated otherwise, all experiments were performed at least three times independently. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software). Statistically significant differences were determined as described in the figure legend. If not noted otherwise, data are represented as mean values ± SEM with the following levels of statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Differences not indicated with asterisks or indicated with ns are not statistically significant ($p > 0.05$).

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the NCBI GEO repository, accession number GSE199963 (transcriptome data)

and the EBI ENA repository, accession number PRJEB51729 (miRNA sequencing) https://www.ebi.ac.uk/ena, PRJEB51729; https://www.ncbi.nlm.nih.gov/geo/, GSE199963.

ETHICS STATEMENT

The animal studies were reviewed and approved by Austrian Ministry for Education, Science and Research.

AUTHOR CONTRIBUTIONS

IR designed and performed the experiments, analyzed the data, and wrote the manuscript. IK, HM, EM, MG, JK, MD, NV, GL, and FZ performed experiments and analyzed data. TS, MK, TM, DK, RM, MS, and RS contributed materials and provided expertise and feedback. AP designed, coordinated, and supervised the project; analyzed the data; and wrote the manuscript.

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REFERENCES

Al-Massadi, O., Porteiro, B., Kuhlow, D., Köhler, M., Gonzalez-Rellan, M. J., Garcia-Lavandeira, M., et al. (2016). Pharmacological and Genetic Manipulation of P53 in Brown Fat at Adult but Not Embryonic Stages Regulates Thermogenesis and Body Weight in Male Mice. *Endocrinology* 157, 2735–2749. doi:10.1210/en.2016-1209

Alkan, H. F., Walter, K. E., Luengo, A., Madreiter-Sokolowski, C. T., Stryeck, S., Lau, A. N., et al. (2018). Cytosolic Aspartate Availability Determines Cell Survival when Glutamine Is Limiting. *Cell Metab.* 28, 706–720. doi:10.1016/j.cmet.2018.07.021

Baker, S. J., Preisinger, A. C., Jessup, J. M., Paraskeva, C., Markowitz, S., Willson, J. K., et al. (1990b). p53 Gene Mutations Occur in Combination with 17p Allelic Deletions as Late Events in Colorectal Tumorigenesis. *Cancer Res.* 50, 7717. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2253215.

Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K. V., and Vogelstein, B. (1990a). Suppression of Human Colorectal Carcinoma Cell Growth by Wild-type P53. *Science* 249, 912–915. doi:10.1126/science.2144057

Bartelt, A., and Heeren, J. (2014). Adipose Tissue Browning and Metabolic Health. *Nat. Rev. Endocrinol.* 10, 24–36. doi:10.1038/nrendo.2013.204

Bazhan, N., Jakovleva, T., Feofanova, N., Denisova, E., Dubinina, A., Sitnikova, N., et al. (2019). Sex Differences in Liver, Adipose Tissue, and Muscle Transcriptional Response to Fasting and Refeeding in Mice. *Cells* 8, 1529. doi:10.3390/cells8121529

Chen, Y., Buyel, J. J., Hanssen, M. J. W., Siegel, F., Pan, R., Naumann, J., et al. (2016). Exosomal microRNA miR-92a Concentration in Serum Reflects Human Brown Fat Activity. *Nat. Commun.* 7, 11420. doi:10.1038/ncomms11420

Cura, A. J., and Carruthers, A. (2012). "Role of Monosaccharide Transport Proteins in Carbohydrate Assimilation, Distribution, Metabolism, and Homeostasis," in *Comprehensive Physiology* (Wiley), 863–914. doi:10.1002/cphy.c110024

de Cabo, R., and Mattson, M. P. (2019). Effects of Intermittent Fasting on Health, Aging, and Disease. *N. Engl. J. Med.* 381, 2541–2551. doi:10.1056/NEJMra1905136

Ding, H., Zheng, S., Garcia-Ruiz, D., Hou, D., Wei, Z., Liao, Z., et al. (2016). Fasting Induces a Subcutaneous-To-Visceral Fat Switch Mediated by microRNA-149-3p and Suppression of PRDM16. *Nat. Commun.* 7, 11533. doi:10.1038/ncomms11533

Dweep, H., Sticht, C., Pandey, P., and Gretz, N. (2011). miRWalk - Database: Prediction of Possible miRNA Binding Sites by "walking" the Genes of Three Genomes. *J. Biomed. Inf.* 44, 839–847. doi:10.1016/j.jbi.2011.05.002

Fischer, M. (2017). Census and Evaluation of P53 Target Genes. *Oncogene* 36, 3943–3956. doi:10.1038/onc.2016.502

Ganeshan, K., and Chawla, A. (2017). Warming the Mouse to Model Human Diseases. *Nat. Rev. Endocrinol.* 13, 458–465. doi:10.1038/nrendo.2017.48

Geiser, F., Currie, S. E., O'Shea, K. A., and Hiebert, S. M. (2014). Torpor and Hypothermia: Reversed Hysteresis of Metabolic Rate and Body Temperature. *Am. J. Physiology-Regulatory, Integr. Comp. Physiology* 307, R1324–R1329. doi:10.1152/ajpregu.00214.2014

Gerhart-Hines, Z., Feng, D., Emmett, M. J., Everett, L. J., Loro, E., Briggs, E. R., et al. (2013). The Nuclear Receptor Rev-Erbα Controls Circadian Thermogenic Plasticity. *Nature* 503, 410–413. doi:10.1038/nature12642

Goeman, F., Strano, S., and Blandino, G. (2017). MicroRNAs as Key Effectors in the P53 Network. *Int. Rev. Cell Mol. Biol.* 33, 51–90. doi:10.1016/bs.ircmb.2017.04.003

Goldstein, I., Baek, S., Presman, D. M., Paakinaho, V., Swinstead, E. E., and Hager, G. L. (2017). Transcription Factor Assisted Loading and Enhancer Dynamics Dictate the Hepatic Fasting Response. *Genome Res.* 27, 427–439. doi:10.1101/gr.212175.116

Goldstein, I., and Hager, G. L. (2015). Transcriptional and Chromatin Regulation during Fasting - the Genomic Era. *Trends Endocrinol. Metabolism* 26, 699–710. doi:10.1016/j.tem.2015.09.005

Gonzalez-Rellan, M. J., Fondevila, M. F., Fernandez, U., Rodríguez, A., Varela-Rey, M., Veyrat-Durebex, C., et al. (2021). O-GlcNAcylated P53 in the Liver Modulates Hepatic Glucose Production. *Nat. Commun.* 12, 5068. doi:10.1038/s41467-021-25390-0

Goody, D., and Pfeifer, A. (2019). MicroRNAs in Brown and Beige Fat. *Biochimica Biophysica Acta (BBA) - Mol. Cell Biol. Lipids* 1864, 29–36. doi:10.1016/j.bbalip.2018.05.003

Harms, M. J., Ishibashi, J., Wang, W., Lim, H.-W., Goyama, S., Sato, T., et al. (2014). Prdm16 Is Required for the Maintenance of Brown Adipocyte Identity and Function in Adult Mice. *Cell Metab.* 19, 593–604. doi:10.1016/j.cmet.2014.03.007

Harms, M., and Seale, P. (2013). Brown and Beige Fat: Development, Function and Therapeutic Potential. *Nat. Med.* 19, 1252–1263. doi:10.1038/nm.3361

Harney, D. J., Cielesh, M., Chu, R., Cooke, K. C., James, D. E., Stöckli, J., et al. (2021). Proteomics Analysis of Adipose Depots after Intermittent Fasting Reveals Visceral Fat Preservation Mechanisms. *Cell Rep.* 34, 108804. doi:10.1016/j.celrep.2021.108804

Hermeking, H. (2012). MicroRNAs in the P53 Network: Micromanagement of Tumour Suppression. *Nat. Rev. Cancer* 12, 613–626. doi:10.1038/nrc3318

Huber, K., Hofer, D. C., Trefely, S., Pelzmann, H. J., Madreiter-Sokolowski, C., Duta-Mare, M., et al. (2019). N-Acetylaspartate Pathway Is Nutrient

Responsive and Coordinates Lipid and Energy Metabolism in Brown Adipocytes. *Biochimica Biophysica Acta (BBA) - Mol. Cell Res.* 1866, 337–348. doi:10.1016/j.bbamcr.2018.08.017

Jang, C., Hui, S., Lu, W., Cowan, A. J., Morscher, R. J., Lee, G., et al. (2018). The Small Intestine Converts Dietary Fructose into Glucose and Organic Acids. *Cell Metab.* 27, 351–361. doi:10.1016/j.cmet.2017.12.016

Jones, R. G., Plas, D. R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., et al. (2005). AMP-activated Protein Kinase Induces a P53-dependent Metabolic Checkpoint. *Mol. Cell* 18, 283–293. doi:10.1016/j.molcel.2005.03.027

Klepac, K., Georgiadi, A., Tschöp, M., and Herzig, S. (2019). The Role of Brown and Beige Adipose Tissue in Glycaemic Control. *Mol. Aspects Med.* 68, 90–100. doi:10.1016/j.mam.2019.07.001

Knehans, A. W., and Romsos, D. R. (1983). Norepinephrine Turnover in Obese (Ob/ob) Mice: Effects of Age, Fasting, and Acute Cold. *Am. J. Physiology-Endocrinology Metabolism* 244, E567–E574. doi:10.1152/ajpendo.1983.244.6.E567

Krstic, J., Reinisch, I., Schupp, M., Schulz, T., and Prokesch, A. (2018). p53 Functions in Adipose Tissue Metabolism and Homeostasis. *Ijms* 19, 2622. doi:10.3390/ijms19092622

Labuschagne, C. F., Zani, F., and Vousden, K. H. (2018). Control of Metabolism by P53 - Cancer and beyond. *Biochimica Biophysica Acta (BBA) - Rev. Cancer* 1870, 32–42. doi:10.1016/j.bbcan.2018.06.001

Lacroix, M., Riscal, R., Arena, G., Linares, L. K., and Le Cam, L. (2020). Metabolic Functions of the Tumor Suppressor P53: Implications in Normal Physiology, Metabolic Disorders, and Cancer. *Mol. Metab.* 33, 2–22. doi:10.1016/j.molmet.2019.10.002

Legeza, B., Marcolongo, P., Gamberucci, A., Varga, V., Bánhegyi, G., Benedetti, A., et al. (2017). Fructose, Glucocorticoids and Adipose Tissue: Implications for the Metabolic Syndrome. *Nutrients* 9, 426. doi:10.3390/nu9050426

Li, L., Li, B., Li, M., and Speakman, J. R. (2019). Switching on the Furnace: Regulation of Heat Production in Brown Adipose Tissue. *Mol. Aspects Med.* 68, 60–73. doi:10.1016/j.mam.2019.07.005

Miranda, K. C., Huynh, T., Tay, Y., Ang, Y.-S., Tam, W.-L., Thomson, A. M., et al. (2006). A Pattern-Based Method for the Identification of MicroRNA Binding Sites and Their Corresponding Heteroduplexes. *Cell* 126, 1203–1217. doi:10.1016/j.cell.2006.07.031

Mueckler, M., and Thorens, B. (2013). The SLC2 (GLUT) Family of Membrane Transporters. *Mol. Aspects Med.* 34, 121–138. doi:10.1016/j.mam.2012.07.001

Nedergaard, J., Cannon, B., and Lindberg, O. (1977). Microcalorimetry of Isolated Mammalian Cells. *Nature* 267, 518–520. doi:10.1038/267518a0

Olivier, M., Eeles, R., Hollstein, M., Khan, M. A., Harris, C. C., and Hainaut, P. (2002). The IARC TP53 Database: New Online Mutation Analysis and Recommendations to Users. *Hum. Mutat.* 19, 607–614. doi:10.1002/humu.10081

Ortega-Prieto, P., and Postic, C. (2019). Carbohydrate Sensing through the Transcription Factor ChREBP. *Front. Genet.* 10, 472. doi:10.3389/fgene.2019.00472

Prokesch, A., Graef, F. A., Madl, T., Kahlhofer, J., Heidenreich, S., Schumann, A., et al. (2017). Liver P53 Is Stabilized upon Starvation and Required for Amino Acid Catabolism and Gluconeogenesis. *FASEB J.* 31, 732–742. doi:10.1096/fj.201600845R

Prokesch, A., Pelzmann, H. J., Pessentheiner, A. R., Huber, K., Madreiter-Sokolowski, C. T., Drougard, A., et al. (2016). N-Acetylaspartate Catabolism Determines Cytosolic Acetyl-CoA Levels and Histone Acetylation in Brown Adipocytes. *Sci. Rep.* 6, 23723. doi:10.1038/srep23723

Rehmsmeier, M., Steffen, P., Höchsmann, M., and Giegerich, R. (2004). Fast and Effective Prediction of microRNA/target Duplexes. *RNA* 10, 1507–1517. doi:10.1261/rna.5248604

Reinisch, I., Schreiber, R., and Prokesch, A. (2020). Regulation of Thermogenic Adipocytes during Fasting and Cold. *Mol. Cell. Endocrinol.* 512, 110869. doi:10.1016/j.mce.2020.110869

Rothwell, N. J., and Stock, M. J. (1997). A Role for Brown Adipose Tissue in Diet-Induced Thermogenesis. *Obes. Res.* 5, 650–656. doi:10.1002/j.1550-8528.1997.tb00591.x

Sanchez-Gurmaches, J., Tang, Y., Jespersen, N. Z., Wallace, M., Martinez Calejman, C., Gujja, S., et al. (2018). Brown Fat AKT2 Is a Cold-Induced Kinase that Stimulates ChREBP-Mediated De Novo Lipogenesis to Optimize Fuel Storage and Thermogenesis. *Cell Metab.* 27, 195–209. doi:10.1016/j.cmet.2017.10.008

Schupp, M., Chen, F., Briggs, E. R., Rao, S., Pelzmann, H. J., Pessentheiner, A. R., et al. (2013). Metabolite and Transcriptome Analysis during Fasting Suggest a Role for the P53-Ddit4 axis in Major Metabolic Tissues. *BMC Genomics* 14, 758. doi:10.1186/1471-2164-14-758

Secor, S. M., and Carey, H. V. (2016). Integrative Physiology of Fasting. *Compr. Physiol.* 6, 773–825. doi:10.1002/cphy.c150013

Sticht, C., De La Torre, C., Parveen, A., and Gretz, N. (2018). miRWalk: An Online Resource for Prediction of microRNA Binding Sites. *PLoS One* 13, e0206239. doi:10.1371/journal.pone.0206239

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., et al. (2005). Gene Set Enrichment Analysis: A Knowledge-Based Approach for Interpreting Genome-wide Expression Profiles. *Proc. Natl. Acad. Sci. U.S.A.* 102, 15545–15550. doi:10.1073/pnas.0506580102

Townsend, K. L., and Tseng, Y.-H. (2014). Brown Fat Fuel Utilization and Thermogenesis. *Trends Endocrinol. Metabolism* 25, 168–177. doi:10.1016/j.tem.2013.12.004

Varma, V., Boros, L. G., Nolen, G. T., Chang, C.-W., Wabitsch, M., Beger, R. D., et al. (2015a). Metabolic Fate of Fructose in Human Adipocytes: a Targeted 13C Tracer Fate Association Study. *Metabolomics* 11, 529–544. doi:10.1007/s11306-014-0716-0

Varma, V., Boros, L., Nolen, G., Chang, C.-W., Wabitsch, M., Beger, R., et al. (2015b). Fructose Alters Intermediary Metabolism of Glucose in Human Adipocytes and Diverts Glucose to Serine Oxidation in the One-Carbon Cycle Energy Producing Pathway. *Metabolites* 5, 364–385. doi:10.3390/metabo5020364

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., et al. (1999). Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1. *Cell* 98, 115–124. doi:10.1016/S0092-8674(00)80611-X

Yoneshiro, T., Wang, Q., and Tajima, K. (2019). BCAA catabolism in brown fat controls energy homeostasis through SLC25A44. *Nature* 572, 614–619. doi:10.1038/s41586-019-1503-x

Zhao, Q., Zhang, Z., Rong, W., Jin, W., Yan, L., Jin, W., et al. (2020). KMT5c Modulates Adipocyte Thermogenesis by Regulating Trp53 Expression. *Proc. Natl. Acad. Sci. U.S.A.* 117, 22413–22422. doi:10.1073/pnas.1922548117

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p53: A Regulator of Ferroptosis Induced by Galectin-1 Derived Peptide 3 in MH7A Cells

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Backgrounds: Rheumatoid arthritis synovial fibroblasts (RASFs) are the primary cells responsible for destruction of marginal cartilage in rheumatoid arthritis (RA). G1dP3, a bioactive peptide derived from galectin-1 domain, possesses potent anti-inflammatory and anti-proliferation properties in RASFs. This study aimed to determine the effects of G1dP3 ferroptosis induction in RASFs and to further clarify the possible mechanisms.

Methods: TNF-α was used to establish a RA model in MH7A cells. Cell Counting Kit-8 assays were employed to detect MH7A cell viability with different treatments. The occurrence of ferroptosis was examined by Lipid ROS assay, cellular labile iron pool measurement, reduced glutathione/oxidized glutathione activity, Gpx4 expression and transmission electron microscopy (TEM) morphology observation. Lentiviral-mediated siRNA interference was used to determine the downstream pathway.

Results: G1dP3 markedly suppressed MH7A cell viability induced by TNF-α. G1dP3-treated MH7A cells presented the morphological features of ferroptosis. Moreover, G1dP3 triggered ferroptosis in MH7A cells by promoting the accumulation of lipid peroxides as well as iron deposition. Inhibition of ferroptosis alleviated G1dP3-mediated suppression of MH7A cell viability. Furthermore, G1dP3 increased p53 expression, which in turn transcriptionally suppressed SLC7A11, a key component of system X_c^- essential for ferroptosis. Knockdown of p53 abrogated the ferroptotic effects of G1dP3 on MH7A cells.

Conclusion: Our findings reveal that the bioactive peptide G1dP3 promotes RASFs ferroptosis cell death via a p53/SLC7A11 axis-dependent mechanism, suggesting its potential role in the treatment of RA.

Keywords: bioactive peptide, MH7A, ferroptosis, p53, synovial fibroblast, ra

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INTRODUCTION

Rheumatoid arthritis (RA) is a common complex systemic disease, afflicting 0.5–1% of populations worldwide (Smolen et al., 2016). This disease is mainly characterized by synovial membrane inflammation and disruption of articular cartilage (Aletaha et al., 2011). It has been reported that synovial fibroblasts are the effector cells of inflammatory responses in RA (Klippel, 2000; Weyand and Goronzy, 2021). Thus, inhibiting synovial fibroblast activation is a potential strategy for RA prevention and treatment.

As a new branch of proteomics, peptidomics can be used to analyze endogenous protein fragments in body fluids and tissues (Baggerman et al., 2005). Biologically active peptides could be a potential biomarker for RA diagnosis, and attenuated inflammation both *in vitro* and *in vivo* (Roark et al., 2016; Smolen, 2020). From a perspective of peptidomics, differentially expressed peptides have been identified between RA and normal synovial tissue groups in our previous study (8). In particular, G1dP3, a galectin-1 derived peptide with two distinct polar/basic residues and two distinct polar/acid residues, could significantly decrease the expression levels of inflammation cytokines (e.g., IL-6, IL-1β, MMP-13, and MMP-1) in MH7A synovial cells induced by TNF-α. In addition, G1dP3 exhibits a proapoptotic effect on MH7A cells, implying its great potential for RA treatment (Hu et al., 2020). However, the underlying mechanism of G1dP3 in inhibiting MH7A cell viability remains unclear.

Ferroptosis is a cell death modality regulated by iron overload and reactive oxygen species (ROS) production (Dixon et al., 2012; Cao and Dixon, 2016). Mechanistic studies have shown that ferroptosis occurs when lipid peroxidation or iron metabolism is dysregulated, together with glutathione (GSH) deletion and glutathione peroxidase (Gpx4) inactivation (Dixon et al., 2012; Cao and Dixon, 2016). Thus, ferroptosis can be induced by decreasing glutamate-cysteine ligase, inhibiting cystine/glutamate antiporter xCT (SLC7A11/system X_c^-), suppressing Gpx4 activity, scavenging free radical (Ferrostatin-1), promoting iron chelation and blocking Fenton reactions (deferoxamine, DFO) (Dixon et al., 2012; Cao and Dixon, 2016; Xie et al., 2016). To our knowledge, the role of ferroptosis in RA synovial fibroblasts has not been clarified, and whether the anti-proliferation properties of G1dP3 are related to ferroptosis in RA synovial fibroblasts remains to be explored.

P53, the most commonly mutated tumour suppressor gene, has been testified to play a dual role in ferroptosis. On the one hand, p53 can induce ferroptosis by upregulating GLS2 and SAT1 expression or downregulating SLC7A11 expression. On the other hand, p53 can suppress ferroptosis by increasing CDKN1A expression or reducing DPP4 activity (Jiang et al., 2015a; Wang et al., 2016; Xie et al., 2017). p53 induces ROS-regulated ferroptosis through GSH depletion caused by xCT downregulation. Besides, SLC7A11 can function a transcriptional target for p53 to bind to its promoter region, which facilitates the cellular uptake of extracellular cysteine, and the intracellular cystine is coverted to cysteine prior to GSH synthesis (Dixon et al., 2012; Guan et al., 2020). These findings imply that p53/SLC7A11 pathways play a vital role in sensitizing synovial fibroblasts to ferroptotic cell death.

In this study, the effect of ferroptosis in a RA model and the underlying mechanism of bioactive peptide G1dP3 in inhibiting MH7A cell activation induced by TNF-α were elucidated for the first time. Furthermore, p53/SLC7A11 axis was identified to exert a crucial effect on G1dP3-regulated ferroptosis in MH7A cells.

MATERIALS AND METHODS

Drugs and Cell Death Inhibitors

Ferrostatin-1 was purchased from Selleckchem Company (Shanghai, China). DFO and z-VAD-fmk were supplied by Sigma-Aldrich (MO, United States). The peptides were obtained from Science Peptide Company (Shanghai, China) after purification.

Cell Culture

MH7A cells (a human RA synovial fibroblast cell line) were obtained from the American Type Culture Collection (VA, United States) and cultured in RPMI 1640 medium (Hyclone, UT, United States) containing 1% penicillin/streptomycin (Invitrogen, CA, United States) and 10% FBS (Gibco, MA, United States) at 37°C and 5% CO_2. The cells were then induced with 50 ng/ml TNF-α.

Cell Transfection

The human siRNA-Caspase3 (sc-29237), siRNA-p53 (sc-29435) and negative controls were supplied by Santa Cruz Biotechnology (Shanghai) Co., Ltd. Cell transfection was conducted with Lipofectamine 3,000 (Invitrogen) for 4 h by following the reagent's protocol. Subsequently, the medium was replenished with a 10% FBS-containing RPMI 1640 medium. After 24 h, the cells were collected. Subsequent western blot was performed to analyze transfection efficiency (**Supplementary Figure SA,B**).

Peptide Synthesis

The galectin-1 derived peptide fragment 3 (G1dP3, ADGDFKIK sequences, 95% purity) and the scrambled peptide (ScP, DAGIDKFK sequences, 95% purity) were synthesized with GRKKRRQRRRPPQ sequences derived from the cell-penetrating peptide (CPP) HIV-1 Tat (48–60) (Hu et al., 2020). The peptides (10 mM) were prepared by dissolving in water and kept in a refrigerator at −80°C. G1dP3 and ScP at a concentration of 20 μM were employed for further analysis.

Cell Viability Assay

Briefly, MH7A cells (5,000 cells/well) were grown in a 96-well plate, and then exposed to the indicated treatment. Cell viability was conducted with Cell Counting Kit-8 (Dojindo Technologies, Kyushu, Japan) by following the kit's protocol. Subsequently, the absorbance of each well was detected at 450 nm.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was isolated with TRIzol reagent (Invitrogen). cDNA synthesis was conducted using a PrimeScript RT Reagent kit (Vazyme Biotech, Nanjing, China) by following the manufacturer's instructions. RT-qPCR was performed on a 7,900 Real-time PCR system (Applied Biosystems, CA, United States) using the ChamQ Universal SYBR qPCR Master Mix (Vazyme). GAPDH was selected as a housekeeping gene. The relative expression levels were

calculated using the 2−ΔΔCT method. The following primer pairs were used:

P53 forward primer: 5′-TTCCCTGGATTGGCCAGACT-3′, reverse primer: 5′-ACCATCGCTATCTGAGCAGC-3'.

GAPDH forward primer: 5′-AGAAGGCTGGGGCTCATT TG-3′, reverse primer: 5′-AGGGGCCATCCACAGTCTTC-3'.

SLC7A11 forward primer: 5′-TGCCCAGATATGCATCGT CC-3′, reverse primer: 5′-TCTTCTTCTGGTACAACTTCC AGT-3'.

GAPDH forward primer: 5′-CAGCCTCAAGATCATCAG CAAT-3′, reverse primer: 5′-AGTCCTTCACGATACCAA AGT-3'.

Western Blot Analysis

The protein expression levels were determined by Western blotting after normalization to GAPDH. Briefly, the protein specimens were incubated overnight at 4°C with primary antibodies against BCL-2 (sc-7382, Santa), Bax (sc-7480, Santa), cleaved-Caspase-3 (sc-6053, Santa), Gpx4 (sc-166570,Santa), p53 (sc-126, Santa), SLC7A11 (ab37185, abcam), and GAPDH (sc-365062, Santa).

Transmission Electron Microscopy (TEM)

TEM analysis was performed according to a previous method (Bao et al., 2021).

Determination of Labile Iron Pool (LIP)

The cells (1×10^6 cells/ml) were incubated with 0.05 μM calcein-acetoxymethyl ester (AnaSpec) at 37°C for 15 min. After rinsing twice with 0.5 ml PBS, the cells were treated with/without 100 μM deferiprone at 37°C for 1 h. Flow cytometric analysis at 525 nm emission and 488 nm excitation. The levels of LIP were determined by comparing the mean fluorescence intensities between deferiprone treated and untreated groups.

Detection of ROS and Lipid ROS

The intracellular ROS levels were measured by DCF-DA (2′,7′-dichlorodihydrofluorescein diacetate; Molecular Probes/ Invitrogen, OR, United States) by following the manufacturer's protocols. Briefly, after incubation with 20 μM DCF-DA at 37°C for 30 min, the fluorescence signals of the cells were determined using a FACStar Flow Cytometer (Beckman Coulter). Lipid ROS levels were evaluated using the BODIPY 581/591 C11 (D3861, Invitrogen) by following the manufacturer's protocols.

Measurement of Reduced Glutathione (GSH)/Oxidized Glutathione (GSSG) Activity

The ratio of GSH/GSSG was detected using the GSH/GSSG-Glo Assay (Promega, United States). After incubation with 50 μl Oxidized Glutathione Lysis Reagent or Total Glutathione Lysis Reagent for 5 min, the cells were incubated again for 30 min with 50 μl Luciferin Generation Reagent. Then, 100 μl Luciferin Detection Reagent was added, and the luminescence signals were detected using a microplate reader (BioTek).

Statistical Analysis

Statistical tests were conducted with SPSS v16.0 (SPSS Inc., United States). All data were presented as mean ± standard deviation. Unpaired Student t-test was applied to compare the difference between two groups. One-way ANOVA was employed to compare the differences among multiple groups. $p < 0.05$ was regarded as statistically significant.

RESULTS

G1dP3 Inhibits MH7A Cell Viability Independent of Apoptosis

In this study, 50 ng/ml TNF-α was used to establish a RA model using the MH7A cells. Consistent with our previous results, treatment of 20 μM G1dP3 for 6 h significantly up-regulated the expression of Bax and cleaved-caspases-3, while down-regulated that of Bcl-2 in MH7A cells (**Figure 1A**). Moreover, G1dP3 treatment resulted in a reduction of cell viability compared to the TNF-α model group (**Figure 1B**). To determine whether G1dP3-suppressed MH7A cell viability was mediated by the apoptotic process, z-VAD-fmk (30 μM) and siRNA-caspase-3 (20 μM) were used to inhibit apoptosis and knockdown the major executors of apoptosis, respectively. Interestingly, the inhibitory effect of G1dP3 on MH7A cell viability could not be reversed by blocking apoptosis (**Figures 1C,D**). These findings suggest that G1dP3 suppresses MH7A cell viability independent of apoptosis.

The Inhibitory Effect of G1dP3 on MH7A Cell Viability Is Correlated With Ferroptosis

Different from necroptosis and apoptosis, nerroptosis is a newly discovered type of regulated cell death (RCD), which is independent of receptor-interacting protein 1 (RIPK1) kinase activity and caspase activity. In the present study, we hypothesize that G1dP3 plays a vital role in regulating MH7A cell death via ferroptosis induction. We firstly examined the levels of ROS in TNF-α-induced MH7A cells following G1dP3 treatment. The results showed that G1dP3 could increase the accumulation of lipid ROS assessed by BODIPY 581/591 C11 (**Figure 2A**). Moreover, G1dP3 markedly reduced the ratio of GSH/GSSG (**Figure 2B**). In addition, G1dP3 could significantly increase free iron levels by measuring the cellular labile iron pool (LIP) (**Figure 2C**). TEM was utilized to observe the cell death mode. Compared to the TNF-α group, G1dP3-treated MH7A cells showed smaller, ruptured mitochondria with increased membrane density and vanished mitochondria cristae, thereby conferring morphological changes in ferroptosis cells (**Figure 2D**). As we expected, Western blot analysis revealed that the protein expression of Gpx4 (a ferroptosis marker) was also decreased after G1dP3 treatment (**Figure 2E**). Collectively, these results indicate that G1dP3 regulates ferroptosis in MH7A cells.

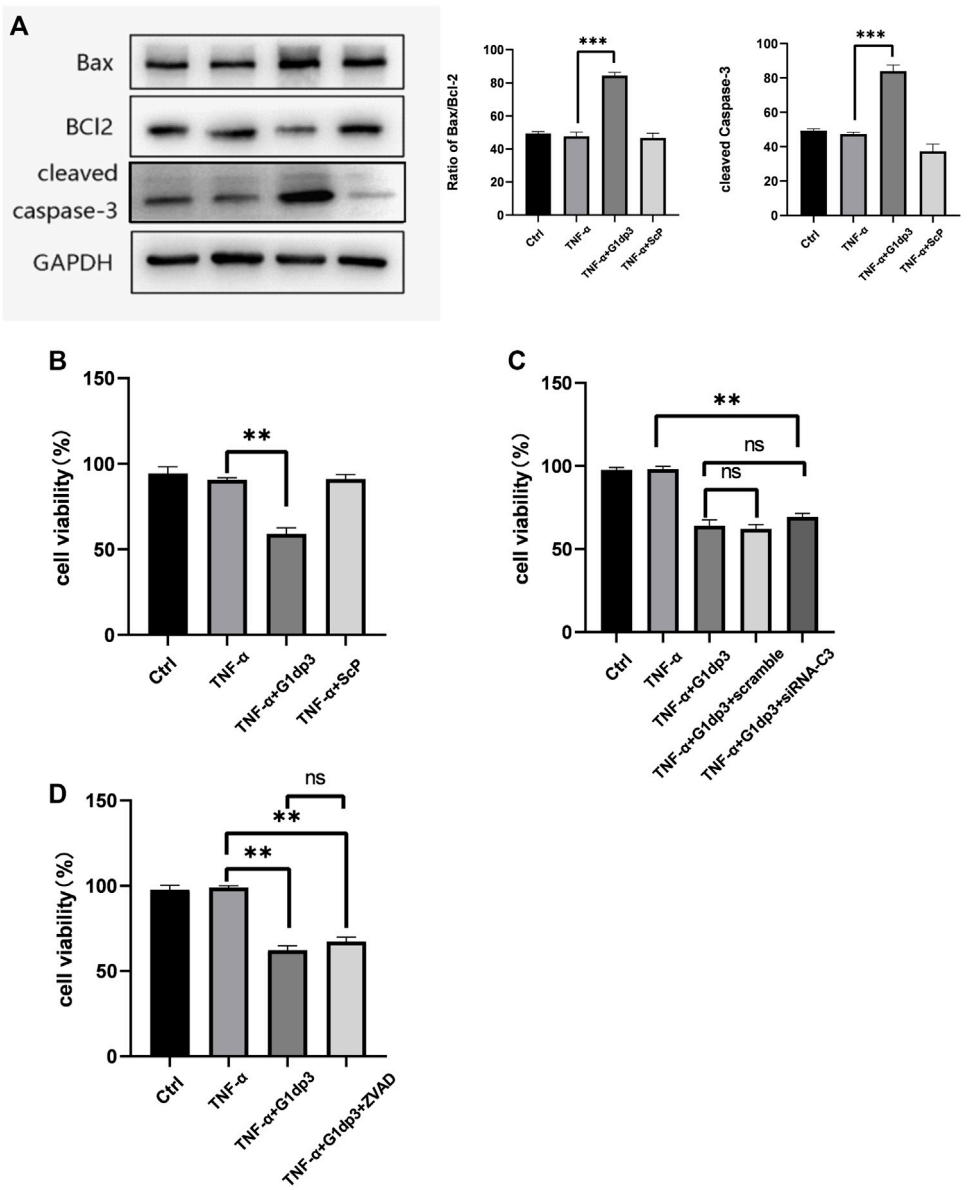

FIGURE 1 | G1dP3 inhibits MH7A cell viability independent of apoptosis. **(A)** The protein levels of Bax, cleaved-caspase-3 and Bcl-2. G1dP3 (20 μM, 6 h) dramatically increased the expression of Bax and cleaved-caspase-3, while decreased that of Bcl-2. **(B)** Cell viability was detected in the indicated MH7A cells. MH7A cell viability was inhibited by G1dP3 (20 μM, 6 h). **(C,D)** The inhibition of G1dP3 in MH7A cell viability could not be reversed by siRNA-Caspase3 (20 μM) or pre-treated z-VAD-fmk (30 μM, 24 h). Graph represents mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns, not significant. Representative data from one of three individual experiments.

Inhibition of Ferroptosis Alleviates G1dP3-Induced Suppression of MH7A Cell Viability

To further investigate whether ferroptosis was involved in G1dP3-mediated MH7A cell viability suppression, we consequently treated MH7A cells with ferroptosis specific inhibitor, ferrostatin-1 (Fer-1, 2 μM) and iron chelators, deferoxamine (DFO, 80 μM) for 24 h to block the initiation of ferroptosis. As shown in **Figure 3A**, G1dP3-induced suppression of MH7A cell viability was remarkably restored by Fer-1 and DFO, along with lipid ROS depletion and GSH generation (**Figures 3A–C**). Likewise, Western blot results indicated that both Fer-1 and DFO could reverse G1dP3-induced Gpx4 down-regulation (**Figure 3D**). Taken together, these findings imply that

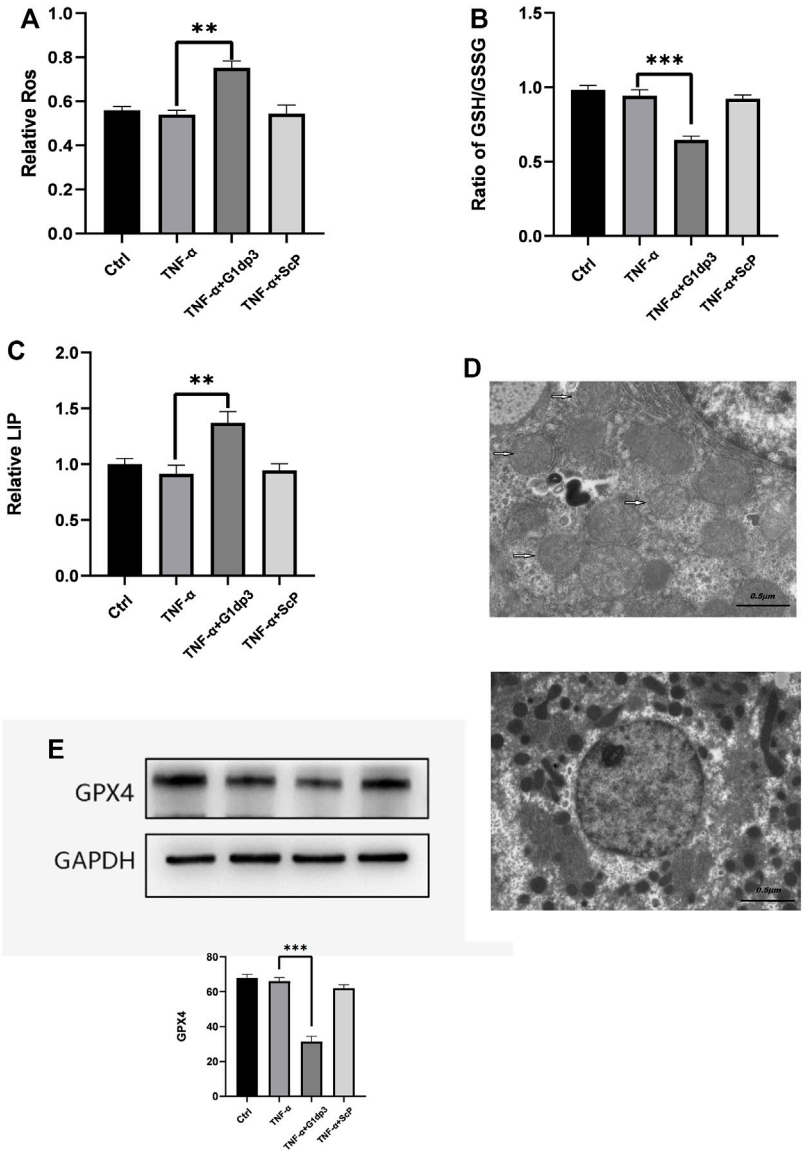

FIGURE 2 | The inhibitory effect of G1dP3 on MH7A cell viability is correlated with ferroptosis **(A)** Relative lipid peroxidation levels in the indicated MH7A cells assessed by BODIPY 581/591 C11. G1dP3 (20 μM, 6 h) could increase the accumulation of lipid ROS. **(B)** Ratio of GSH/GSSG in the indicated MH7A cells. G1dP3 reduced the ratio of GSH/GSSG. **(C)** Relative cellular labile iron pool (LIP). G1dP3 could significantly increase free iron levels in MH7A cells. **(D)** Representative TEM images illustrating that G1dP3 alters mitochondrial morphology in MH7A cells. Scale bars are displayed in each image. **(E)** The protein expression of Gpx4. Graph represents mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns, not significant. Representative data from one of three individual experiments.

the suppression of MH7A cell activity is associated with G1dP3-induced ferroptosis.

G1dP3 Regulates Ferroptosis Through p53/SLC7A11 Axis

P53 can serve as an essential factor for regulating ferroptosis in response to different types of damage. SLC7A11 is a target gene of p53. xCT, the predominant transporter of cystine, is encoded by the SLC7A11 gene. In the cells, cystine is reduced to cysteine, which is a key molecule for GSH synthesis. Depletion of GSH by xCT inhibition could serve as a crucial pathway for the development of ferroptosis. To determine whether p53/SLC7A11 axis can govern ferroptotic activity in G1dP3-treated MH7A cells, the expression levels of p53 and SLC7A11 were detected. As shown in **Figure 4A**, G1dP3 increased p53 but decreased SLC7A11 expression at both mRNA and protein levels. To confirm the role of p53 in G1dP3-induced ferroptosis, p53 was knocked down through specific small interfering RNA. It was found that siRNA-p53 could significantly increase SLC7A11 expression (**Figure 4B**). Furthermore, knockdown of p53 markedly decreased G1dP3-

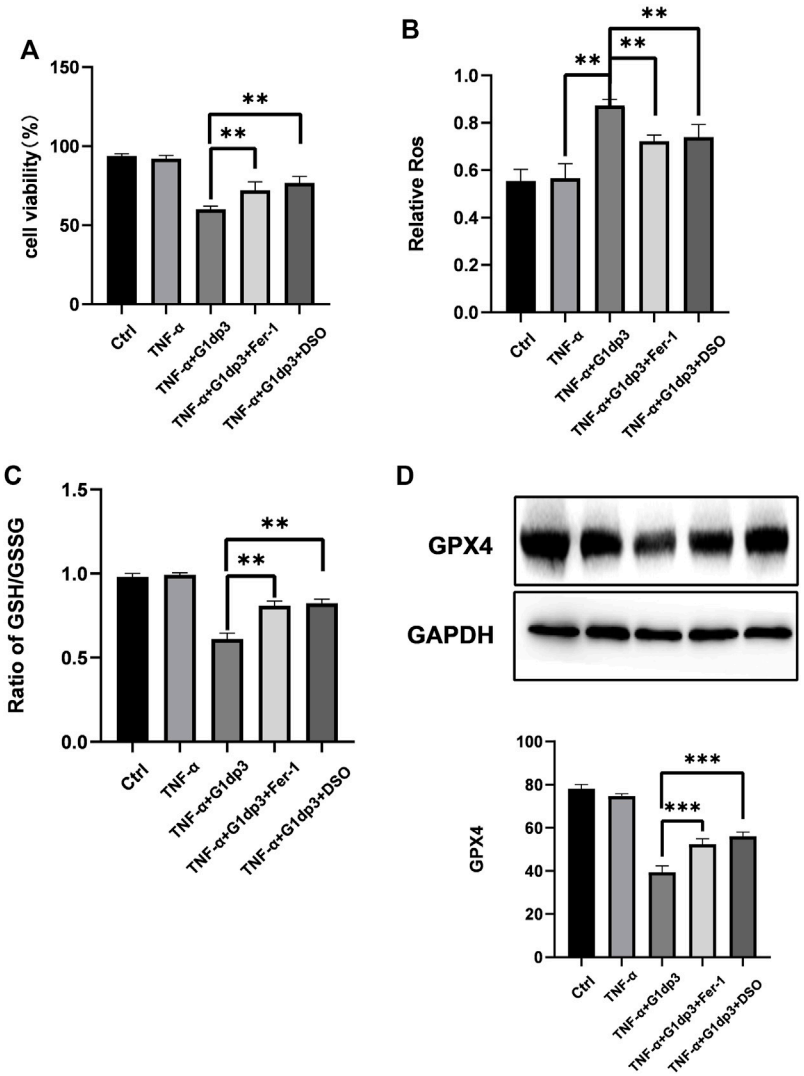

FIGURE 3 | Inhibition of ferroptosis alleviates G1dP3-induced suppression of MH7A cell viability MH7A cells were pretreated with the ferroptosis-specific inhibitor Fer-1 (2 μM), iron chelator DFO (80 μM) for 24 h, and then coincubated with G1dP3. **(A)** Cell viability was detected in the indicated MH7A cells. **(B)** Relative lipid peroxidation levels in the indicated MH7A cells. **(C)** Ratio of GSH/GSSG in the indicated MH7A cells. **(D)** Protein levels of Gpx4 in the indicated treatment. Graph represents mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns, not significant. Representative data from one of three individual experiments.

induced free iron levels (**Figure 4C**). On the contrary, siRNA-p53 significantly enhanced cell viability and GSH/GSSG ratio in G1dP3-treated MH7A cells (**Figures 4D,E**). These data reveal that blockade of p53 activation markedly abrogates the ferroptotic effects of G1dP3, and p53/SLC7A11 axis plays a pivotal role in regulating G1dP3-induced ferroptosis in MH7A cells.

DISCUSSION

RA is a complex autoimmune disorder characterized by chronic synovitis and gradually leads to the destruction of articular cartilage, even the corruption of bone and the impairment of joints. Although the etiology of RA remains unclear, recent evidence has suggested that activation of RA synovial fibroblasts, a complex network of cell regulation, plays a vital role in the pathogenesis of RA (Pap et al., 2000; Turner and Filer, 2015). Thus, inhibiting RA synovial fibroblast proliferation and blocking its effects can significantly reduce RA symptoms and prevent bone destruction (Panipinto et al., 2021).

As a new branch of proteomics, peptidomics can be used to analyze endogenous protein fragments in body fluids and tissues (Smolen et al., 2010). Over the past decades, biologically active peptides have gained considerable interest as a therapeutic agent, since their are highly selective, efficacious, relatively safe and well-tolerated (Fosgerau and Hoffmann, 2015; Zorko et al., 2022). Although bioactive peptides are responsible for nearly all physiological processes such as tumor formation, immune regulation and cell differentiation, the potential role of

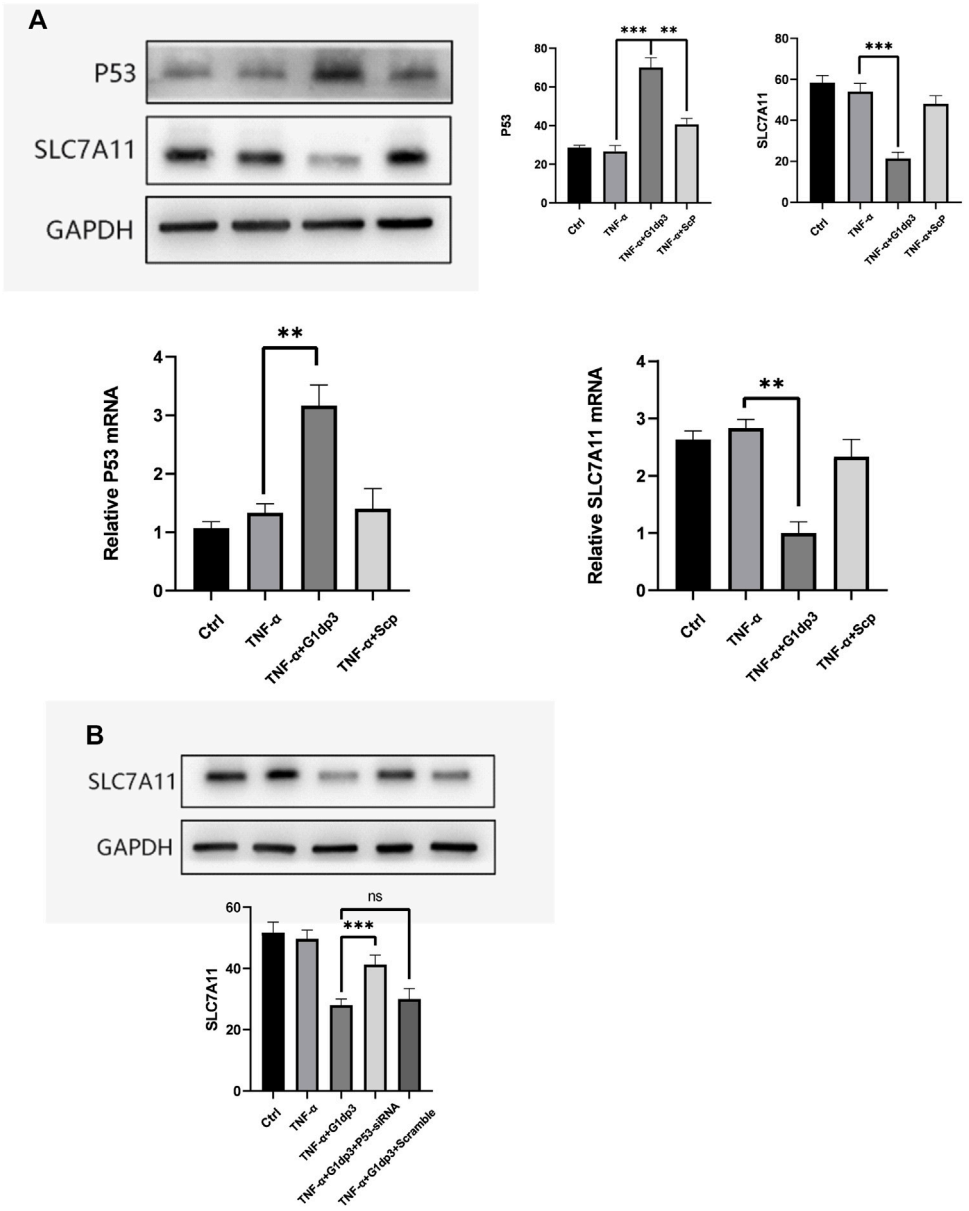

FIGURE 4 | (Continued).

endogenous peptides in the pathophysiology of RA synovial fibroblasts remains unclear. At present, there is a lack of peptidomics studies on RA synovial tissues. Our previous study showed that G1dP3 had potent anti-proliferation and anti-inflammation effects on MH7A synovial cells induced by TNF-α, indicating that it has great potential for the treatment of RA. In this study, the underlying mechanisms of G1dP3 in inhibiting MH7A cell viability was elucidated. Given that the peptides are unable to cross cell membranes and exert their functions, G1dP3 with a CPP attached to its N-terminal was chemically synthesized for further research (8). G1dP3 treatment remarkably up-regulated the expression of Bax and cleaved-caspase-3, while down-regulated that of Bcl-2 in MH7A cells induced by TNF-α. Caspase-3 is the major executor of apoptosis (Lim et al., 2010), and the balance of protein Bcl-2 and Bax plays an essential role in regulating mitochondrial-dependent apoptotic pathways (Tian et al., 2016), Therefore, we primarily assume that apoptotic process is the potential mechanism of G1dP3 in reducing MH7A cell viability. To validate this conjecture, z-VAD-fmk, an inhibitor of apoptosis, was employed. Moreover, the expression of caspase-3 was knocked down with siRNA-caspase3 to prevent apoptosis execution. However, blocking apoptosis induction did not attenuate the anti-proliferation effect of G1dP3 on MH7A cells, suggesting that

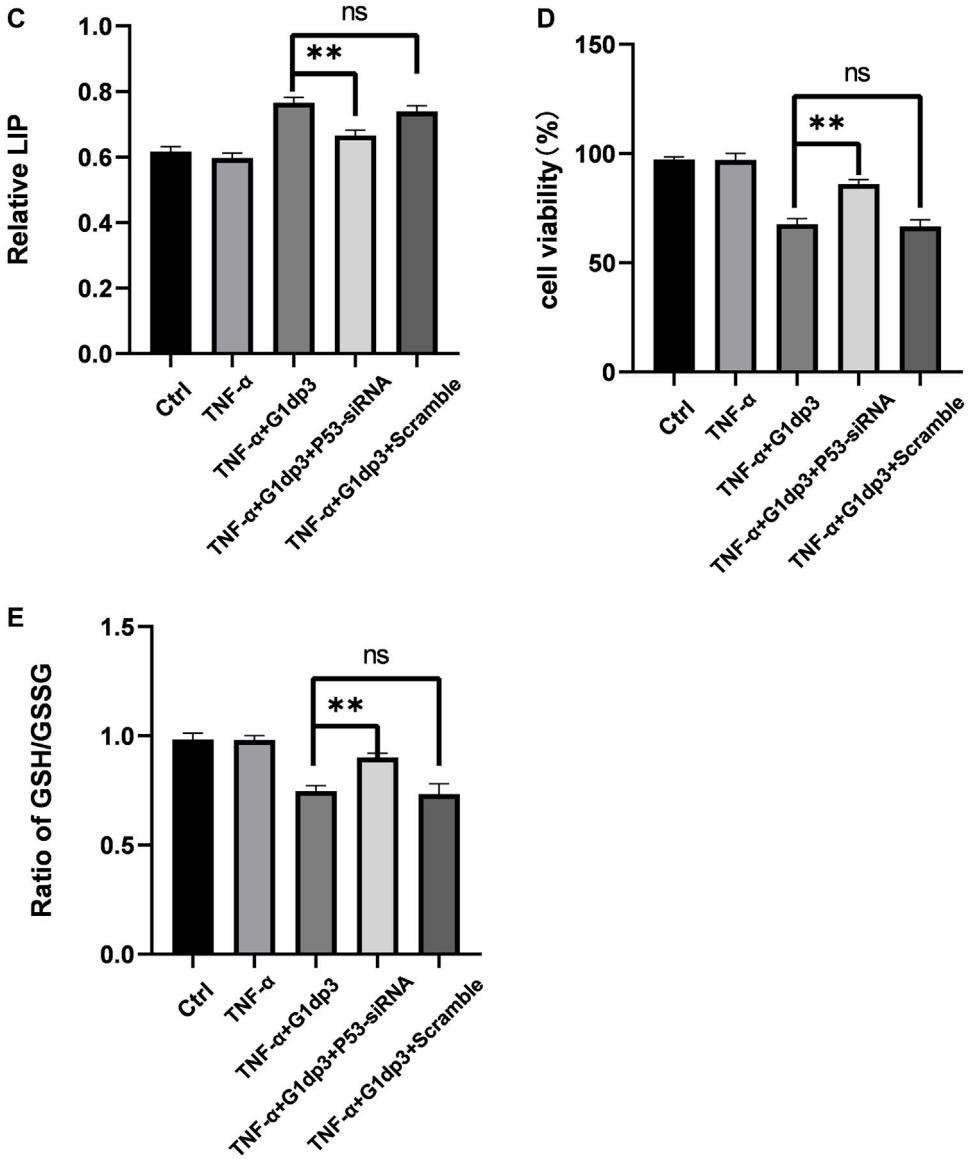

FIGURE 4 | (Continued). G1dP3 regulates ferroptosis through p53/SLC7A11 axis. **(A)** p53 and SLC7A11 expression levels were determined by RT-qPCR and Western blotting. G1dP3 increased p53 but reduced SLC7A11 at both mRNA and protein levels. To explore the role of p53 in G1dP3-regulated ferroptosis, siRNA knockdown of p53 was carried out. **(B)** SLC7A11 protein level was determined by Western blotting. **(C)** Relative LIP levels in the indicated MH7A cells. **(D)** Cell viability were detected in the indicated MH7A cells. **(E)** Ratio of GSH/GSSG in the indicated MH7A cells. Graph represents mean ± SD; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, ns, not significant. Representative data from one of three individual experiments.

G1dP3 could inhibit MH7A cell viability independent of apoptosis.

Ferroptosis is a newly recognized form of RCD discovered by Brent R. Stockwell's lab in 2012. Distinct from other types of RCD (e.g., autophagy, necrosis and apoptosis) at morphological, genetical and mechanistic levels, ferroptosis is defined as an iron-dependent form of non-apoptotic cell death (Dixon et al., 2012; Jiang et al., 2021). Mechanistically, ferroptosis arises from dysregulated lipid peroxidation or iron metabolism, along with GSH depletion and Gpx4 inactivation (Dixon et al., 2012; Jiang et al., 2021). Neither suppression of apoptosis, necrosis nor autophagy by small molecules can reverse ferroptosis. Meanwhile, cellular morphological manifestations in ferroptosis cells are also different from normal features. More importantly, rupture of the mitochondrial outer membrane and deposition of copious lipid droplets in the cytoplasm are dispensable for ferroptosis (Jiang et al., 2015b). A growing body of research has pointed out the function of ferroptosis in degenerative diseases (Stockwell et al., 2017). For instance, inhibition of ferroptosis could alleviate traumatic and hemorrhagic brain injury and other neurodegeneration syndromes (Stockwell et al., 2017; Bao et al., 2021). Besides, ferroptosis may be a potential therapeutic target for ameliorating

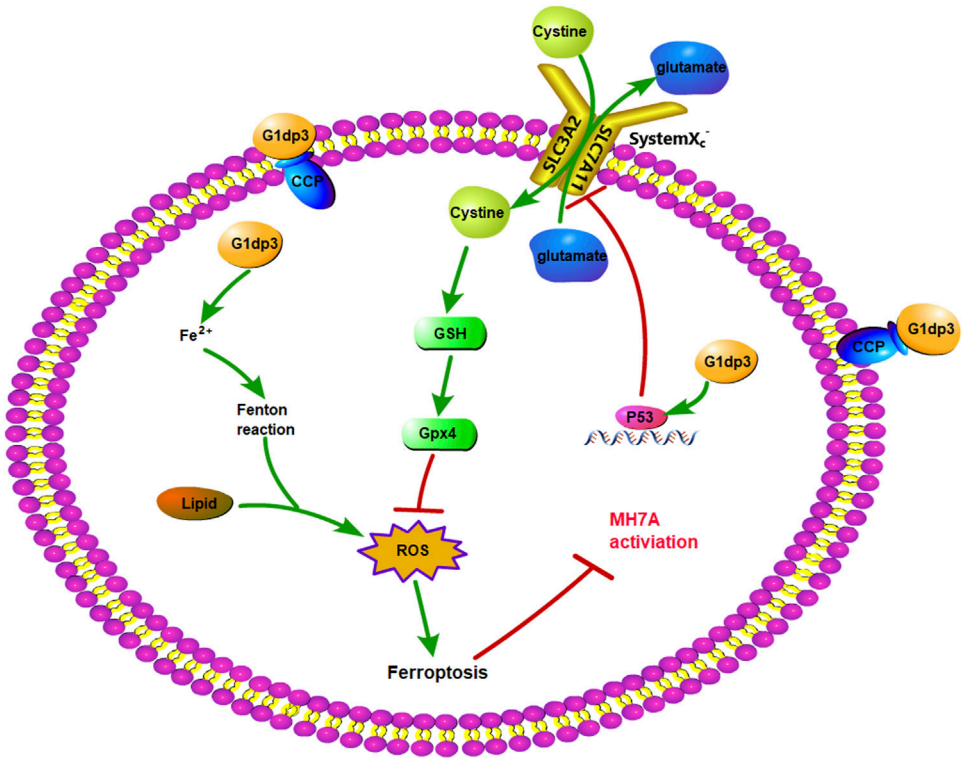

FIGURE 5 | p53/SLC7A11 pathways play an essential role in G1dP3-regulated MH7A ferroptosis. G1dP3 treatment induced p53 overexpression, SLC7A11 inactivation, GSH depletion, Gpx4 deficiency, ROS production, ferroptosis activation, and ultimately suppressed MH7A cell activation.

hemochromatosis-related tissue damage and liver fibrosis (Wang et al., 2017, 2019). It seems that degenerative diseases are induced by disorders mainly in repairing peroxidized lipids, which subsequently lead to cell death (Viswanathan et al., 2017; Tarangelo et al., 2018). Thus, ferroptosis might serve as a suppressor or inducer in degenerative disorders by removing cells lacking access to seize crucial nutrients, including chondrocyte death and bone erosion in RA (Maiorino et al., 2018). To our surprise, transmission electron microscopy showed smaller, ruptured mitochondria with increased membrane density and vanished mitochondria cristae, which conferred morphological changes in ferroptosis cells. These findings implicated that G1dP3-mediated MH7A cell viability suppression was associated with cellular ferroptosis. As expected, the enhanced lipid peroxidation, elevated iron levels, depleted GSH levels and reduced Gpx4 activity were also observed in G1dP3-mediated MH7A cells. Furthermore, the ferroptosis specific inhibitor Fer-1 and iron chelators DFO alleviated G1dP3-induced decrease of MH7A cell viability. Taken together, these data support our hypothesis that the suppression of MH7A cell viability is associated with G1dP3-regulated ferroptosis. To our knowledge, this is the first time report of ferroptosis in RA synovial fibroblasts.

Commonly referred to as the "guardian of the genome", p53 has been considered a powerful tumour suppressor through the induction of apoptosis, senescence and cell cycle arrest (Levine and Oren, 2009; Levine, 2020). However, the single function of p53 responsible for its tumor suppressive effects remains to be identified (Kaiser and Attardi, 2018). Recently, Gu et al. revealed that p53 suppressed tumour growth through its metabolism-regulatory ability, instead of its DNA-damage response (Liu and Gu, 2022). p53 can regulate a cascade of metabolic pathways including amino acids, lipid, ROS and iron metabolism, which is tightly bound to ferroptosis (Stockwell et al., 2020; Liu and Gu, 2021). Furthermore, p53 was proposed to positively regulate ferroptosis by downregulating SLC7A11 expression to induce the levels of ROS (Jiang et al., 2015b; Wang et al., 2019). SLC7A11 is an active constituent of the system X_c^-, which is a disulfide-bonded heterodimer generated via SLC3A2 and SLC7A11 (Sato et al., 1999). The system X_c^- has antioxidant properties by supplying cysteine for GSH synthesis and maintaining redox balance across the plasma membranes (Conrad and Sato, 2012). As a consequence, SLC7A11 is mainly involved in the negative modulation of ferroptosis (Jiang et al., 2015a; Wang et al., 2016). Additionally, $p53^{K101R}$ mutant has lost its ability to induce SLC7A11 expression (Liu et al., 2019), indicating that p53/SLC7A11 signaling may govern ferroptotic activity in MH7A cells. Our findings demonstrated that G1dP3 increased the expression and nuclear transportation of p53, accompanied by the downregulated expression of SLC7A11 in both mRNA and protein levels. Knockdown of p53 abrogated the ferroptotic effects of G1dP3 on MH7A cells by decreasing free iron levels, promoting GSH synthesis, and in turn enhancing MH7A cell viability. Overall, these findings revealed that G1dP3 mediated ferroptosis in TNF-α-induced MH7A cells via a p53/SLC7A11 axis-dependent mechanism (**Figure 5**).

Nevertheless, some limitations exist in our study. Firstly, although the inhibitory effect of G1dP3 on MH7A cell viability is independent

of apoptosis, the proapoptotic familiy gene members were indeed upregulated and the executor of apoptosis Caspase-3 cleaved after G1dP3 treatment. This indicates that G1dP3 induces ferroptosis, accompanying with apoptotic regulation. Hence, other metabolic mechanisms of RA synovial fibroblasts should be explored in further studies. What's more, as a classic apoptosis-promoting gene, the increased expression and nuclear transportation of p53 following the treatment of G1dP3 may account for the upregulation of apoptotic genes (**Figure 1**). Our findings also confirmed that blockade of p53 activation markedly abrogated the ferroptotic effects of G1dP3 by promoting the expression of SLC7A11. However, the expression levels of apoptosis-related genes after p53 knockdown have not been detected. Moreover, ferroptosis proceeds even in the absence of key apoptotic effectors, (e.g., caspases and BAX/BAK), which has been regarded as an non-apoptotic form of RCD driven by lipid peroxidation and iron deposition. It has been previously suggested that the inhibition of caspase or deletion of BAX/BAK should be examined to confirm the effect of apoptosis or ferroptosis, as both apoptosis and ferroptosis may simultaneously occur during the pathological process of cell death (Stockwell et al., 2017). Ferroptotic cells are characterized by smaller, ruptured mitochondria with increased membrane density and vanished mitochondria cristae, which may be regulated by the proapoptotic BCL2 family members such as BCL2-binding component 3 (BBC3, also known as PUMA) (Hong et al., 2017) and BH3-interacting domain death agonist (BID) (Neitemeier et al., 2017), indicating the possibilities of interaction between ferroptosis and apoptosis in cell metabolism. Furthermore, necroptotic pathways may be revealed by the suppression of apoptosis via caspase inhibition, and similar backup systems will come into action when other RCD modalities are suppressed (Tang et al., 2019). Thus, more research is needed to explore the interplay between ferroptosis and apoptosis and identify the unique molecular effectors of ferroptosis following apoptosis suppression.

CONCLUSION

Our data demonstrated for the first time that the bioactive peptide G1dP3 could sensitize RA synovial fibroblasts to ferroptotic cell death via a p53/SLC7A11 axis-dependent mechanism, thereby providing novel opportunities and future directions for RA treatment. However, further studies are required to validate our findings in terms of RA prevention through an *in vivo* model.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JH: Methodology, Writing—original draft, Data curation, Writing—review editing. RZ: Formal analysis. QC and MJ: Methodology, Software. HZ: Validation, Software. RG: Methodology, Formal analysis. CL: Software. ZW: Conceptualization, Supervision, Project administration, Funding acquisition.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.920273/full#supplementary-material

Supplementary Figure SA | Western blot analysis of protein expression of p53. MH7A were pretreated with p53-siRNA or siRNA. **p<0.01.

Supplementary Figure SB | Western blot analysis of protein expression of Caspase3. MH7A were pretreated with Caspase3-siRNA or siRNA. **p<0.01.

REFERENCES

Aletaha, D., Funovits, J., and Smolen, J. S. (2011). Physical Disability in Rheumatoid Arthritis Is Associated with Cartilage Damage rather Than Bone Destruction. *Ann. rheumatic Dis.* 70 (5), 733–739. doi:10.1136/ard.2010.138693

Baggerman, G., Boonen, K., Verleyen, P., De Loof, A., and Schoofs, L. (2005). Peptidomic Analysis of the larvalDrosophila Melanogaster Central Nervous System by Two-Dimensional Capillary Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry. *J. Mass Spectrom.* 40 (2), 250–260. doi:10.1002/jms.744

Bao, Z., Liu, Y., Chen, B., Miao, Z., Tu, Y., Li, C., et al. (2021). Prokineticin-2 Prevents Neuronal Cell Deaths in a Model of Traumatic Brain Injury. *Nat. Commun.* 12 (1), 4220. doi:10.1038/s41467-021-24469-y

Cao, J. Y., and Dixon, S. J. (2016). Mechanisms of Ferroptosis. *Cell. Mol. Life Sci.* 73 (11-12), 2195–2209. doi:10.1007/s00018-016-2194-1

Conrad, M., and Sato, H. (2012). The Oxidative Stress-Inducible Cystine/glutamate Antiporter, System X C − : Cystine Supplier and beyond. *Amino acids* 42 (1), 231–246. doi:10.1007/s00726-011-0867-5

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: an Iron-dependent Form of Nonapoptotic Cell Death. *Cell.* 149 (5), 1060–1072. doi:10.1016/j.cell.2012.03.042

Fosgerau, K., and Hoffmann, T. (2015). Peptide Therapeutics: Current Status and Future Directions. *Drug Discov. today* 20 (1), 122–128. doi:10.1016/j.drudis.2014.10.003

Guan, Z., Chen, J., Li, X., and Dong, N. (2020). Tanshinone IIA Induces Ferroptosis in Gastric Cancer Cells through P53-Mediated SLC7A11 Down-Regulation. *Biosci. Rep.* 40 (8). doi:10.1042/BSR20201807

Hong, S. H., Lee, D.-H., Lee, Y.-S., Jo, M. J., Jeong, Y. A., Kwon, W. T., et al. (2017). Molecular Crosstalk between Ferroptosis and Apoptosis: Emerging Role of ER Stress-Induced P53-independent PUMA Expression. *Oncotarget* 8 (70), 115164–115178. doi:10.18632/oncotarget.23046

Hu, J., Lu, J., Zhang, X., Wang, C., Ren, K., Chang, Q., et al. (2020). Peptidomic Analysis on Synovial Tissue Reveals Galectin-1 Derived Peptide as a Potential

Bioactive Molecule against Rheumatoid Arthritis. *Cytokine* 131, 155020. doi:10.1016/j.cyto.2020.155020

Jiang, L., Hickman, J. H., Wang, S.-J., and Gu, W. (2015a). Dynamic Roles of P53-Mediated Metabolic Activities in ROS-Induced Stress Responses. *Cell. cycle* 14 (18), 2881–2885. doi:10.1080/15384101.2015.1068479

Jiang, L., Kon, N., Li, T., Wang, S.-J., Su, T., Hibshoosh, H., et al. (2015b). Ferroptosis as a P53-Mediated Activity during Tumour Suppression. *Nature* 520 (7545), 57–62. doi:10.1038/nature14344

Jiang, X., Stockwell, B. R., and Conrad, M. (2021). Ferroptosis: Mechanisms, Biology and Role in Disease. *Nat. Rev. Mol. Cell. Biol.* 22 (4), 266–282. doi:10.1038/s41580-020-00324-8

Kaiser, A. M., and Attardi, L. D. (2018). Deconstructing Networks of P53-Mediated Tumor Suppression *In Vivo*. *Cell. Death Differ.* 25 (1), 93–103. doi:10.1038/cdd.2017.171

Klippel, J. H. (2000). Biologic Therapy for Rheumatoid Arthritis. *N. Engl. J. Med.* 343 (22), 1640–1641. doi:10.1056/nejm200011303432210

Levine, A. J. (2020). p53: 800 Million Years of Evolution and 40 Years of Discovery. *Nat. Rev. Cancer* 20 (8), 471–480. doi:10.1038/s41568-020-0262-1

Levine, A. J., and Oren, M. (2009). The First 30 Years of P53: Growing Ever More Complex. *Nat. Rev. Cancer* 9 (10), 749–758. doi:10.1038/nrc2723

Lim, C. T., Kola, B., and Korbonits, M. (2010). AMPK as a Mediator of Hormonal Signalling. *J. Mol. Endocrinol.* 44 (2), 87–97. doi:10.1677/jme-09-0063

Liu, Y., and Gu, W. (2022). p53 in Ferroptosis Regulation: the New Weapon for the Old Guardian. *Cell. death Differ.* 29 (5), 895–910. doi:10.1038/s41418-022-00943-y

Liu, Y., and Gu, W. (2021). The Complexity of P53-Mediated Metabolic Regulation in Tumor Suppression. *Seminars cancer Biol.* 27, S1044–579X. doi:10.1016/j.semcancer.2021.03.010

Liu, Y., Tavana, O., and Gu, W. (2019). p53 Modifications: Exquisite Decorations of the Powerful Guardian. *J. Mol. Cell. Biol.* 11 (7), 564–577. doi:10.1093/jmcb/mjz060

Maiorino, M., Conrad, M., and Ursini, F. (2018). GPx4, Lipid Peroxidation, and Cell Death: Discoveries, Rediscoveries, and Open Issues. *Antioxidants redox Signal.* 29 (1), 61–74. doi:10.1089/ars.2017.7115

Neitemeier, S., Jelinek, A., Laino, V., Hoffmann, L., Eisenbach, I., Eying, R., et al. (2017). BID Links Ferroptosis to Mitochondrial Cell Death Pathways. *Redox Biol.* 12, 558–570. doi:10.1016/j.redox.2017.03.007

Panipinto, P. M., Singh, A. K., Shaikh, F. S., Siegel, R. J., Chourasia, M., and Ahmed, S. (2021). Takinib Inhibits Inflammation in Human Rheumatoid Arthritis Synovial Fibroblasts by Targeting the Janus Kinase-Signal Transducer and Activator of Transcription 3 (JAK/STAT3) Pathway. *Int. J. Mol. Sci.* 22 (22). doi:10.3390/ijms222212580

Pap, T., Müller-Ladner, U., Gay, R. E., and Gay, S. (2000). Fibroblast biologyRole of Synovial Fibroblasts in the Pathogenesis of Rheumatoid Arthritis. *Arthritis Res.* 2 (5), 361–367. doi:10.1186/ar113

Roark, C. L., Anderson, K. M., Aubrey, M. T., Rosloniec, E. F., and Freed, B. M. (2016). Arthritogenic Peptide Binding to DRB1*01 Alleles Correlates with Susceptibility to Rheumatoid Arthritis. *J. Autoimmun.* 72, 25–32. doi:10.1016/j.jaut.2016.04.006

Sato, H., Tamba, M., Ishii, T., and Bannai, S. (1999). Cloning and Expression of a Plasma Membrane Cystine/glutamate Exchange Transporter Composed of Two Distinct Proteins. *J. Biol. Chem.* 274 (17), 11455–11458. doi:10.1074/jbc.274.17.11455

Smolen, J. S., Aletaha, D., Bijlsma, J. W. J., Breedveld, F. C., Boumpas, D., Burmester, G., et al. (2010). Treating Rheumatoid Arthritis to Target: Recommendations of an International Task Force. *Ann. rheumatic Dis.* 69 (4), 631–637. doi:10.1136/ard.2009.123919

Smolen, J. S., Aletaha, D., and McInnes, I. B. (2016). Rheumatoid Arthritis. *Lancet* 388 (10055), 2023–2038. doi:10.1016/s0140-6736(16)30173-8

Smolen, J. S. (2020). Insights into the Treatment of Rheumatoid Arthritis: A Paradigm in Medicine. *J. Autoimmun.* 110, 102425. doi:10.1016/j.jaut.2020.102425

Stockwell, B. R., Friedmann Angeli, J. P., Bayir, H., Bush, A. I., Conrad, M., Dixon, S. J., et al. (2017). Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell.* 171 (2), 273–285. doi:10.1016/j.cell.2017.09.021

Stockwell, B. R., Jiang, X., and Gu, W. (2020). Emerging Mechanisms and Disease Relevance of Ferroptosis. *Trends Cell. Biol.* 30 (6), 478–490. doi:10.1016/j.tcb.2020.02.009

Tang, D., Kang, R., Berghe, T. V., Vandenabeele, P., and Kroemer, G. (2019). The Molecular Machinery of Regulated Cell Death. *Cell. Res.* 29 (5), 347–364. doi:10.1038/s41422-019-0164-5

Tarangelo, A., Magtanong, L., Bieging-Rolett, K. T., Li, Y., Ye, J., Attardi, L. D., et al. (2018). p53 Suppresses Metabolic Stress-Induced Ferroptosis in Cancer Cells. *Cell. Rep.* 22 (3), 569–575. doi:10.1016/j.celrep.2017.12.077

Tian, X.-Q., Yang, Y.-J., Li, Q., Huang, P.-S., Li, X.-D., Jin, C., et al. (2016). Globular Adiponectin Inhibits the Apoptosis of Mesenchymal Stem Cells Induced by Hypoxia and Serum Deprivation via the AdipoR1-Mediated Pathway. *Cell. Physiol. Biochem.* 38 (3), 909–925. doi:10.1159/000443044

Turner, J. D., and Filer, A. (2015). The Role of the Synovial Fibroblast in Rheumatoid Arthritis Pathogenesis. *Curr. Opin. rheumatology* 27 (2), 175–182. doi:10.1097/bor.0000000000000148

Viswanathan, V. S., Ryan, M. J., Dhruv, H. D., Gill, S., Eichhoff, O. M., Seashore-Ludlow, B., et al. (2017). Dependency of a Therapy-Resistant State of Cancer Cells on a Lipid Peroxidase Pathway. *Nature* 547 (7664), 453–457. doi:10.1038/nature23007

Wang, H., An, P., Xie, E., Wu, Q., Fang, X., Gao, H., et al. (2017). Characterization of Ferroptosis in Murine Models of Hemochromatosis. *Hepatology* 66 (2), 449–465. doi:10.1002/hep.29117

Wang, L., Zhang, Z., Li, M., Wang, F., Jia, Y., Zhang, F., et al. (2019). P53-dependent Induction of Ferroptosis Is Required for Artemether to Alleviate Carbon Tetrachloride-Induced Liver Fibrosis and Hepatic Stellate Cell Activation. *IUBMB life* 71 (1), 45–56. doi:10.1002/iub.1895

Wang, S.-J., Li, D., Ou, Y., Jiang, L., Chen, Y., Zhao, Y., et al. (2016). Acetylation Is Crucial for P53-Mediated Ferroptosis and Tumor Suppression. *Cell. Rep.* 17 (2), 366–373. doi:10.1016/j.celrep.2016.09.022

Weyand, C. M., and Goronzy, J. J. (2021). The Immunology of Rheumatoid Arthritis. *Nat. Immunol.* 22 (1), 10–18. doi:10.1038/s41590-020-00816-x

Xie, Y., Hou, W., Song, X., Yu, Y., Huang, J., Sun, X., et al. (2016). Ferroptosis: Process and Function. *Cell. Death Differ.* 23 (3), 369–379. doi:10.1038/cdd.2015.158

Xie, Y., Zhu, S., Song, X., Sun, X., Fan, Y., Liu, J., et al. (2017). The Tumor Suppressor P53 Limits Ferroptosis by Blocking DPP4 Activity. *Cell. Rep.* 20 (7), 1692–1704. doi:10.1016/j.celrep.2017.07.055

Zorko, M., Jones, S., and Langel, Ü. (2022). Cell-penetrating Peptides in Protein Mimicry and Cancer Therapeutics. *Adv. drug Deliv. Rev.* 180, 114044. doi:10.1016/j.addr.2021.114044

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Case Report: Pulmonary sarcomatoid carcinoma complicating TP53 mutation treated successfully with Tislelizumab combined with Anlotinib—a case report

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Pulmonary sarcomatoid carcinoma (PSC) is a rare subtype of lung malignant tumor. Conventional chemotherapy has a suboptimal effectiveness. PSC has the characteristics of rapid disease progression and poor prognosis. We herein report a 56-year-old male patient with substantial smoking history was pathologically diagnosed as PSC, cT4N0M0 IIIA stage. Peripheral blood NGS showed TP53 mutation. The patient had poor tolerance to the first-line chemotherapy regimen "albumin paclitaxel + cisplatin," but the severe anemia was significantly improved after 5 days of anti-angiogenic therapy with Anlotinib. At this time, the patient received anti-PD-1 immunotherapy with Tislelizumab. Half a month later, degree III liver injury occurred repeatedly. After excluding drug-induced liver injury, we found that HCV-RNA 3.10×10^5 IU/ml and suspended all anti-tumor therapy. After the start of anti-HCV treatment with Epclusa, the treatment of Tislelizumab combined with Anlotinib was restarted, and there was no liver injury after that. The patient received monthly maintenance therapy with Tislelizumab combined with Anlotinib to the present. The pulmonary lesions continued to decrease, and only one lung cavity is left. The patient has achieved clinical complete remission (CCR) with PSF over 20 months. Our findings suggest that Tislelizumab combined with Anlotinib may be a preferred strategy in PSC complicating TP53 mutation. Core tip: Immune-check point inhibitors (ICIs) have been reported for the treatment of PSC in a small number of case reports and retrospective analysis, but there are few reports of ICIs combined with anti-angiogenic drugs. This patient was diagnosed as locally advanced PSC complicated with TP53 mutation and hepatitis C. After 14 cycles of Tislelizumab combined with Anlotinib treatment (during the course of treatment, several courses were not treated on time for economic reasons, rather than adverse reactions), the patient has achieved CCR. III degree liver injury occurred during the treatment, and the liver function returned to normal range after anti-hepatitis C treatment, which did not affect the continued treatment of this regimen.

KEYWORDS

Tislelizumab, Anlotinib, pulmonary sarcomatoid carcinoma, TP53 mutation, hepatitis C, case report

Introduction

Pulmonary sarcomatoid carcinoma (PSC) is a rare kind of lung malignant tumor, and its incidence accounts for about 0.1–0.4% of the total number of lung malignant tumors. It is common in elderly men with a large history of smoking (Mehrad et al., 2018). It features high degree of malignancy, rapid disease progression, and poor prognosis (Shum et al., 2016). Radical surgery can be selected for early PSC, but most patients miss the optimal time of surgery because of the late diagnosis (Tulsi et al., 2021). PSC shows insensitivity to traditional radiotherapy and chemotherapy (Gu et al., 2015).

With the wide application of next-generation sequencing (NGS), targeted therapy, and immunotherapy (Wang et al., 2021) in recent years, there are more options for the treatment of PSC (Li X. et al., 2020). A Chinese NGS study of 32 PSC patients showed that TP53 (69%) was the most common mutated tumor suppressor gene (Liang et al., 2019). The tumor protein TP53 gene, encoding the cellular tumor antigen p53, is the single most frequently mutated gene in human cancers (Vaddavalli and Schumacher, 2022). However, there are no clinically approved drugs targeting TP53 mutations currently (Ku et al., 2017). In addition, Nalan A. Babacan's summary analysis showed that nearly 90% of PSC patients had PD-L1 ≥ 1%, and the level of PD-L1 expression was significantly correlated with therapeutic effect of immune-check point inhibitors (ICIs) (Babacan et al., 2020). The therapeutic effect of selective application of ICIs in patients with related tests is much better than that of traditional radiotherapy and chemotherapy (Li X. et al., 2020). However, due to the low incidence of PSC, the application of ICIs is mostly reported in case reports, retrospective summary analysis, and small sample stage II clinical trials. Further attention needs to be paid to its adverse reactions, combination treatments, treatment course, and so on.

We herein report a case of advanced PSC treated with first-line conventional chemotherapy (albumin paclitaxel + cisplatin) and then adjusted to Anlotinib combined with Tislelizumab, which is the first case reporting the effectiveness of Anlotinib combined with Tislelizumab in the treatment of PSC complicating TP53 mutation.

Case report

A 56-year-old man with a history of 30 pack-years smoking and drug use complained of chest tightness, asthma for 1 week. A physical examination revealed the following: ECOG 4 score, height 176 cm, weight 46 kg. The conjunctiva of the mouth and lower eyelid was pale.

A laboratory examination revealed the following: White blood cell count 64.9 × 10^9/L, neutrophil absolute value 59.95 × 10^9/L, hemoglobin 57 g/L, platelet count 377 × 10^9/L, HBsAb 153.2mIU/ml, HBcAb 8.52S/CO, HCV-Ab 9.29S/CO. There was no obvious abnormality in quantitative detection of hepatitis B DNA, liver function and autoimmune antibody.

Chest CT showed that the left lung occupied a large space, with a size of about 13.2*11.1 cm, uneven density, local invasion of adjacent bronchial branches, narrowing and occlusion of adjacent bronchial branches, and interstitial changes of the left lung.

Under CT guidance, the tip was determined to be located in the lesion (Figure 1). A 18G cutting needle was inserted, four fish-like tissues were cut, and the specimens were fixed with formalin. The biopsy pathology of the left lung mass showed poorly differentiated carcinoma. Immunohistochemistry showed: tumor cells Vimentin (+), Ki67 (about 50%+), CK-pan (scattered weak +), CK-L (+), CKH (−), CK7 (scattered weak +), NapsinA (−), TTF1 (−), CK5/6 (−), P63 (−), P40 (−), Syn (−), EMA (−), CD34 (−), Desmin (−), S100 (−), MelanA (−), FLI1 (−), SOX10 (−), INI-1 (scattered weak +), and tended to sarcomatoid carcinoma (Figure 2). We performed targeted enrichment using a GenCap Custom Exome Enrichment kit (MyGenostics, Beijing, China) for the patient's peripheral blood. This enrichment kit contains 562 tumor-driven genes, 45 chemotherapy genes, 90 genetic risk-related genes, and 35 HRR-related genes (Supplementary Table S1). The results of peripheral blood NGS (including 595 tumor-related genes) showed mutation in exon 4 of TP53 (TP53 p.T125K mutation); TMB was 7.7 mutations/Mb, moderate TMB; MSS was stable; and MMR gene had no mutation. According to the patient's will, we did not perform PD-L1 test. And tumor tissue NGS could not be performed because of limited biopsy tissue. Therefore, the patient was diagnosed with sarcomatoid carcinoma of the left lung in stage IIIA (cT4N0M0).

The patient received multiple red blood cell suspension transfusions and parenteral nutrition support. In September 2020, he was treated with albumin paclitaxel (150 mg, d1/d8) combined with cisplatin (20 mg, d1-3). IV degree myelosuppression followed, and the treatment was abandoned in the second half of the cycle. After 5 days of oral administration of Anlotinib (12 mg qd), the general condition of the patient was significantly improved and he did not need to rely on blood transfusion. He received 200 mg immunotherapy with Tislelizumab on September 29.

Half a month later, the patient found III degree liver injury, accompanied by positive antinuclear antibody. After the recovery of liver function, patients were treated with albumin paclitaxel (150 mg D1) combined with cisplatin (20 mg D1-3) and

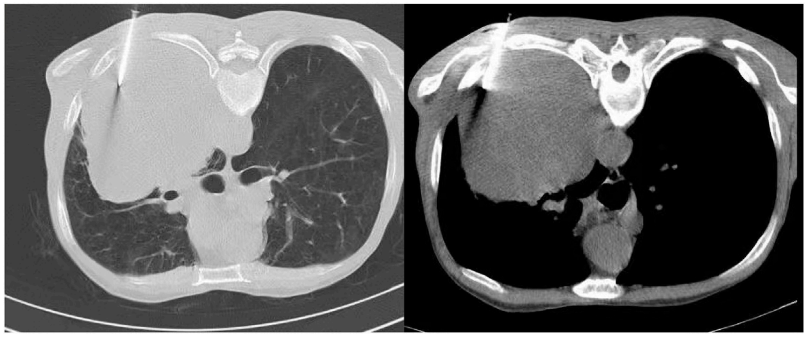

FIGURE 1
CT-guided percutaneous lung biopsy (CT-PTNB). CT = computed tomography.

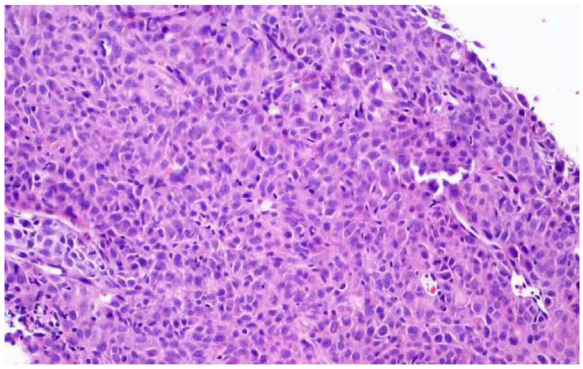

FIGURE 2
Microscopic findings of lung biopsy specimens. Routine biopsy showed: poorly differentiated adenocarcinoma. Immunohistochemistry showed: tumor cells Vimentin (+), Ki67 (about 50%+), CK-pan (scattered weak +), CK-L (+), CKH (–), CK7 (scattered weak+), NapsinA (–), TTF1 (–), CK5/6 (–), P63 (–), P40 (–), Syn (–), EMA (–), CD34 (–), Desmin (–), S100 (–), MelanA (–), FLI1 (–), SOX10 (–), INI-1 (scattered weak +), and tended to sarcomatoid carcinoma.

Anlotinib on October 23. II degree myelosuppression occurred in the follow-up.

The III degree liver injury was found again in November 2020, and the autoantibodies were all negative. CT evaluation was PR. After the recovery of liver function, patients were treated with Anlotinib combined with Tislelizumab for the second cycle on November 24.

Unfortunately, II degree liver injury occurred again on December 22, and the HCV-RNA of hepatitis C was 3.10×10^5 IU/ml, so we suspended all antineoplastic therapy. However, the quantity of HCV-RNA was negative on 6 January 2021.

On 20 January 2021, he began to receive antiviral therapy with sufosbuvir and velpatasvir tablets (Epclusa), and was treated with Anlotinib combined with Tislelizumab for the third cycle. There was no liver injury in the follow-up, and the patient was treated with Anlotinib combined with Tislelizumab every month (Figure 3). His last reexamination was on 23 June 2022. A physical examination revealed the following: ECOG 1 score, weight 50 kg. There was no obvious abnormality in tumor index, blood routine, and biochemical index. CT shows that the cavity on the lung shrinks again.

According to the patient's recovery condition, conversion therapy can be considered at present. However, he is unwilling to undergo surgical treatment, thus we keep him on maintenance medication and closely monitor adverse reactions.

Discussion

At present, ICIS targeting PD-1/PD-L1 has become an indispensable part of the treatment of non-small cell lung cancer (NSCLC), and the expression level of PD-L1 is a common marker for predicting the efficacy of immunotherapy (Doroshow et al., 2019). Previous studies have shown that PD-L1 is highly expressed in PSC. Velchet reported that 69.2% (9/13) of the patients were positive for PD-L1 (Velcheti et al., 2013), and 36.5% (54/148) of the patients were positive in the Yang Z study (Yang et al., 2019). Charlotte Domblides's retrospective evaluation of the efficacy of second- and third-line ICI treatment in 37 patients with PSC showed that regardless of the expression level of PD-L1, the ORR of patients after immunotherapy was 40.5%, the DCR was 64.8%, and the median OS was 12.7 months (Domblides et al., 2020). Patients with PSC who only received routine chemotherapy had 5–7.7 months of OS and 0–16.5% of ORR (Vieira et al., 2013; Bonomi et al., 2019). These data show that immunotherapy is effective in patients with PSC.

The tumor suppressor protein p53, a transcription product of the anti-oncogene TP53, is a critical factor in preventing cellular cancerization and killing cancer cells by inducing apoptosis. As a result, p53 is often referred to as the "guardian of the genome"

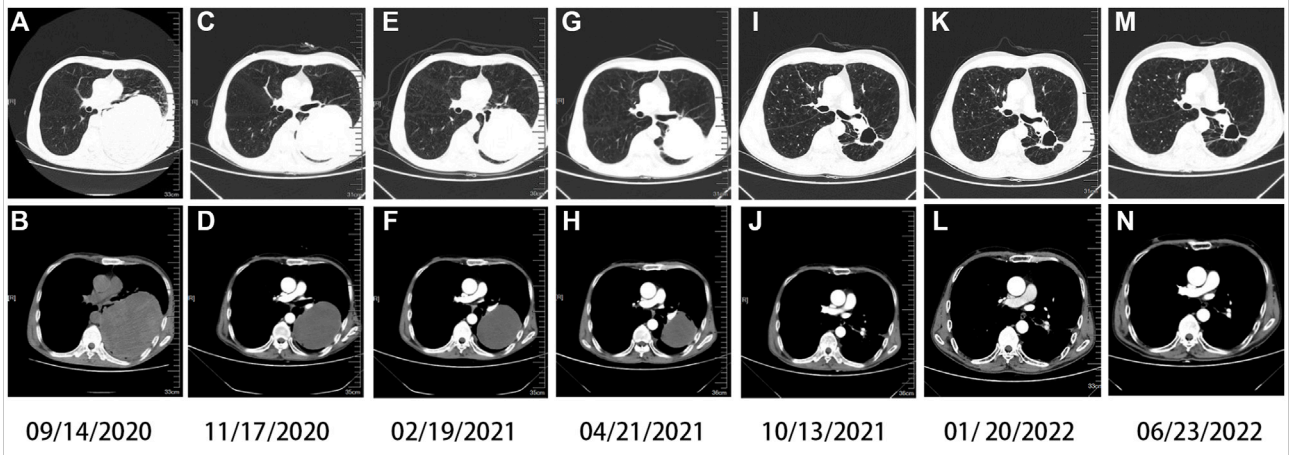

FIGURE 3
Chest computed tomography (CT). Imaging findings during the patient's course **(A,B)** at baseline (solid tumor, dmax = 13.2 cm × 11.1 cm), **(C,D)** after one cycle of Tislelizumab and two cycles of chemotherapy (solid tumor, dmax = 11 cm × 8.7 cm), **(E,F)** after three cycles of Tislelizumab (solid tumor, dmax = 9.5 cm × 8.1 cm), **(G,H)** after five cycles of Tislelizumab (solid tumor, dmax = 7.4 cm × 7.0 cm), **(I,J)** after ten cycles of Tislelizumab (cavity, dmax = 3.9 cm × 3.2 cm), and **(K,L)** after thirteen cycles of Tislelizumab (cavity, dmax = 3.6 cm × 2.8 cm); **(M,N)** in recent recheck (cavity, dmax = 3.3 cm × 2.5 cm), Anlotinib is in continuous use.

(Hibino and Hiroaki, 2022). Mutation of the TP53 tumor suppressor gene is the most common genetic alteration in cancer, and almost 1,000 alleles have been identified in human tumors. Virtually all TP53 mutations are thought to compromise wild-type p53 activity, thus producing mutant p53 protein, which results in the dysfunction of wild-type p53 and promotes malignant transformation of cells (Kennedy and Lowe, 2022). Lysine(K) residue acetylation is a critical epigenetic modification to influence protein structure and gene expression (Verdone et al., 2006). On the DBD (central deoxyribonucleic acid (DNA)-binding domain)of p53, there is an important acetylation site K120, which is catalyzed by three members (Tip60, MOF, and MOZ) of the MYST HAT family (Liu et al., 2019). Tip60 and MOF acetylates p53 at K120 to induce the expression of proapoptotic genes (like PUMA and Bax) (Li et al., 2009; Liu et al., 2019). MOZ-mediated K120 acetylation of p53 specifically enhances its antiproliferative activity. In cancers, K120 is often mutated. The tumor-derived mutant p53 (K120R) is defective for Tip60-mediated acetylation, thus abrogating p53-dependent activation of apoptosis but having no significant effect on cell growth arrest (Li et al., 2009). In addition to K120 acetylation, the multimodular structure of p53 makes it a perfect platform to undergo a multitude of covalent modifications. Actually, in most cases, there is widespread crosstalk between modifications (Gu and Zhu, 2012). In this case, our patient's mutation occurred at amino acid 125, which is close to the K120 site, and there may be nearby modification crosstalk (one modification affects another in its local area). Therefore, the mutation at amino acid 125 may produce similar results as the K120 mutation, accordingly preventing the activation of apoptosis and promoting cancer progression.

Preclinical studies have shown that patients with TP53 mutations may benefit from Wee1 inhibitor AZD1775 (Ku et al., 2017). However, no targeted therapy for TP53 has been approved. Fortunately, studies have shown that TP53 mutation can significantly activate immune-checkpoints, initiate effector T cells, and increase immune factors expression levels. In lung cancer immunotherapy, TP53 mutation may be a positive predictor of immunotherapy (Cui et al., 2021). What's more, studies have shown that smokers are more likely to respond to ICI treatment (Li J. J. N. et al., 2020). Based on these evidences, we chose anti-PD-1 immunotherapy.

Tislelizumab is a humanized IgG4 monoclonal antibody against PD-1, which is different from other PD-1 antibodies in that it can avoid binding to Fc receptors on macrophages through unique modification of the Fc segment, thus eliminating the ADCP effect and avoiding the anti-tumor effect due to the decrease of the number of T cells (Liu and Wu, 2020). In lung cancer, it has been approved by the National Medical Products Administration (NMPA) for combined chemotherapy in the treatment of advanced NSCLC. It has been reported that PD-1 inhibitors such as Nivolumab, Pembrolizumab, and Toripalimab have been used in the treatment of PSC (Cimpeanu et al., 2020; Jin and Yang, 2020; Jiao et al., 2021). Here, we report for the first time the use of Tislelizumab in the treatment of advanced PSC, and the tumor retraction has reached almost complete remission.

The patient had a history of injection drug use, and the baseline showed that HBV-DNA was negative and HCV-Ab

9.29S/CO↑. Before anti-tumor therapy, the screening rate of hepatitis C was low (Hwang et al., 2014; Mohamed et al., 2020). The baseline liver function of this patient was not abnormal, and he denied the history of hepatitis, so no HCV-RNA test was performed. It has been reported that HCV reactivation can occur after chemotherapy (Talima et al., 2019; Xu et al., 2020), but HCV infection has no adverse effect on chemotherapy patients in general (Liu et al., 2017). There are few studies on the effect of HCV on the treatment of ICI, and the reactivation of HCV during ICI treatment is rare (Pertejo-Fernandez et al., 2020; Alkrekshi and Tamaskar, 2021). A retrospective study by Professor Zhang showed that 6 of the 114 HBsAg (+) cancer patients developed viral reactivation after receiving ICI treatment (5.3%). Prophylactic antiviral therapy significantly reduced the risk of viral reactivation (1.2 vs.17.2%, $p = 0.004$) and reduced the incidence of HBV-related hepatitis (1.2 vs.13.8%, $p = 0.019$) (Zhang et al., 2019). It has been reported that a 55-year-old male liver cancer patient with baseline HCV-RNA 152 × 105 IU/ml showed elevated transaminase and second-degree liver injury after the first Nivolumab treatment. The liver injury was relieved after prednisone treatment, and the subsequent use of Nivolumab was not affected (Alkrekshi and Tamaskar, 2021). Therefore, in patients with chemotherapy and immunotherapy, attention should be paid to the reexamination of HBV-DNA and HCV-RNA when liver injury occurs (Gomes et al., 2020).

It has been reported that patients with PSC tried antivascular therapy (Jin and Yang, 2020; Kong et al., 2020). This patient had severe anemia at the time of first admission, and it was difficult to improve by RBC transfusion. After the use of Anlotinib, the anemia was significantly improved, which won the opportunity for subsequent treatment. Anlotinib is a novel multi-target tyrosine kinase inhibitor, which mainly targets VEGFR, EGFR and PDGFR to inhibit tumor angiogenesis. Anlotinib has been approved for third-line treatment of NSCLC, SCLC, second-line treatment of soft tissue sarcoma, and medullary thyroid carcinoma (Syed, 2018; Gao et al., 2020). In our case, the patient was well tolerated with Anlotinib, and no adverse side effects were observed in the follow-ups.

Anti-angiogenic drugs combined with ICI can be synergistic. Anti-angiogenic drugs improve tumor microenvironment by resisting tumor angiogenesis, while anti-PD1 immunotherapy can activate immune cells and promote vascular normalization, both of which form a positive feedback circulatory mechanism. Therefore, anti-angiogenic therapy combined with immunotherapy can act synergistically with tumor cells and improve the curative effect (Huang et al., 2018). After the patient gave up chemotherapy, the patient was treated with Anlotinib combined with Tislelizumab to the present.

As far as we know, this is the first report showing that Tislelizumab combined with Anlotinib is effective in the treatment of PSC complicating TP53 mutation, and this is one of the few reported cases of chemotherapy and immunotherapy in tumor patients with HBV and HCV infection. Immunotherapy combined with anti-angiogenic drugs may be a potential and promising strategy for the treatment of PSC, but its effectiveness and safety need to be further verified in more cases, and more accurate populations need to be selected. In the future, we will continue to follow up the patient to observe the adverse events of ICI and related drug resistance. Moreover, in a variety of clinical cases, we will strive to select more accurate people for accurate treatment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HS and C-YY have contributed significantly in the conception and design of the work. Y-FL has contributed mainly in drafting the work and revising it critically for important intellectual content. X-FZ, YT, and X-YX have contributed in collecting and organizing clinical data.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.949989/full#supplementary-material

References

Alkrekshi, A., and Tamaskar, I. (2021). Safety of immune checkpoint inhibitors in patients with cancer and hepatitis C virus infection. *Oncologist* 26 (5), e827–e830. doi:10.1002/onco.13739

Babacan, N. A., Pina, I. B., Signorelli, D., Prelaj, A., Garassino, M. C., Tanvetyanon, T., et al. (2020). Relationship between programmed death receptor-ligand 1 expression and response to checkpoint inhibitor immunotherapy in pulmonary sarcomatoid carcinoma: a pooled analysis. *Clin. Lung Cancer* 21 (5), e456–e463. doi:10.1016/j.cllc.2020.02.022

Bonomi, P., Fidler, M. J., Shah, P., and Borgia, J. (2019). Theoretical and practical implications of treating cachexia in advanced lung cancer patients. *Cancers (Basel)* 11 (11), 1619. doi:10.3390/cancers11111619

Cimpeanu, E., Ahmed, J., Zafar, W., DeMarinis, A., Bardarov, S. S., Salman, S., et al. (2020). Pembrolizumab-emerging treatment of pulmonary sarcomatoid carcinoma: a case report. *World J. Clin. Cases* 8 (1), 97–102. doi:10.12998/wjcc.v8.i1.97

Cui, J., Zheng, L., Zhang, Y., and Xue, M. (2021). Bioinformatics analysis of DNMT1 expression and its role in head and neck squamous cell carcinoma prognosis. *Sci. Rep.* 11 (1), 2267. doi:10.1038/s41598-021-81971-5

Domblides, C., Leroy, K., Monnet, I., Mazieres, J., Barlesi, F., Gounant, V., et al. (2020). Efficacy of immune checkpoint inhibitors in lung sarcomatoid carcinoma. *J. Thorac. Oncol.* 15 (5), 860–866. doi:10.1016/j.jtho.2020.01.014

Dong, Z. Y., Zhong, W. Z., Zhang, X. C., Su, J., Xie, Z., Liu, S. Y., et al. (2017). Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. *Clin. Cancer Res.* 23 (12), 3012–3024. doi:10.1158/1078-0432.CCR-16-2554

Doroshow, D. B., Sanmamed, M. F., Hastings, K., Politi, K., Rimm, D. L., Chen, L., et al. (2019). Immunotherapy in non-small cell lung cancer: facts and hopes. *Clin. Cancer Res.* 25 (15), 4592–4602. doi:10.1158/1078-0432.CCR-18-1538

Gao, Y., Liu, P., and Shi, R. (2020). Anlotinib as a molecular targeted therapy for tumors. *Oncol. Lett.* 20 (2), 1001–1014. doi:10.3892/ol.2020.11685

Gomes, C., Ginzberg, D., and Wong, R. J. (2020). Delays and gaps in progressing through the hepatitis C virus cascade of care: an underserved safety-net hospital experience. *J. Transl. Int. Med.* 8 (4), 261–267. doi:10.2478/jtim-2020-0039

Gu, B., and Zhu, W. G. (2012). Surf the post-translational modification network of p53 regulation. *Int. J. Biol. Sci.* 8 (5), 672–684. doi:10.7150/ijbs.4283

Gu, L., Xu, Y., Chen, Z., Pan, Y., and Lu, S. (2015). Clinical analysis of 95 cases of pulmonary sarcomatoid carcinoma. *Biomed. Pharmacother.* 76, 134–140. doi:10.1016/j.biopha.2015.10.009

Hibino, E., and Hiroaki, H. (2022). Potential of rescue and reactivation of tumor suppressor p53 for cancer therapy. *Biophys. Rev.* 14 (1), 267–275. doi:10.1007/s12551-021-00915-5

Huang, Y., Kim, B. Y. S., Chan, C. K., Hahn, S. M., Weissman, I. L., Jiang, W., et al. (2018). Improving immune-vascular crosstalk for cancer immunotherapy. *Nat. Rev. Immunol.* 18 (3), 195–203. doi:10.1038/nri.2017.145

Hwang, J. P., Suarez-Almazor, M. E., Torres, H. A., Palla, S. L., Huang, D. S., Fisch, M. J., et al. (2014). Hepatitis C virus screening in patients with cancer receiving chemotherapy. *J. Oncol. Pract.* 10 (3), e167–74. doi:10.1200/JOP.2013.001215

Jiao, Y., Liu, M., Luo, N., Guo, H., and Li, J. (2021). Successful treatment of advanced pulmonary sarcomatoid carcinoma with the PD-1 inhibitor toripalimab: a case report. *Oral Oncol.* 112, 104992. doi:10.1016/j.oraloncology.2020.104992

Jin, C., and Yang, B. (2020). Dramatic response of pulmonary sarcomatoid carcinoma to nivolumab combined with anlotinib: a case report. *Case Rep. Oncol.* 13 (2), 601–605. doi:10.1159/000507568

Kennedy, M. C., and Lowe, S. W. (2022). Mutant p53: it's not all one and the same. *Cell Death Differ.* 29 (5), 983–987. doi:10.1038/s41418-022-00989-y

Kong, F. W., Wang, W. M., Liu, L., Wu, W. B., Wang, X., Zhang, M., et al. (2020). First-line albumin-bound paclitaxel/carboplatin plus apatinib in advanced pulmonary sarcomatoid carcinoma: a case series and review of the literature. *Med. Baltim.* 99 (23), e20667. doi:10.1097/MD.0000000000020667

Ku, B. M., Bae, Y. H., Koh, J., Sun, J. M., Lee, S. H., Ahn, J. S., et al. (2017). Mutational status of TP53 defines the efficacy of Wee1 inhibitor AZD1775 in KRAS-mutant non-small cell lung cancer. *Oncotarget* 8 (40), 67526–67537. doi:10.18632/oncotarget.18728

Li, X., Wu, L., Corsa, C. A., Kunkel, S., and Dou, Y. (2009). Two mammalian MOF complexes regulate transcription activation by distinct mechanisms. *Mol. Cell* 36 (2), 290–301. doi:10.1016/j.molcel.2009.07.031

Li, X., Wu, D., Liu, H., and Chen, J. (2020). Pulmonary sarcomatoid carcinoma: progress, treatment and expectations. *Ther. Adv. Med. Oncol.* 12, 1758835920950207. doi:10.1177/1758835920950207

Li, J. J. N., Karim, K., Sung, M., Le, L. W., Lau, S. C. M., Sacher, A., et al. (2020). Tobacco exposure and immunotherapy response in PD-L1 positive lung cancer patients. *Lung Cancer* 150, 159–163. doi:10.1016/j.lungcan.2020.10.023

Liang, X., Li, Q., Xu, B., Hu, S., Wang, Q., Li, Y., et al. (2019). Mutation landscape and tumor mutation burden analysis of Chinese patients with pulmonary sarcomatoid carcinomas. *Int. J. Clin. Oncol.* 24, 1061–1068. doi:10.1007/s10147-019-01454-6

Liu, S. Y., and Wu, Y. L. (2020). Tislelizumab: an investigational anti-PD-1 antibody for the treatment of advanced non-small cell lung cancer (NSCLC). *Expert Opin. Investig. Drugs* 29 (12), 1355–1364. doi:10.1080/13543784.2020.1833857

Liu, Y., Li, Z. Y., Wang, J. N., Li, X., Huang, Q. A., Huang, Y., et al. (2017). Effects of hepatitis C virus infection on the safety of chemotherapy for breast cancer patients. *Breast Cancer Res. Treat.* 164 (2), 379–383. doi:10.1007/s10549-017-4259-8

Liu, Y., Tavana, O., and Gu, W. (2019). p53 modifications: exquisite decorations of the powerful guardian. *J. Mol. Cell Biol.* 11 (7), 564–577. doi:10.1093/jmcb/mjz060

Mehrad, M., Roy, S., LaFramboise, W. A., Petrosko, P., Miller, C., Incharoen, P., et al. (2018). KRAS mutation is predictive of outcome in patients with pulmonary sarcomatoid carcinoma. *Histopathology* 73 (2), 207–214. doi:10.1111/his.13505

Mohamed, A. A., Omar, A. A. A., El-Awady, R. R., Hassan, S. M. A., Eitah, W. M. S., Ahmed, R., et al. (2020). MiR-155 and MiR-665 role as potential non-invasive biomarkers for hepatocellular carcinoma in Egyptian patients with chronic hepatitis C virus infection. *J. Transl. Int. Med.* 8 (1), 32–40. doi:10.2478/jtim-2020-0006

Pertejo-Fernandez, A., Ricciuti, B., Hammond, S. P., Marty, F. M., Recondo, G., Rangachari, D., et al. (2020). Safety and efficacy of immune checkpoint inhibitors in patients with non-small cell lung cancer and Hepatitis B or hepatitis C infection. *Lung Cancer* 145, 181–185. doi:10.1016/j.lungcan.2020.02.013

Shum, E., Stuart, M., Borczuk, A., Wang, F., Cheng, H., Halmos, B., et al. (2016). Recent advances in the management of pulmonary sarcomatoid carcinoma. *Expert Rev. Respir. Med.* 10 (4), 407–416. doi:10.1586/17476348.2016.1157475

Syed, Y. Y. (2018). Anlotinib: first global approval. *Drugs* 78 (10), 1057–1062. doi:10.1007/s40265-018-0939-x

Talima, S., Kassem, H., and Kassem, N. (2019). Chemotherapy and targeted therapy for breast cancer patients with hepatitis C virus infection. *Breast Cancer* 26 (2), 154–163. doi:10.1007/s12282-018-0904-2

Tulsi, R., Ul Haque, M. M., Hanif, F. M., Devi, A., Mubarak, M., Hassan Luck, N., et al. (2021). Metastasis of duodenal adenocarcinoma to the urinary bladder presenting as hematuria. *J. Transl. Int. Med.* 9 (2), 143–145. doi:10.2478/jtim-2021-0010

Vaddavalli, P. L., and Schumacher, B. (2022). The p53 network: cellular and systemic DNA damage responses in cancer and aging. *Trends Genet.* 38 (6), 598–612. doi:10.1016/j.tig.2022.02.010

Velcheti, V., Rimm, D. L., and Schalper, K. A. (2013). Sarcomatoid lung carcinomas show high levels of programmed death ligand-1 (PD-L1). *J. Thorac. Oncol.* 8 (6), 803–805. doi:10.1097/JTO.0b013e318292be18

Verdone, L., Agricola, E., Caserta, M., and Di Mauro, E. (2006). Histone acetylation in gene regulation. *Brief. Funct. Genomic. Proteomic.* 5 (3), 209–221. doi:10.1093/bfgp/ell028

Vieira, T., Girard, N., Ung, M., Monnet, I., Cazes, A., Bonnette, P., et al. (2013). Efficacy of first-line chemotherapy in patients with advanced lung sarcomatoid carcinoma. *J. Thorac. Oncol.* 8 (12), 1574–1577. doi:10.1097/01.JTO.0000437008.00554.90

Wang, Y., Hou, K., Jin, Y., Bao, B., Tang, S., Qi, J., et al. (2021). Lung adenocarcinoma-specific three-integrin signature contributes to poor outcomes by metastasis and immune escape pathways. *J. Transl. Int. Med.* 9 (4), 249–263. doi:10.2478/jtim-2021-0046

Xu, J., Zhang, J., and Wang, J. (2020). The application of traditional Chinese medicine against the tumor immune escape. *J. Transl. Int. Med.* 8 (4), 203–204. doi:10.2478/jtim-2020-0032

Yang, Z., Xu, J., Li, R., Gao, Y., and He, J. (2019). PD-L1 and CD47 co-expression in pulmonary sarcomatoid carcinoma: a predictor of poor prognosis and potential targets of future combined immunotherapy. *J. Cancer Res. Clin. Oncol.* 145 (12), 3055–3065. doi:10.1007/s00432-019-03023-w

Zhang, X., Zhou, Y., Chen, C., Fang, W., Cai, X., Zhang, X., et al. (2019). Hepatitis B virus reactivation in cancer patients with positive Hepatitis B surface antigen undergoing PD-1 inhibition. *J. Immunother. Cancer* 7 (1), 322. doi:10.1186/s40425-019-0808-5

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REVIEW
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STAT3 and p63 in the Regulation of Cancer Stemness

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Signal transducer and activator of transcription 3 (STAT3) is a transcription factor with many important functions in normal and transformed cells. STAT3 regulatory activities are highly complex as they are involved in various signaling pathways in different cell types under different conditions. Biologically, STAT3 is a regulative factor for normal and cancer stem cells (CSCs). Tumor protein p63 (p63), a member of the p53 protein family, is involved in these biological processes and is also physically and functionally associated with STAT3. STAT3 activation occurs during various aspects of carcinogenesis, including regulation of CSCs properties. In combination with p63, STAT3 is a possible biological marker of CSCs and a major regulator of maintenance of stemness in CSCs. We summarized the STAT3 functions and regulation and its role in CSC properties and highlight how these are affected by its associations with p63.

Keywords: STAT3 (signal transducer and activator of transcription 3), P63 (TP63), cancer, stemness, cancer stem cell

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1 INTRODUCTION

Tumor protein p63 (p63), a member of the p53 protein family, has been associated with Signal transducer and activator of transcription 3 (STAT3), which is one of the seven members of the STATs family of transcription factors (Galoczova et al., 2018a; Wei, 2020). As essential cell survival and proliferation regulators, both STAT3 and p63 have crucial functions in maintenance of stem cell stemness and differentiation. Moreover, they are involved in carcinogenesis of numerous cell types. STAT3 regulates cancer suppressor genes and oncogenes and influences tumor microenvironments (Zou et al., 2020). P63 is commonly associated with epithelial malignancies, particularly squamous cancers (Gatti et al., 2022). Functionally, p63 is also essential for cell adhesion and motility and plays significant roles in regulating various genes that are involved in tumor proliferation, survival, and differentiation (Gatti et al., 2019). In this review, STAT3, its regulation, its roles in cancer stemness maintenance, and its relationship with p63 are elucidated.

2 STRUCTURE AND REGULATION OF STAT3

The STATs family members have similar functional domains, that is, N-terminal domain, central DNA-binding domain, and coiled-coil domain that can achieve protein-protein interactions as well as linker domain that can influence DNA-binding stability and classic SRC homology2 (SH2) domain. STAT3 has two significant phosphorylation positions, a tyrosine residue at amino acid position 705 (Tyr705) within the SH2 domain and a serine phosphorylation site at position 727 (Ser727) within the C-terminal transactivation domain, that are however absent in alternatively spliced STAT3-β variants (**Figure 1**) (Galoczova et al., 2018a).

Functional diversity of STAT3 in different cell types is demonstrated by its regulation of numerous activators and negative regulators. Phosphorylation of Tyr705 rather than Ser727 by

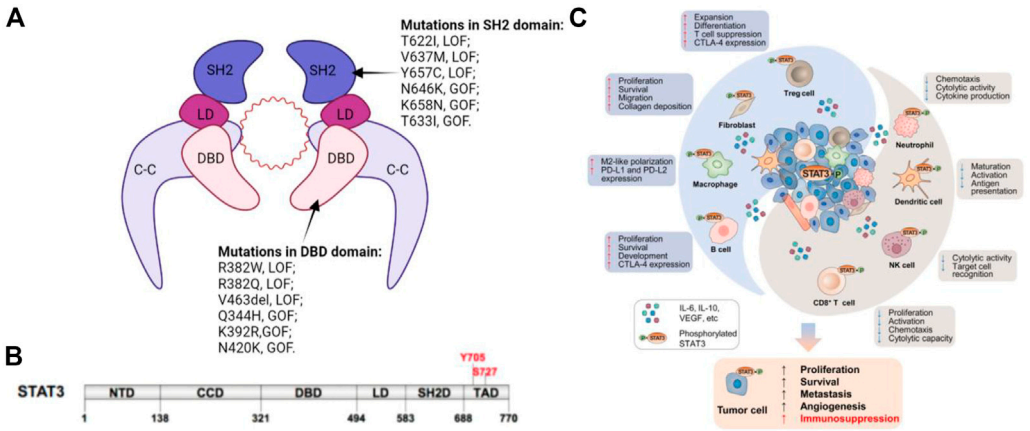

FIGURE 1 | (A) Schematic illustration of STAT3 dimer bound to DNA. This is a representation of the major STAT3-DNA binding complex (resolved in the X-ray crystal structure). **(B)** Primary structure of STAT3 showing domain organization along the polypeptide chain. **(C)** STAT3 induces immunosuppression in the TME. STAT3 activities in tumor cells augment cancer hallmarks, including increased secretion of immunosuppressive factors, such as IL-6, IL-10, and EGFR, which can activate STAT3 in innate and adaptive immune cell subsets as well as CAFs in the TME. Immune cells and CAFs within the TME can release certain factors, including IL-6, which enhances STAT3 signaling in tumor cells. Elevated STAT3 in the TME has dual effects.

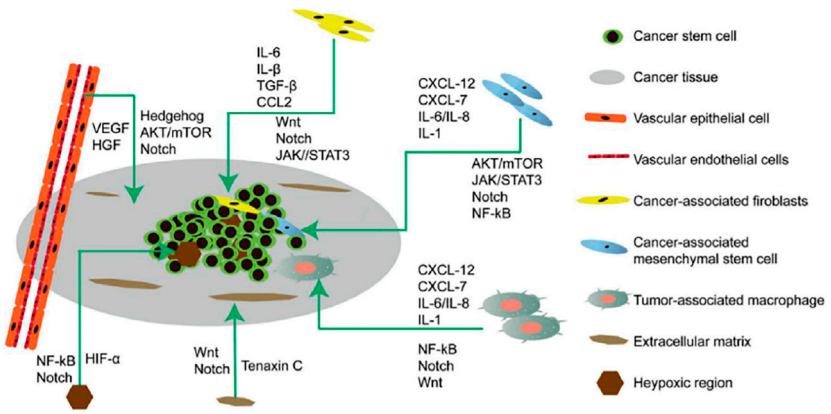

FIGURE 2 | Microenvironment of cancer stem cells (CSCs) illustrating their proliferation, self-renewal, differentiation, metastasis, and tumorigenesis. The CSC microenvironment is mainly composed of vascular niches, hypoxia, tumor-associated macrophages, cancer-associated fibroblasts, cancer-associated mesenchymal stem cells, and an extracellular matrix. These cells, in response to hypoxic stress and matrices, induce growth factors and cytokines (such as IL-6 and VEGF) to regulate CSC growth *Via* Wnt, Notch, and other signaling pathways.

upstream kinases is the main mechanism of STAT3 activation. Non-phosphorylation mechanisms through which STAT3 activation is regulated include post-translational modifications such as acetylation, methylation, and ubiquitination. Negative regulation of STAT3 activation occurs via protein phosphatases, specific protein inhibitors-suppressors of cytokine signaling (SOCS), protein inhibitors of activated STAT (PIAS), and several miRNAs (Butturini et al., 2020). Activation and expressions of STAT3 are regulated by various signals, which function in multiple signaling pathways, thereby making STAT3 a flexible and adaptable regulator that directly and indirectly regulates gene expressions in different types of cells under diverse conditions (Tošić and Frank, 2021).

3 STAT3, CSCS, AND CANCER STEMNESS

Cancer stem cells (CSCs) were first isolated from leukemia cells and identified as therapeutic targets for cancer. CSCs undergo self-renewal, are pluripotent, and are involved in recurrence, metastasis, heterogeneity, and drug and radiation resistance of tumors (Yang et al., 2020). CSCs are regulated by pluripotent transcription factors (OCT4, Sox2, Nanog, KLF4, and MYC). They are also regulated by intracellular signaling pathways, including Wnt, NF-κB, Notch, Hedgehog, JAK-STAT, PI3K/AKT/mTOR, TGF/SMAD, PPAR, and extracellular factors, such as vascular niches, hypoxia, tumor-associated macrophages, cancer-associated fibroblasts, cancer-associated mesenchymal stem cells, extracellular matrices, and exosomes. Thus, to potentially destroy

CSCs, drugs, vaccines, antibodies and CAR-T cells that target these pathways are effective (**Figure 2**) (Liu et al., 2019).

Some of the STAT3-associated signaling pathways have been reported in some hematological malignancies and solid tumors (Butturini et al., 2020) and in facilitation of cancer proliferation via regulation of CSC activities. Activation of STAT3 is predictive of poor prognostic outcomes in many cancers. Even though STAT3 is rarely altered by changes in gene expressions, its mRNA levels usually present a similar trend with its activation (Heichler et al., 2020; Ma et al., 2020). STAT3 is involved in maintenance of expressions of genes that encode the stem cell phenotype and is, therefore, used as a CSC maker. Hence, the STAT3 pathway is active in cells that are rich in other CSC markers, and its inhibition—proteins that facilitate cell growth and proliferation reduced activities on the STAT3 pathway—potentially downregulates cell viability and tumorsphere formation (Gatti et al., 2019; Siersbæk et al., 2020).

The relationship between epithelial-mesenchymal transition (EMT) and tumor microenvironments is based on plasticity between CSCs and their more differentiated derivatives, in which differentiation occurs from CSCs to non-CSCs and vice versa with the reverse requiring EMT. STAT3 activation is involved in induction of EMT and such activation is via either IL-6 dependent or independent procedures, for instance, non-canonical Frizzled 2 pathway/TGF-β/LIF pathway (Jin, 2020). EMT induction and proliferation of CSCs, post STAT3 activation, were associated with resistance to trastuzumab or cisplatin (Chung et al., 2014). An IL-6 loop, in which IL-6 activates Akt, STAT3, and NF- κB pathways, and inhibits PTEN expressions has been documented (Galoczova et al., 2018a; Ma et al., 2020; Wei, 2020).

In the tumor microenvironment, STAT3 regulates NF-κB signaling in tumor and non-transformed stromal cells. Physically, STAT3 interacts with NF in tumor and tumor-associated immune cells (Sun et al., 2021), wherein lactoglobule-EGF Factor 8 (MGF-E8)/STAT3, Sonic Hedgehog/EGFR/STAT3/Sox2 pathways, and CSC-like phenotypes were promoted by tumor-associated macrophages (Wei, 2020; Gatti et al., 2022).

In conclusion, STAT3 has a complex role in maintaining and promoting CSC characteristics. Directly, STAT3 interacts with transmembrane glycoproteins expressed by normal stem cells and is a biomarker for CSC identification and isolation. EMT, as a mechanism by which CSCs are engendered, is correlated with STATs-associated pathways. STAT3 is critical in angiogenesis and regulation of the tumor microenvironment, which provides signals for differentiation or proliferation via inflammation, for instance, in the NF-κB pathway. Moreover, feedback activation of STAT3 is involved in anticancer drug resistance (Johnson et al., 2018; Zou et al., 2020).

4 STRUCTURE AND REGULATION OF P63

P63, also known as tumor protein 63 (TP63) or transformation-related protein 63 (Trp63) or amplified in squamous cell carcinoma (AIS), is a member of the p53 transcription factor family and the corresponding p53/p63/p73 gene family that encodes p53, p63 and p73 proteins (Soares and Zhou, 2018; Fisher et al., 2020). These family members contribute to tumorigenesis as well as morphogenesis and have a similar domain organization—DNA binding domain (DBD), C-terminal oligomeric domain (OD), and N-terminal transcriptional activation domain (TAD). Structurally, they are tetramers and may form heterotetramers, because of their partial homology in oligomeric domains. P63 has numerous isoforms that are linked to stem cell development and differentiation, aging, proliferation, stem cell maintenance, senescence, and apoptosis (Galoczova et al., 2018b).In addition, by directly regulating chromatin-modulating factors, engaging and opening chromatin regions, p63 is essential for adjusting the chromatin landscape in epidermal keratinocytes (Galoczova et al., 2018b). P63 can bind target genes of p53 and p73 since all three have highly homologous DNA binding domains (Peng et al., 2021).

The TP63 gene encodes two types of isoforms and consists of 15 exons distributed across 270 kb of chromosome 3q27. Exon 1, which is found upstream of p63, is a transcription promoter of TAp63. TAp63 transcripts contain three TA-specific exons (Exons 1, 2, and 3) that encode N-terminal transactivation domains, homologous to those of p53 (Murray-Zmijewski et al., 2006; Tošić and Frank, 2021). β and δ isoforms lack exon 13 while γ lacks exons 11-14 but has a γ-specific exon 10′. Premature stop codon on exon 10 generates an ε isoform. The above isoforms have DNA-binding and oligomerization domains. TAp63 and ΔNp63 isoforms differ in organ sites of transcription, such that TAp63 transcripts are prevalent in the heart, kidney, brain, thymus, testis, and cerebellum, whereasΔNp63 transcripts are highly detected in the epithelia, spleen, kidney, and thymus (Mangiulli et al., 2009; Jin, 2020).

P63 regulates DNA damage responses, and this is both isoform and cell type specific. For instance, in response to DNA damage, TAp63 is expressed in epithelial tissues, neurons, and germlines. Expressions of ΔNp63 are induced by tyrosine kinase receptor epidermal growth factor receptors (EGFR). Unlike EGFR activation of phosphatidylinositol 3-kinase (PI3K) signaling in keratinocytes, EGFR activation of ΔNp63α is mediated by STAT3 in cancer, and these two pathways are potentially linked via the mammalian target of rapamycin (mTOR) as PI3K activation of mTOR results in mTOR-dependent activation of the STAT3/p63/Jagged pathway. Apart from EGFR-mediated activation of ΔNp63α, ΔNp63α expressions are induced by interactions of α6β4 integrin and specific proteins, such as transglutaminase 2 (TG2) (Fisher et al., 2016; Wei, 2020; Sun et al., 2021).

Wnt/β-catenin signaling is a highly conserved pathway that is involved in regulation of cellular proliferation, differentiation, migration, apoptosis, and stem cell renewal. Through the Wnt/β-catenin pathway, binding lymphoid enhancer-binding factor 1 (Lef1) to β-catenin between TAp63 and ΔNp63 promoters directly regulates p63 (Ferretti et al., 2011). As a downstream target of p63, Hedgehog signals are involved in embryonic development, organismal polarity formatting, wound healing, maintenance of somatic stems, and pluripotent cells. Reduced

expressions of p63 are due to overexpression of the p65 subunit of NF-κB, possibly via NF-κB-mediated enhancement of expressions of the zinc finger E-box binding homeobox (ZEB1) (Wu et al., 2009).

5 ASSOCIATIONS BETWEEN STAT3 AND P63

The *TP63* gene is localized on chromosome 3 and gives rise to multiple isoforms due to differential promoter selection (full-length TA and N-terminal truncated ΔNp63) and alternative splicing of the 3′end of the mRNA (α, β, γ, δ, ε) (Yang et al., 1998). ΔNp63 isoforms lack the N-terminal transactivation domain, hence they are able to antagonize full-length isoforms of p63 as well as other p53 family members and act like dominant negative transcription inhibitors. However, they also have transactivation activities that are attributed to the presence of an alternative TAD (Yang et al., 1998; Chu et al., 2008). Among the C-terminal isoforms, p63α isoforms have a sterile alpha motif (SAM) that is involved in protein-protein interactions and have a transcription inhibitory domain (TID), which inhibits its transcriptional activities (Sayan et al., 2007; Rufini et al., 2011).

P63 is involved in epidermal development because it is highly expressed in basal cells of various epithelial tissues, thereby conferring them with stem-cell-like properties. Δ p63 mice mutants either lack stratified squamous epithelia and their derivatives or have stratified but disrupted epidermis. Truncated or lost limbs and craniofacial abnormalities also characterize Δ p63 mice mutants (Yang et al., 1998). Thus, basal cells are multipotent tissue-specific epithelial progenitors that express p63, cytokeratin 5, and cytokeratin 14. Like stem cells, these basal cells quickly proliferate in response to epithelial damage and contribute to the repair of damaged epithelium in both mouse and human trachea. P63 is a critical mediator of normal epithelial development, maintenance and homeostasis.ΔNp63α is a predominant p63 isoform in epithelial tissues and is highly expressed in basal cells of stratified and glandular epithelia, including epidermis. Its levels decrease with cellular differentiation. Conversely, TAp63 positive cells are lowly expressed in the stratified epithelia, indicating a switch between isoforms during differentiation (Galoczova et al., 2018a; Liu et al., 2019; Wei, 2020). As expressions of ΔNp63 proteins are only observed in other basal cells, such as breasts, prostate, bladder, and colorectum, they have been used as basal cell markers (Galoczova et al., 2018a; Wei, 2020). A possible association between p63 and activated STAT3 is the function of the latter in promoting airway ciliated cell regeneration from basal stem cells and malignant transformation of foregut basal progenitor cells. In addition, p63 works in tandem with STAT3 in human keratinocytes, as revealed via ChIP-Seq analysis (Sethi et al., 2014; Zou et al., 2020).

Since TAp63 and Δ Np63 oppose each other's regulatory roles, P63 exhibits bidirectional regulation of tumorigenesis. Like STAT3, p63 is a highly conserved protein and its mutations, such as gene amplification, result in cancerous cells, in which p63 activities are increased (Koster et al., 2006). ΔNp63 is a putative oncoprotein whose expression is upregulated in squamous cell carcinomas, triple-negative basal-like breast tumors, and other tumor types. It also affects the various signaling pathways that contribute to development of CSC phenotypes (Gatti et al., 2022; Butturini et al., 2020). Moreover, apart from p53, TAp63 induces cell cycle arrest and apoptosis (**Figure 3**). ΔNp63 enhances the expressions of Wnt receptor Frizzled 7, thereby promoting Wnt signaling, leading to promotion of normal mammary stem cell activities and tumor-initiating activities in the basal-like subtype of breast cancer (Chakrabarti et al., 2014). Memmi et al. (Jin, 2020) revealed positive modulation of the Hedgehog signaling pathway by ΔNp63 that maintains the self-renewal potential of mammary CSCs. Autoregulation of ΔNp63 gene transcription is mediated by activation of STAT3 and its subsequent binding to STAT3RE. Since STAT3 activation by interleukin-6 also leads to Δ Np63 upregulation and blockade of either DeltaNp63 or STAT3 expressions by siRNA, it results in cell growth suppression. Thus, the identified regulatory pathway is of probable cell physiological significance.

There are various associations between STAT3 andΔNp63. First, both are CSC markers that are associated with triple-negative breast tumors, which, when compared to non-triple negative types, had more CSC markers (Wei, 2020; Galoczova et al., 2018a; Gatti et al., 2022). Second, STAT3 is constitutively activated in squamous cell carcinomas, in which ΔNp63 is overexpressed. Third, both are the main regulators of CSC maintenance (Gerbe et al., 2019). Fourth, dual-regulatory effects of ΔNp63 on its own promoter are dependent on STAT3 activation, which binds the ΔNp63 promoter. Fifth, since the expressions of ΔNp63 are regulated by the EGFR/STAT3 axis that is vital for the proliferation of CSCs, activations, and expressions of both STAT3 andΔNp63 are possibly regulated in part by the EGFR signaling pathway (Ma et al., 2010). Sixth, we previously determined that peroxisome proliferator-activated receptor-γ(PPAR-γ) agonists inhibit stemness of lung adenocarcinoma cancer stem cells (CSCs) by downregulating the expressions of Janus tyrosine kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway and elucidated p63-STATS connections are illustrated (**Figure 4**) (Wei, 2020).

Seventh, mTOR activates STAT3 to induce p63 expressions, which then activates Notch signaling by stimulating Jag1 gene expressions that inhibit murine and human cell differentiation (Lin et al., 2021). Peng et al. (2021) found that a sharp rise of IL-6 boosted STAT3 phosphorylation and then restrained p63 expression in bleomycin-induced acute lung injury. Further results suggested that mesenchymal stem cells' supernatant lyophilized powder activated p63 by inhibiting the IL-6-p-STAT3 pathway. Then, to clarify the role of IL-6, the researchers instilled rh IL-6 into the airways of mice. ELISA demonstrated that rh IL-6 was enriched in the lung tissues on day 1, after which it was absorbed and removed on day 2. Consistently, rh IL-6 administration promoted STAT3 phosphorylation and decreased p63 expression. Despite these close associations between STAT3 and ΔNp63, only ΔNp63 is involved in the inflammatory NF-κB pathway, angiogenesis

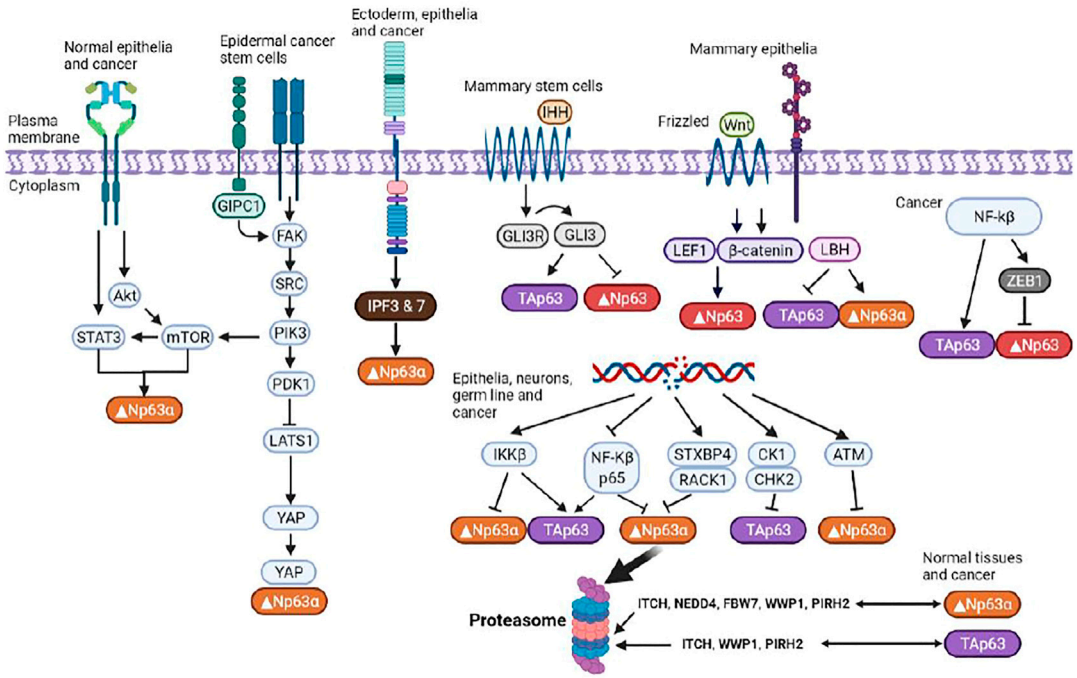

FIGURE 3 | Regulation of p63 isoforms. P63 is involved in modulation of the chromatin landscape in epidermal keratinocytes by directly regulating chromatin-modulating factors and engaging and opening chromatin regions. Multiple isoforms with distinct, often opposing, functions enable p63 to exert an array of effects on essential cellular functions.

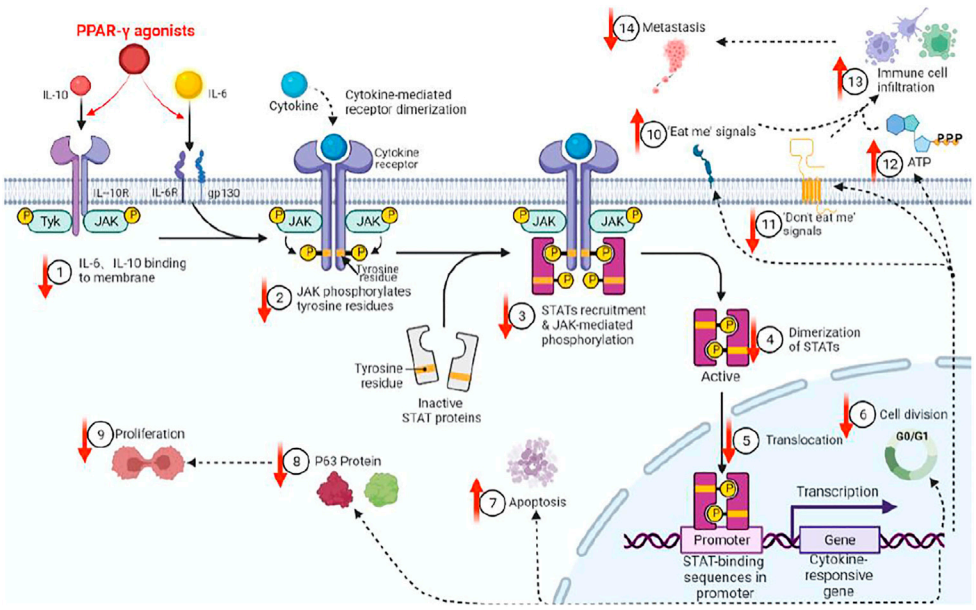

FIGURE 4 | PPAR-γ agonist inhibits JAK-STAT signaling pathway activations through molecules such as IL6/IL10, thereby inhibiting p63 protein expressions in CSCs. We previously showed that PPAR-γ agonists target and inhibit CSC proliferation by downregulating the phosphorylation of the JAK-STATs pathway and p63 protein expressions, etc. (↓=downregulated, ↑=upregulated).

through VEGF, and EMT—Δ Np63 increased EMT and reduced the opposing process of mesenchymal-epithelial transformation (MET). Hypothetically, p63 activates the Notch signaling pathway in adjacent cells, which increases EMT. Relatedly, TAp63 possibly contributes to cancer cell transitions to tumor-initiating cells.

Due to the abundance of possible interactions among p53, p63, and p73, studies on relationships between p63 and STAT3 and the role of p63 in cancer should also consider the expressions of the p63 isoforms and the relationship of these isoforms to other p53 family members.

6 CONCLUSION

STAT3 signaling is the main regulatory pathway for the fate of embryonic stem cells and the limiting factor of human somatic cell reprogramming. During carcinogenesis, STAT3 signaling activation is involved in many aspects, including regulating the properties of CSCs. STAT3 and p63 are the main regulators of CSC maintenance. Apart from being biomarkers of CSC, STAT3, and ΔNp63 interact directly and, through numerous ways, regulate CSC properties. More studies should be performed to elucidate on the associations between the two.

AUTHOR CONTRIBUTIONS

SW and JL are responsible for writing the article. KZ and XG are responsible for literature search. LF, MT, and WL are responsible for revising the article.

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REFERENCES

Butturini, E., Carcereri de Prati, A., and Mariotto, S. (2020). Redox Regulation of STAT1 and STAT3 Signaling. *Int. J. Mol. Sci.* 21 (19), 7034. doi:10.3390/ijms21197034

Chakrabarti, R., Wei, Y., Hwang, J., Hang, X., Andres Blanco, M., Choudhury, A., et al. (2014). ΔNp63 Promotes Stem Cell Activity in Mammary Gland Development and Basal-like Breast Cancer by Enhancing Fzd7 Expression and Wnt Signalling. *Nat. Cell. Biol.* 16 (10), 10041–101513. doi:10.1038/ncb3040

Chu, W.-K., Dai, P.-M., Li, H.-L., and Chen, J.-K. (2008). Transcriptional Activity of the ΔNp63 Promoter Is Regulated by STAT3. *J. Biol. Chem.* 283 (12), 7328–7337. doi:10.1074/jbc.M800183200

Chung, S. S., Giehl, N., Wu, Y., and Vadgama, J. V. (2014). STAT3 Activation in HER2-Overexpressing Breast Cancer Promotes Epithelial-Mesenchymal Transition and Cancer Stem Cell Traits. *Int. J. Oncol.* 44 (2), 403–411. doi:10.3892/ijo.2013.2195

Ferretti, E., Li, B., Zewdu, R., Wells, V., Hebert, J. M., Karner, C., et al. (2011). A Conserved Pbx-Wnt-P63-Irf6 Regulatory Module Controls Face Morphogenesis by Promoting Epithelial Apoptosis. *Dev. Cell.* 21 (4), 627–641. doi:10.1016/j.devcel.2011.08.005

Fisher, M. L., Balinth, S., and Mills, A. A. (2020). p63-related Signaling at a Glance. *J. Cell. Sci.* 133 (17), jcs228015. doi:10.1242/jcs.228015

Fisher, M. L., Kerr, C., Adhikary, G., Grun, D., Xu, W., Keillor, J. W., et al. (2016). Transglutaminase Interaction with α6/β4-Integrin Stimulates YAP1-dependent ΔNp63α Stabilization and Leads to Enhanced Cancer Stem Cell Survival and Tumor Formation. *Cancer Res.* 76 (24), 7265–7276. doi:10.1158/0008-5472.CAN-16-2032

Galoczova, M., Coates, P., and Vojtesek, B. (2018). STAT3, Stem Cells, Cancer Stem Cells and P63. *Cell. Mol. Biol. Lett.* 23, 12. doi:10.1186/s11658-018-0078-0

Galoczova, M., Coates, P., and Vojtesek, B. (2018). STAT3, Stem Cells, Cancer Stem Cells and P63. *Cell. Mol. Biol. Lett.* 23, 12. doi:10.1186/s11658-018-0078-0

Gatti, V., Bongiorno-Borbone, L., Fierro, C., Annicchiarico-Petruzzelli, M., Melino, G., and Peschiaroli, A. (2019). p63 at the Crossroads between Stemness and Metastasis in Breast Cancer. *Ijms* 20 (11), 2683. doi:10.3390/ijms20112683

Gatti, V., Fierro, C., Compagnone, M., La Banca, V., Mauriello, A., Montanaro, M., et al. (2022). ΔNp63-Senataxin Circuit Controls Keratinocyte Differentiation by Promoting the Transcriptional Termination of Epidermal Genes. *Proc. Natl. Acad. Sci. U.S.A.* 119 (10), e2104718119. doi:10.1073/pnas.2104718119

Gerbe, A., Alame, M., Dereure, O., Gonzalez, S., Durand, L., Tempier, A., et al. (2019). Systemic, Primary Cutaneous, and Breast Implant-Associated ALK-Negative Anaplastic Large-Cell Lymphomas Present Similar Biologic Features Despite Distinct Clinical Behavior. *Virchows Arch.* 475 (2), 163–174. doi:10.1007/s00428-019-02570-4

Heichler, C., Scheibe, K., Schmied, A., Geppert, C. I., Schmid, B., Wirtz, S., et al. (2020). STAT3 Activation through IL-6/IL-11 in Cancer-Associated Fibroblasts Promotes Colorectal Tumour Development and Correlates with Poor Prognosis. *Gut* 69 (7), 1269–1282. doi:10.1136/gutjnl-2019-319200

Jin, W. (2020). Role of JAK/STAT3 Signaling in the Regulation of Metastasis, the Transition of Cancer Stem Cells, and Chemoresistance of Cancer by Epithelial-Mesenchymal Transition. *Cells* 9 (1), 217. doi:10.3390/cells9010217

Johnson, D. E., O'Keefe, R. A., and Grandis, J. R. (2018). Targeting the IL-6/JAK/STAT3 Signalling axis in Cancer. *Nat. Rev. Clin. Oncol.* 15 (4), 234–248. doi:10.1038/nrclinonc.2018.8

Koster, M. I., Lu, S.-L., White, L. D., Wang, X.-J., and Roop, D. R. (2006). Reactivation of Developmentally Expressed P63 Isoforms Predisposes to Tumor Development and Progression. *Cancer Res.* 66 (8), 3981–3986. doi:10.1158/0008-5472.CAN-06-0027

Lin, W., Wan, X., Sun, A., Zhou, M., Chen, X., Li, Y., et al. (2021). RUNX1/EGFR Pathway Contributes to STAT3 Activation and Tumor Growth Caused by Hyperactivated mTORC1. *Mol. Ther. - Oncolytics* 23, 387–401. doi:10.1016/j.omto.2021.10.009

Liu, B., Yan, L., and Zhou, M. (2019). Target Selection of CAR T Cell Therapy in Accordance with the TME for Solid Tumors. *Am. J. Cancer Res.* 9 (2), 228–241.

Ma, J.-h., Qin, L., and Li, X. (2020). Role of STAT3 Signaling Pathway in Breast Cancer. *Cell. Commun. Signal* 18 (1), 33. doi:10.1186/s12964-020-0527-z

Ma, J., Meng, Y., Kwiatkowski, D. J., Chen, X., Peng, H., Sun, Q., et al. (2010). Mammalian Target of Rapamycin Regulates Murine and Human Cell Differentiation through STAT3/p63/Jagged/Notch Cascade. *J. Clin. Invest.*. 120 (1), 103–114. doi:10.1172/JCI37964

Mangiulli, M., Valletti, A., Caratozzolo, M. F., Tullo, A., Sbisà, E., Pesole, G., et al. (2009). Identification and Functional Characterization of Two New Transcriptional Variants of the Human P63 Gene. *Nucleic Acids Res.* 37 (18), 6092–6104. doi:10.1093/nar/gkp674

Murray-Zmijewski, F., Lane, D. P., and Bourdon, J.-C. (2006). p53/p63/p73 Isoforms: an Orchestra of Isoforms to Harmonise Cell Differentiation and Response to Stress. *Cell. Death Differ.* 13 (6), 962–972. doi:10.1038/sj.cdd.4401914

Peng, W., Chang, M., Wu, Y., Zhu, W., Tong, L., Zhang, G., et al. (2021). Lyophilized Powder of Mesenchymal Stem Cell Supernatant Attenuates Acute Lung Injury through the IL-6-p-STAT3-p63-JAG2 Pathway. *Stem Cell. Res. Ther.* 12 (1), 216. doi:10.1186/s13287-021-02276-y

Rufini, S., Lena, A. M., Cadot, B., Mele, S., Amelio, I., Terrinoni, A., et al. (2011). The Sterile Alpha-Motif (SAM) Domain of P63 Binds *In Vitro*

Monoasialoganglioside (GM1) Micelles. *Biochem. Pharmacol.* 82 (10), 1262–1268. doi:10.1016/j.bcp.2011.07.087

Sayan, B. S., Sayan, A. E., Yang, A. L., Aqeilan, R. I., Candi, E., Cohen, G. M., et al. (2007). Cleavage of the Transactivation-Inhibitory Domain of P63 by Caspases Enhances Apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* 104 (26), 10871–10876. doi:10.1073/pnas.0700761104

Sethi, I., Sinha, S., and Buck, M. J. (2014). Role of Chromatin and Transcriptional Co-regulators in Mediating P63-Genome Interactions in Keratinocytes. *BMC Genomics* 15 (1), 1042. doi:10.1186/1471-2164-15-1042

Siersbæk, R., Scabia, V., Nagarajan, S., Chernukhin, I., Papachristou, E. K., Broome, R., et al. (2020). IL6/STAT3 Signaling Hijacks Estrogen Receptor α Enhancers to Drive Breast Cancer Metastasis. *Cancer Cell.* 38 (3), 412–423. doi:10.1016/j.ccell.2020.06.007

Soares, E., and Zhou, H. (2018). Master Regulatory Role of P63 in Epidermal Development and Disease. *Cell.. Mol. Life Sci.* 75 (7), 1179–1190. doi:10.1007/s00018-017-2701-z

Sun, H.-J., Xiong, S.-P., Cao, X., Cao, L., Zhu, M.-Y., Wu, Z.-Y., et al. (2021). Polysulfide-mediated Sulfhydration of SIRT1 Prevents Diabetic Nephropathy by Suppressing Phosphorylation and Acetylation of P65 NF-Kb and STAT3. *Redox Biol.* 38, 101813. doi:10.1016/j.redox.2020.101813

Tošić, I., and Frank, D. A. (2021). STAT3 as a Mediator of Oncogenic Cellular Metabolism: Pathogenic and Therapeutic Implications. *Neoplasia* 23 (12), 1167–1178. doi:10.1016/j.neo.2021.10.003

Wei, S. X. (2020). Research Progress on Mechanism and Clinical Evidence of Peroxisome Proliferator Activated Receptor Gamma Agonist in the Treatment of Lung Cancer[J]. *Pract. J. Cardiac Cereb. Pneumal Vasc. Dis.* 28(8):136–140. doi:10.3969/j.issn.1008-5971.2020.08.027

Wu, J., Bergholz, J., Lu, J., Sonenshein, G. E., and Xiao, Z.-X. J. (2009). TAp63 Is a Transcriptional Target of NF-Îob. *J. Cell.. Biochem.* 109 (4), a–n. doi:10.1002/jcb.22449

Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M. D., Dötsch, V., et al. (1998). p63, a P53 Homolog at 3q27-29, Encodes Multiple Products with Transactivating, Death-Inducing, and Dominant-Negative Activities. *Mol. Cell.* 2 (3), 305–316. doi:10.1016/s1097-2765(00)80275-0

Yang, L., Shi, P., Zhao, G., Xu, J., Peng, W., Zhang, J., et al. (2020). Targeting Cancer Stem Cell Pathways for Cancer Therapy. *Sig Transduct. Target Ther.* 5 (1), 8. doi:10.1038/s41392-020-0110-5

Zou, S., Tong, Q., Liu, B., Huang, W., Tian, Y., and Fu, X. (2020). Targeting STAT3 in Cancer Immunotherapy. *Mol. Cancer* 19 (1), 145. doi:10.1186/s12943-020-01258-7

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Molecular mechanism of ferroptosis and its role in the occurrence and treatment of diabetes

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Ferroptosis is an iron-dependent programmed cell death, which is different from apoptosis, necrosis, and autophagy. Specifically, under the action of divalent iron or ester oxygenase, unsaturated fatty acids that are highly expressed on the cell membrane are catalyzed to produce lipid peroxidation, which induces cell death. In addition, the expression of the antioxidant system [glutathione (GSH) and glutathione peroxidase 4 (GPX4)] is decreased. Ferroptosis plays an important role in the development of diabetes mellitus and its complications. In this article, we review the molecular mechanism of ferroptosis in the development of diabetes mellitus and its complications. We also summarize the emerging questions in this particular area of research, some of which remain unanswered. Overall, this is a comprehensive review focusing on ferroptosis-mediated diabetes and providing novel insights in the treatment of diabetes from the perspective of ferroptosis.

KEYWORDS

ferroptosis, diabetes, ROS, GPX4, lipid peroxide, ferroptosis inhibitor

Introduction

In 2012, Brent R. Stockwell and his team found that erastin, a selectively lethal small molecule of oncogenic RAS, triggered a unique form of iron-dependent nonapoptotic cell death—a new form of cell death, called ferroptosis (Dixon et al., 2012). Ferroptosis is a kind of regulated cell death that primarily relies on iron-mediated oxidative damage and follows cell membrane damage (Chen et al., 2021a). Unlike previous forms of cell death, cells that experience ferroptosis exhibit atypical features of apoptosis. Morphologically, in erastin-induced cell death, mitochondria become smaller with increasing membrane density and the number of mitochondrial cristae decreases, without swelling of the cytoplasm and organelles (Lei et al., 2022). Increased iron accumulation, free-radical production, fatty-acid supply, and lipid peroxidation are crucial for the development of ferroptosis (Chen et al., 2021a). Key

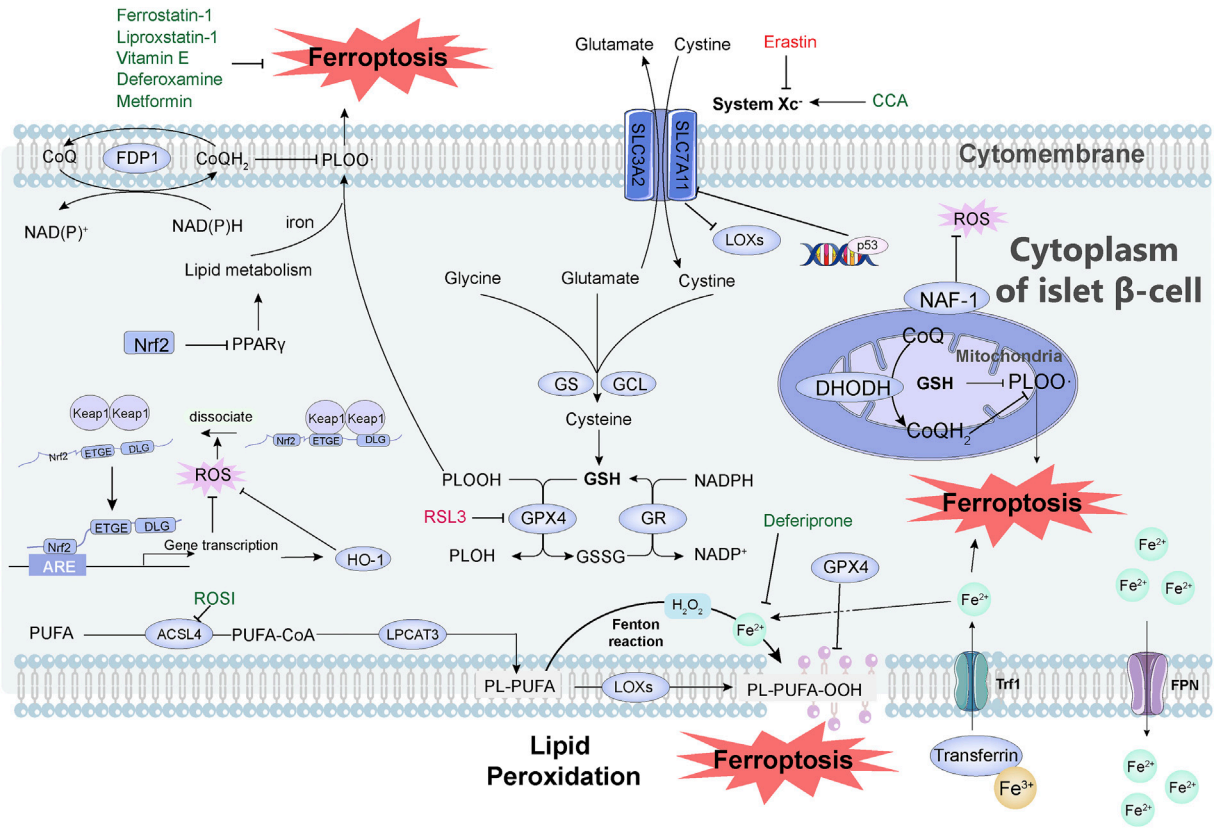

FIGURE 1
Mechanisms of how ferroptosis occurs and how it is inhibited in pancreatic islet cells. System Xc^- is a cystine/glutamate antiporter system consisting of SLC7A11 and SLC3A2. After cystine enters the cell, it is reduced to cysteine, which becomes the raw material for the synthesis of GSH, and GSH becomes the reducing cofactor of GPX4. Glutamate cysteine ligase (GCL) and GSH synthase (GS) are the rate-limiting enzymes in the GSH biosynthesis pathway. DHODH is involved in the regulation of ferroptosis independent of GSH pathway. FSP1 reduces CoQ through NAD(P)H, thereby reducing lipid free radicals and thereby inhibiting ferroptosis. Iron ions entering the cells undergo the Fenton reaction, which causes the peroxidation of PUFAs to generate lipid peroxides, resulting in the destruction of the cell membrane structure and the occurrence of ferroptosis. This peroxidation is regulated by three synthetases: ACSL4, LPCAT3, and LOX. Transferrin imports iron from the extracellular environment to the intracellular state through TfR1 recognition, and excess iron is stored in the form of bound ferritin and transported to cells under the action of FPN1. Transferrin imports Fe^{3+} (yellow ball) from the extracellular environment to the cytoplasm of islet β-cell through TfR1 recognition, and then Fe^{3+} is converted to Fe^{2+} (light green ball). The excess iron is stored in the form of bound ferritin and finally effluxed by FPN. Activation of p53 severely reduced the protein level of SLC7A11 and thus mediated ferroptosis. When the organism is under oxidative stress, Nrf2 dissociates from Keap1 and interacts with the ARE of target genes to maintain cellular redox homeostasis. The Nrf2-HO-1 pathway can modulate intracellular iron concentration to suppress ferroptosis. Nrf2 inhibits ferroptosis directly through the PPARγ pathway. Small molecules in green in the figure can inhibit ferroptosis, including: Vitamin E, Metformin, Deferoxamine, Liproxstatin-1, Ferrostatin-1, Deferiprone, CCA, ROSI. Small molecules in red can induce ferroptosis, including Erastin and RSL3. Abbreviation, System Xc^-, a cystine/glutamate antiporter system; GSH, glutathione; GPX4, glutathione peroxidase; GCL, glutamate cysteine ligase; GS, GSH synthase; RSL3, RAS-selective lethal 3; DHODH, dihydroorotate dehydrogenase; FSP1, ferroptosis suppressor protein 1; CoQ, coenzyme Q; PUFAs, polyunsaturated fatty acids; ACSL4, acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; LOX, lipoxygenase; TfR1, transferrin receptor 1; FPN1, ferroportin; ARE, antioxidant response element; CCA, cryptochlorhydric acid; ROSI, rosiglitazone.

proteins in ferroptosis-related signaling pathways can be targeted by drugs or some small-molecule inhibitors.

Since the discovery of ferroptosis, many studies have confirmed that ferroptosis is related to the occurrence and development of many diseases, such as cancer, diabetes, and ischemia–reperfusion injury (Stockwell et al., 2017; Wei et al., 2020). According to the 10th edition of the International Diabetes Federation (IDF) diabetes atlas, diabetes is one of the fastest growing global health emergencies in the 21st century, and the number of people with diabetes worldwide is expected to reach 643 million by 2030 (Oka et al., 2021). Absolute or relative insufficiency of insulin secretion from pancreatic β-cells is the culprit of diabetes. Pancreatic islet cells are more sensitive to oxygen than other cells and have relatively weak antioxidant capacity. Pancreatic islets are prone to death when attacked by free radicals or when oxidative stress occurs in the islet microenvironment (Bottino et al., 2004; Wang and Wang, 2017). A number of

small molecules have been found to improve β-cell function by inhibiting certain pathways of ferroptosis (Bannai et al., 1977; Zhou, 2020), which provides more possibilities for clinical treatment of diabetes. This article reviews the general mechanism of ferroptosis and the role of ferroptosis in the development of diabetes, thereby providing a comprehensive understanding for early diagnosis, treatment, and prognosis of diabetes.

Molecular mechanisms of ferroptosis

As a new form of cell death, ferroptosis was originally discovered by the selective inhibition of erastin and RAS-selective lethal 3 (RSL3) (Yang and Stockwell, 2008). These two compounds are known to target RAS-mutant tumors (Dolma et al., 2003). Thereafter, detailed research has been performed on ferroptosis, demonstrating that compared with other forms of cell death, ferroptosis is a new, nonapoptotic programmed cell death (PCD).

Inhibition of system Xc^-–GSH–GPX4 activity promotes ferroptosis

System Xc^-, a cystine/glutamate antiporter system, is an antiporter made up of two subunits, SLC7A11 (solute carrier family 7 member 11), which is the light chain of the subunit, and SLC3A2 (solute carrier family 3 member 2), which is the heavy chain of the subunit (Koppula et al., 2018). SLC7A11 is responsible for the main transport activity and is profoundly specific for cystine and glutamate, while SLC3A2 acts as a chaperonin. System Xc^- transports a molecule of glutamate out of the cell for every molecule of cystine delivered into the cell (Liu et al., 2021). Cystine is reduced to cysteine, which becomes the raw material for the synthesis of glutathione (GSH). Subsequently, GSH becomes a reducing cofactor for glutathione peroxidase, including glutathione peroxidase 4 (GPX4) and phospholipid hydroperoxide glutathione peroxidase (PHGPx).

GSH, which is mainly distributed in the cytoplasm, can scavenge reactive oxygen species (ROS) and play an important role in inhibiting ferroptosis (Wu et al., 2004). Inhibition of GSH synthesis leading to its depletion can lead to ferroptosis. L-glutamic acid, cysteine, and glycine are needed to synthesize GSH with energy provided by adenosine triphosphate (ATP). When erastin inhibits system Xc^-, the source of cysteine is reduced and the amount of GSH synthesis decreases, which in turn leads to the accumulation of lipid peroxides, triggers protein and membrane damage, and initiates ferroptosis. Glutamate cysteine ligase (GCL) and GSH synthase (GS) are the rate-limiting enzymes in the GSH biosynthesis pathway. Inhibition of GCL and GS can lead to depletion of GSH and result in ferroptosis (Lu, 2009).

GPX inactivation or depletion induces ferroptosis

GPX4 is the fourth member of the selenium-containing GPX family. In mammals, GPX4 is the only GPX family member with the ability to resist peroxide damage (Brigelius-Flohé and Maiorino, 2013). GPX4 reduces oxidative stress damage by converting the peroxy bonds of lipid peroxidation into hydroxyl groups so as to reduce lipid peroxides to lipid alcohols, despite the fact that these lipids have become a part of cell membranes or lipoproteins (Brigelius-Flohé and Maiorino, 2013; Seibt et al., 2019). As one of the mediators of oxidative stress, GPX4 is a core regulator of ferroptosis (Bersuker et al., 2019). PL-peroxidase activity of GPX4 is inhibited by RSL3 ((1S,3R)-RSL3), a ferroptosis inducer, by its binding to GPX4 selenocysteine (Sec) site (Yang et al., 2016). Boyi Gan's team from the University of Texas MD Anderson Cancer Center found that uridine 5′-monophosphate (UMP) synthesis was significantly increased after treating cells with GPX4 inhibitors such as RSL3. This suggests that there may be a relationship between ferroptosis and pyrimidine nucleotide synthesis. It was next found that dihydroorotate dehydrogenase (DHODH) may be involved in the regulation of ferroptosis. Namely, the ferroptosis defense system located in mitochondria can regulate ferroptosis independently of the GSH pathway (Mao et al., 2021), and DHODH may serve as a novel target to restore cell death induced by ferroptosis.

Depletion of reduced coenzyme Q10 (CoQ10) increases cellular susceptibility to ferroptosis

According to previous studies, ferroptosis suppressor protein 1 (FSP1) has a proapoptotic effect under certain conditions. FSP1 could translocate into the nucleus and trigger DNA degradation. However, recent studies have shown that FSP1 has an anti-ferroptosis mechanism parallel to the Cyst(e)ine-GSH-GPX4 axis. FSP1 reduces CoQ10 through NAD(P)H, which reduces lipid free radicals, thereby inhibiting lipid peroxidation and consequently inhibiting ferroptosis (Bersuker et al., 2019).

Unlike the Cyst(e)ine/GSH/GPX4 axis, which is dependent on the cysteine anti-transport system Xc^- and the cysteine-providing supersulfur pathway, the FSP1/CoQ10 axis is dependent on the mevalonate pathway. The mevalonate pathway involves the production of isopentenyl

pyrophosphate (IPP), squalene (both IPP and squalene are precursors of CoQ10), CoQ10, and cholesterol (Moosmann and Behl, 2004). Inhibition of CoQ10 biosynthesis through the mevalonate pathway or inactivation of CoQ10 reductases such as FSP1 or DHODH can induce ferroptosis by increasing cellular susceptibility to ferroptosis in the absence of GPX4. Conversely, tetrahydrobiopterin (BH4) inhibits ferroptosis by converting phenylalanine to tyrosine to promote the synthesis of CoQ10 (Kraft et al., 2020).

Induction of lipid ROS generation by means of iron and/or polyunsaturated fatty acid overload

As an iron-dependent form of cell death, ferroptosis requires high levels of iron. In the 1870s, Henry Fenton discovered that iron salts can react with peroxides to generate hydroxyl radicals, and proposed the famous fenton reaction (Fe^{2+} + HOOH $\rightarrow Fe^{3+}$ + OH^- + OH·). This reaction is also the basic principle of the lipid peroxidation step in ferroptosis. Namely, iron ions entering the cell undergo a Fenton reaction and peroxidize polyunsaturated fatty acids (PUFAs) to generate lipid peroxides (Li and Li, 2020). This process, regulated by three synthases, including acyl CoA synthase long-chain family member 4 (ACSL4), lysophosphatidylcholine acyltransferase 3 (LPCAT3), and lipoxygenase (LOX) (Doll et al., 2017), results in damage to the cell membrane structure and eventually leads to cell death. Therefore, iron metabolism is closely related to the occurrence of ferroptosis in cells, and it incorporates at least the following four aspects: intake, storage, utilization, and efflux (Chen et al., 2021b). Transferrin imports iron from the extracellular environment to intracellular space through recognition by transferrin receptor 1 (TfR1), while excess iron is bound to ferritin and transported outside of the cell under the action of ferroportin (FPN1), where the whole process normally maintains a dynamic balance (He et al., 2022).

Ferroptosis in diabetes

On the basis of the abovementioned molecular mechanisms of ferroptosis, multiple pathways are involved in the regulation of ferroptosis. We summarize the relationship between some pathways and diabetes, and reveal their potential applications in the diagnosis, treatment, and prognosis of diabetes (Figure 1).

Crucial role of p53 in ferroptosis-mediated diabetes

p53 is a tumor-suppressor gene in humans that causes cell cycle arrest, apoptosis, and/or senescence (Liu et al., 2019). One of the methods to activate the p53 protein signaling pathway is to disrupt the integrity of the DNA template (Liu and Gu, 2021). As shown by western blot (WB) analysis, the activation of p53 severely reduces the protein level of SLC7A11, and the flanking region of the SLC7A11 gene has a site that exactly matches the p53-binding sequence, indicating that the SLC7A11 gene is one of the downstream targets of p53 (Jiang et al., 2015; Liu and Gu, 2022). Gu's team found that ALOX12 (arachidonate 12-lipoxygenase, 12S type) depletion had no significant effect on the expression of p53 and its downstream targets, but was able to rescue p53-mediated ferroptosis, indicating that ALOX12 is necessary for the p53-mediated ferroptosis pathway under ROS stress (Chu et al., 2019). They further constructed an ACSL4/GPX4 double-gene knockout ($ACSL4^{-/-}$/$GPX4^{-/-}$) human osteosarcoma cell line U2OS. The levels of p53 and its downstream targets were unaffected, but the cells underwent ferroptosis when exposed to TBH (tert-butyl hydroperoxide, mimicking ROS environment) and Nutlin (p53 activator), suggesting that p53 induces ferroptosis in a GPX4-independent manner (Chen et al., 2021c).

Minamino et al. (2009) demonstrated that upregulation of p53 in adipose tissue causes an inflammatory response that leads to insulin resistance. When β-cells were treated with free fatty acids (FFAs), the production of ROS increased and p53 was activated (Yuan et al., 2010). β-cell mitosis was reduced and their apoptosis was increased. Induction of the downstream microRNA miR34a sensitized β-cells to apoptosis and restrained insulin secretion. Akt plays an important role in promoting pancreatic β-cell survival by inhibiting the proapoptotic proteins such as glycogen synthase kinase 3α/β (GSK3α/β), Forkhead Box O1 (FoxO1), and p53 (Wrede et al., 2002; Lovis et al., 2008). Similar to Mdm2, ARF-BP1 acts as a ubiquitin ligase to control p53 stability and activity. The stability of p53 is tightly regulated by ARF-BP1 *in vivo*. Researchers constructed a mouse model with a specific deletion in pancreatic cells (*arf-bp1*$^{FL/Y}$/*RIP-cre*) and found that the mice died of severe diabetes as they matured; in contrast, when the p53 deletion was reversed (*p53*$^{LFL/FL}$/*arf-bp1*$^{FL/Y}$/*RIP-cre*), the mice lived longer (Kon et al., 2012). T-cell factor 7-like 2 (TCF7L2) is a key transcriptional effector of the Wnt/β-catenin signaling pathway, and leads to type 2 diabetes mainly by reducing β-cell survival and impairing insulin secretion. In glucose-stimulated INS-1 cells, TCF7L2 binds to gene promoters such as p53 and Fto. When TCF7L2 is silenced in INS-1 cells, p53INP1 protein expression is reduced, and apoptosis of INS-1 cells is decreased (Zhou et al., 2012). P53 is a key molecule for ferroptosis, which is linked to β-cell apoptosis and survival. Since p53 plays an important role in regulating physiological processes such as apoptosis, inflammation, and aging, targeting p53 in diabetes treatment may offer additional benefits.

Nrf2 in ferroptosis-mediated diabetes

Nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that plays an important role in cellular antioxidant activity, regulates transcription of components of the GSH antioxidant systems, and is involved in phase I and phase II detoxification of exogenous and endogenous products, NADPH regeneration, and heme metabolism enzymes (Tonelli et al., 2018). As many of its downstream genes are associated with ferroptosis, Nrf2 is considered an important regulator of ferroptosis. When the organism is in a state of oxidative stress, Nrf2 dissociates from Keap1 and is transferred to the nucleus, where it interacts with antioxidant response elements (ARE) of target genes, activates transcriptional pathways, and maintains cellular redox homeostasis (Zhang, 2006). Selvakumar et al. found that the Nrf2-HO-1 pathway can regulate ferritin and thus intracellular iron concentration (Selvakumar et al., 2019). A protein–protein interaction network analysis revealed that Nrf2 mainly regulates ferroptosis by directly affecting the synthesis and function of Gpx4 and the PPARγ pathway (Song and Long, 2020).

Patients with type 2 diabetes mellitus commonly experience hyperglycemia, insulin resistance, inflammation, and dyslipidemia, all of which cause intracellular oxidative stress and inflammation (Karam et al., 2017). Flavin adenine dinucleotide hydroquinone form (FADH2) and nicotinamide adenine dinucleotide (NADH) generated by glucose or fatty acid oxidation undergo oxidative phosphorylation in the mitochondrial electron transport chain. When a large amount of oxidative phosphorylation occurs, the electron transport chain becomes congested, and the electrons return to complex I to generate ROS with oxygen (Fisher-Wellman and Neufer, 2012). Redox stress is a producer of diabetes-related tissue injury and causes serious complications.

Antioxidative enzymes are generally expressed at low levels in pancreatic endocrine cells, and treatment of islet cells with antioxidants rescues oxidative damage to β-cells in diabetic mice (Kaneto et al., 1999). The antioxidant ability of the Nrf2 pathway can maintain the body's glucose level by saving the oxidative stress status of β-cells and maintaining insulin secretion. Cryptochlorhydric acid (CCA), a standout among the active components in mulberry leaf extract, has been shown to improve inflammation and insulin resistance (Tian et al., 2019). It inhibits ferroptosis by activating cystine/system x_c^-/Gpx4/Nrf2 and inhibiting NCOA4 in diabetes, thereby exerting a strong antidiabetic effect (Zhou, 2020).

ACSL4 mediated-ferroptosis in β-cells

Acyl-CoA synthetase long-chain family member 4 (ACSL4) is an important enzyme in lipid metabolism, which catalyzes the reaction between long-chain fatty acids and coenzyme A to generate acyl-CoA. ACSL4 activates arachidonic acid to arachidonyl-CoA, which is further esterified to phospholipid (Liao et al., 2022). The oxidation of the endoplasmic reticulum–associated compartment involved in ferroptosis has been found to occur only on one class of phospholipids [phosphatidylethanolamines (PEs)] and targets two fatty acids [arachidonoyl (AA) and adrenoyl (AdA)] (Kagan et al., 2017). Doll et al. found that exogenous arachidonic acid enhanced RSL3-induced ferroptosis, and ACSL4 was a key enzyme in arachidonic acid–induced ferroptosis in synergy with IFNγ (Doll et al., 2017; Liao et al., 2022). The expression of ACSL4 can reflect the sensitivity of cells to ferroptosis (Yuan et al., 2016).

The ACSL4 protein exists in the β-cells of human and rat islets, and its distribution site suggests that ACSL4 is involved in insulin secretion by modifying fatty acids in insulin secretion granules and mitochondria (Ansari et al., 2017). Upregulation of ACSL4 expression was observed in mice fed a high-fat diet, and when ACSL4 was specifically knocked out in adipocytes in mice fed a high-fat diet, the mice were protected from insulin resistance (Killion et al., 2018). Some studies have found that when the ACSL4 inhibitor rosiglitazone (ROSI) is used in diabetic nephropathy mice, it may inhibit the inflammatory response, thereby inhibiting ferroptosis, and finally improving the damage of renal tubular cells in a high-glucose environment (Wang et al., 2020), which also provides some ideas for ACSL4 as a potential therapeutic target for diabetes.

WFS-T2 in β-cells

WFS-T2 encodes the protein NAF-1. NAF-1 is a member of the NEET protein family, a highly conserved [2Fe-2S] protein. NAF-1 localizes to mitochondria, the endoplasmic reticulum (ER), and the mitochondria-associated membrane connecting these organelles, and its unique [2Fe-2S] cluster structure makes NAF-1 essential for autophagy, ferroptosis, redox, and oxidative stress. It plays a key regulatory role in the process of cell proliferation (Nechushtai et al., 2020). Researchers built an NAF-1 knockout INS-1 cell model, and the cells developed ferroptosis-like features such as enhanced lipid peroxidation, mitochondrial atrophy, and GPX4 expression. Insulin secretion was impaired in NFA-1 knockout INS-1 cells. When this cell model was treated with the iron chelator deferiprone, the GSH precursor N-acetyl cysteine, and the ferroptosis inhibitor ferrostatin-1, the levels of ROS generated by the cells were reduced, the mitochondrial and endoplasmic reticulum structures were improved, and cellular insulin secretion function was repaired (Agrawal et al., 2018; Ommati et al., 2021).

Conclusion and perspectives

This article reviews the mechanism of ferroptosis and the role of ferroptosis in diabetes. At present, diabetes is one of the most prevalent diseases in the world, and its complex pathogenesis and systemic complications make it a very difficult problem. As a new form of PCD, ferroptosis has different characteristics from other cell deaths, and has shown great potential in the diagnosis, treatment, and prognosis of diabetes. Four mechanisms for the induction of ferroptosis have been identified: (Dixon et al., 2012): inhibition of the system Xc^-, (Chen et al., 2021a) inhibition/degradation/inactivation of GPX4, (Lei et al., 2022) depletion of reduced CoQ10, and (Stockwell et al., 2017) lipid peroxidation *via* iron or polyunsaturated fatty acid overload.

Given the association between diabetes and ferroptosis, starting from the key targets of ferroptosis might improve diabetes. Improvements in insulin secretion and insulin sensitivity along with better control of blood glucose have been observed after reducing iron storage levels in the body (Houschyar et al., 2012). Although serum ferritin levels can be for early diagnosis of type 2 diabetes mellitus and gestational diabetes mellitus (Wang et al., 2018), the use of serum ferritin levels to calculate iron stores in the body appears unreliable because ferritin is also elevated in other diseases such as cancer and liver disease (Wang et al., 2010). In the treatment of diabetes, many specific ferroptosis inhibitors have been identified, such as ferrostatin-1, liproxstatin-1, vitamin E, and deferoxamine (Ju et al., 2021). Although many drugs are still in the preclinical stage, some drugs that have been marketed (such as metformin) have been shown to inhibit ferroptosis and may be beneficial for diabetes and its complications (Ma et al., 2021). This can provide ideas for the application of ferroptosis in diabetes treatment.

However, there are still some unanswered questions about the occurrence and development of ferroptosis in diabetes. For example, as there are no markers of ferroptosis *in vivo*, we cannot be sure whether ferroptosis occurs during cell growth and differentiation. In addition, ferroptosis is caused by phospholipid peroxidation, and ROS are also closely related to ferroptosis. Considering the oxygen demand of islet cells, it is unclear whether the ferroptosis process is dependent on the concentration of oxygen. In conclusion, although ferroptosis has not been thoroughly studied and its molecular mechanism in diabetes and diabetic complications needs to be further explored, ferroptosis is a potential target for the therapeutics of diabetes.

Author contributions

YW and XH contributed to the conception and design of the work. GD drafted the manuscript. QZ prepared the figure. YW and XH substantively revised the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Agrawal, S., Fox, J., Thyagarajan, B., and Fox, J. H. (2018). Brain mitochondrial iron accumulates in Huntington's disease, mediates mitochondrial dysfunction, and can be removed pharmacologically. *Free Radic. Biol. Med.* 120, 317–329. doi:10.1016/j.freeradbiomed.2018.04.002

Ansari, I. H., Longacre, M. J., Stoker, S. W., Kendrick, M. A., O'Neill, L. M., Zitur, L. J., et al. (2017). Characterization of Acyl-CoA synthetase isoforms in pancreatic beta cells: Gene silencing shows participation of ACSL3 and ACSL4 in insulin secretion. *Arch. Biochem. Biophys.* 618, 32–43. doi:10.1016/j.abb.2017.02.001

Bannai, S., Tsukeda, H., and Okumura, H. (1977). Effect of antioxidants on cultured human diploid fibroblasts exposed to cystine-free medium. *Biochem. Biophys. Res. Commun.* 74 (4), 1582–1588. doi:10.1016/0006-291x(77)90623-4

Bersuker, K., Hendricks, J. M., Li, Z., Magtanong, L., Ford, B., Tang, P. H., et al. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 575 (7784), 688–692. doi:10.1038/s41586-019-1705-2

Bottino, R., Balamurugan, A. N., Tse, H., Thirunavukkarasu, C., Ge, X., Profozich, J., et al. (2004). Response of human islets to isolation stress and the effect of antioxidant treatment. *Diabetes* 53 (10), 2559–2568. doi:10.2337/diabetes.53.10.2559

Brigelius-Flohé, R., and Maiorino, M. (2013). Glutathione peroxidases. *Biochim. Biophys. Acta* 1830 (5), 3289–3303. doi:10.1016/j.bbagen.2012.11.020

Chen, D., Chu, B., Yang, X., Liu, Z., Jin, Y., Kon, N., et al. (2021). iPLA2β-mediated lipid detoxification controls p53-driven ferroptosis independent of GPX4. *Nat. Commun.* 12 (1), 3644. doi:10.1038/s41467-021-23902-6

Chen, X., Kang, R., Kroemer, G., and Tang, D. (2021). Broadening horizons: The role of ferroptosis in cancer. *Nat. Rev. Clin. Oncol.* 18 (5), 280–296. doi:10.1038/s41571-020-00462-0

Chen, X., Li, J., Kang, R., Klionsky, D. J., and Tang, D. (2021). Ferroptosis: Machinery and regulation. *Autophagy* 17 (9), 2054–2081. doi:10.1080/15548627.2020.1810918

Chu, B., Kon, N., Chen, D., Li, T., Liu, T., Jiang, L., et al. (2019). ALOX12 is required for p53-mediated tumour suppression through a distinct ferroptosis pathway. *Nat. Cell. Biol.* 21 (5), 579–591. doi:10.1038/s41556-019-0305-6

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell.* 149 (5), 1060–1072. doi:10.1016/j.cell.2012.03.042

Doll, S., Proneth, B., Tyurina, Y. Y., Panzilius, E., Kobayashi, S., Ingold, I., et al. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* 13 (1), 91–98. doi:10.1038/nchembio.2239

Dolma, S., Lessnick, S. L., Hahn, W. C., and Stockwell, B. R. (2003). Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. *Cancer Cell.* 3 (3), 285–296. doi:10.1016/s1535-6108(03)00050-3

Fisher-Wellman, K. H., and Neufer, P. D. (2012). Linking mitochondrial bioenergetics to insulin resistance via redox biology. *Trends Endocrinol. Metab.* 23 (3), 142–153. doi:10.1016/j.tem.2011.12.008

He, J., Li, Z., Xia, P., Shi, A., FuChen, X., Zhang, J., et al. (2022). Ferroptosis and ferritinophagy in diabetes complications. *Mol. Metab.* 60, 101470. doi:10.1016/j.molmet.2022.101470

Houschyar, K. S., Lüdtke, R., Dobos, G. J., Kalus, U., Broecker-Preuss, M., Rampp, T., et al. (2012). Effects of phlebotomy-induced reduction of body iron stores on metabolic syndrome: Results from a randomized clinical trial. *BMC Med.* 10, 54. doi:10.1186/1741-7015-10-54

Jiang, L., Kon, N., Li, T., Wang, S. J., Su, T., Hibshoosh, H., et al. (2015). Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520 (7545), 57–62. doi:10.1038/nature14344

Ju, J., Song, Y. N., and Wang, K. (2021). Mechanism of ferroptosis: A potential target for cardiovascular diseases treatment. *Aging Dis.* 12 (1), 261–276. doi:10.14336/AD.2020.0323

Kagan, V. E., Mao, G., Qu, F., Angeli, J. P., Doll, S., Croix, C. S., et al. (2017). Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat. Chem. Biol.* 13 (1), 81–90. doi:10.1038/nchembio.2238

Kaneto, H., Kajimoto, Y., Miyagawa, J., Matsuoka, T., Fujitani, Y., Umayahara, Y., et al. (1999). Beneficial effects of antioxidants in diabetes: Possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 48 (12), 2398–2406. doi:10.2337/diabetes.48.12.2398

Karam, B. S., Chavez-Moreno, A., Koh, W., Akar, J. G., and Akar, F. G. (2017). Oxidative stress and inflammation as central mediators of atrial fibrillation in obesity and diabetes. *Cardiovasc. Diabetol.* 16 (1), 120. doi:10.1186/s12933-017-0604-9

Killion, E. A., Reeves, A. R., El Azzouny, M. A., Yan, Q. W., Surujon, D., Griffin, J. D., et al. (2018). A role for long-chain acyl-CoA synthetase-4 (ACSL4) in diet-induced phospholipid remodeling and obesity-associated adipocyte dysfunction. *Mol. Metab.* 9, 43–56. doi:10.1016/j.molmet.2018.01.012

Kon, N., Zhong, J., Qiang, L., Accili, D., and Gu, W. (2012). Inactivation of arf-bp1 induces p53 activation and diabetic phenotypes in mice. *J. Biol. Chem.* 287 (7), 5102–5111. doi:10.1074/jbc.M111.322867

Koppula, P., Zhang, Y., Zhuang, L., and Gan, B. (2018). Amino acid transporter SLC7A11/xCT at the crossroads of regulating redox homeostasis and nutrient dependency of cancer. *Cancer Commun.* 38 (1), 12. doi:10.1186/s40880-018-0288-x

Kraft, V. A. N., Bezjian, C. T., Pfeiffer, S., Ringelstetter, L., Müller, C., Zandkarimi, F., et al. (2020). GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. *ACS Cent. Sci.* 6 (1), 41–53. doi:10.1021/acscentsci.9b01063

Lei, G., Zhuang, L., and Gan, B. (2022). Targeting ferroptosis as a vulnerability in cancer. *Nat. Rev. Cancer* 22 (7), 381–396. doi:10.1038/s41568-022-00459-0

Li, D., and Li, Y. (2020). The interaction between ferroptosis and lipid metabolism in cancer. *Signal Transduct. Target. Ther.* 5 (1), 108. doi:10.1038/s41392-020-00216-5

Liao, P., Wang, W., Wang, W., Kryczek, I., Li, X., Bian, Y., et al. (2022). CD8(+) T cells and fatty acids orchestrate tumor ferroptosis and immunity via ACSL4. *Cancer Cell.* 40 (4), 365–378.e6. e6. doi:10.1016/j.ccell.2022.02.003

Liu, M. R., Zhu, W. T., and Pei, D. S. (2021). System xc(-): A key regulatory target of ferroptosis in cancer. *Invest. New Drugs* 39 (4), 1123–1131. doi:10.1007/s10637-021-01070-0

Liu, Y., and Gu, W. (2022). p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell. Death Differ.* 29 (5), 895–910. doi:10.1038/s41418-022-00943-y

Liu, Y., and Gu, W. (2021). The complexity of p53-mediated metabolic regulation in tumor suppression. *Semin. Cancer Biol.* S1044-579X (21), 00060–00062. doi:10.1016/j.semcancer.2021.03.010

Liu, Y., Tavana, O., and Gu, W. (2019). p53 modifications: exquisite decorations of the powerful guardian. *J. Mol. Cell. Biol.* 11 (7), 564–577. doi:10.1093/jmcb/mjz060

Lovis, P., Roggli, E., Laybutt, D. R., Gattesco, S., Yang, J. Y., Widmann, C., et al. (2008). Alterations in microRNA expression contribute to fatty acid-induced pancreatic beta-cell dysfunction. *Diabetes* 57 (10), 2728–2736. doi:10.2337/db07-1252

Lu, S. C. (2009). Regulation of glutathione synthesis. *Mol. Asp. Med.* 30 (1-2), 42–59. doi:10.1016/j.mam.2008.05.005

Ma, W. Q., Sun, X. J., Zhu, Y., and Liu, N. F. (2021). Metformin attenuates hyperlipidaemia-associated vascular calcification through anti-ferroptotic effects. *Free Radic. Biol. Med.* 165, 229–242. doi:10.1016/j.freeradbiomed.2021.01.033

Mao, C., Liu, X., Zhang, Y., Lei, G., Yan, Y., Lee, H., et al. (2021). DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature* 593 (7860), 586–590. doi:10.1038/s41586-021-03539-7

Minamino, T., Orimo, M., Shimizu, I., Kunieda, T., Yokoyama, M., Ito, T., et al. (2009). A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat. Med.* 15 (9), 1082–1087. doi:10.1038/nm.2014

Moosmann, B., and Behl, C. (2004). Selenoproteins, cholesterol-lowering drugs, and the consequences: Revisiting of the mevalonate pathway. *Trends cardiovasc. Med.* 14 (7), 273–281. doi:10.1016/j.tcm.2004.08.003

Nechushtai, R., Karmi, O., Zuo, K., Marjault, H. B., Darash-Yahana, M., Sohn, Y. S., et al. (2020). The balancing act of NEET proteins: Iron, ROS, calcium and metabolism. *Biochim. Biophys. Acta. Mol. Cell. Res.* 1867 (11), 118805. doi:10.1016/j.bbamcr.2020.118805

Oka, M., Xu, L., Suzuki, T., Yoshikawa, T., Sakamoto, H., Uemura, H., et al. (2021). Aberrant splicing isoforms detected by full-length transcriptome sequencing as transcripts of potential neoantigens in non-small cell lung cancer. *Genome Biol.* 22 (1), 9. doi:10.1186/s13059-020-02240-8

Ommati, M. M., Amjadinia, A., Mousavi, K., Azarpira, N., Jamshidzadeh, A., and Heidari, R. (2021). N-acetyl cysteine treatment mitigates biomarkers of oxidative stress in different tissues of bile duct ligated rats. *Stress* 24 (2), 213–228. doi:10.1080/10253890.2020.1777970

Seibt, T. M., Proneth, B., and Conrad, M. (2019). Role of GPX4 in ferroptosis and its pharmacological implication. *Free Radic. Biol. Med.* 133, 144–152. doi:10.1016/j.freeradbiomed.2018.09.014

Selvakumar, G. P., Ahmed, M. E., Raikwar, S. P., Thangavel, R., Kempuraj, D., Dubova, I., et al. (2019). CRISPR/Cas9 editing of glia maturation factor regulates mitochondrial dynamics by attenuation of the NRF2/HO-1 dependent ferritin activation in glial cells. *J. Neuroimmune Pharmacol.* 14 (4), 537–550. doi:10.1007/s11481-019-09833-6

Song, X., and Long, D. (2020). Nrf2 and ferroptosis: A new research direction for neurodegenerative diseases. *Front. Neurosci.* 14, 267. doi:10.3389/fnins.2020.00267

Stockwell, B. R., Friedmann Angeli, J. P., Bayir, H., Bush, A. I., Conrad, M., Dixon, S. J., et al. (2017). Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. *Cell.* 171 (2), 273–285. doi:10.1016/j.cell.2017.09.021

Tian, S., Wang, M., Liu, C., Zhao, H., and Zhao, B. (2019). Mulberry leaf reduces inflammation and insulin resistance in type 2 diabetic mice by TLRs and insulin Signalling pathway. *BMC Complement. Altern. Med.* 19 (1), 326. doi:10.1186/s12906-019-2742-y

Tonelli, C., Chio, I. I. C., and Tuveson, D. A. (2018). Transcriptional regulation by Nrf2. *Antioxid. Redox Signal.* 29 (17), 1727–1745. doi:10.1089/ars.2017.7342

Wang, J., and Wang, H. (2017). Oxidative stress in pancreatic beta cell regeneration. *Oxid. Med. Cell. Longev.* 2017, 1930261. doi:10.1155/2017/1930261

Wang, W., Knovich, M. A., Coffman, L. G., Torti, F. M., and Torti, S. V. (2010). Serum ferritin: Past, present and future. *Biochim. Biophys. Acta* 1800 (8), 760–769. doi:10.1016/j.bbagen.2010.03.011

Wang, Y., Bi, R., Quan, F., Cao, Q., Lin, Y., Yue, C., et al. (2020). Ferroptosis involves in renal tubular cell death in diabetic nephropathy. *Eur. J. Pharmacol.* 888, 173574. doi:10.1016/j.ejphar.2020.173574

Wang, Z., Fan, H. B., Yang, W. W., Mao, X. D., Xu, S. H., Ma, X. P., et al. (2018). Correlation between plasma ferritin level and gestational diabetes mellitus and its impact on fetal macrosomia. *J. Diabetes Investig.* 9 (6), 1354–1359. doi:10.1111/jdi.12836

Wei, S., Qiu, T., Yao, X., Wang, N., Jiang, L., Jia, X., et al. (2020). Arsenic induces pancreatic dysfunction and ferroptosis via mitochondrial ROS-autophagy-

lysosomal pathway. *J. Hazard. Mat.* 384, 121390. doi:10.1016/j.jhazmat.2019.121390

Wrede, C. E., Dickson, L. M., Lingohr, M. K., Briaud, I., and Rhodes, C. J. (2002). Protein kinase B/Akt prevents fatty acid-induced apoptosis in pancreatic beta-cells (INS-1). *J. Biol. Chem.* 277 (51), 49676–49684. doi:10.1074/jbc.M208756200

Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R., and Turner, N. D. (2004). Glutathione metabolism and its implications for health. *J. Nutr.* 134 (3), 489–492. doi:10.1093/jn/134.3.489

Yang, W. S., Kim, K. J., Gaschler, M. M., Patel, M., Shchepinov, M. S., and Stockwell, B. R. (2016). Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* 113 (34), E4966–E4975. doi:10.1073/pnas.1603244113

Yang, W. S., and Stockwell, B. R. (2008). Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. *Chem. Biol.* 15 (3), 234–245. doi:10.1016/j.chembiol.2008.02.010

Yuan, H., Li, X., Zhang, X., Kang, R., and Tang, D. (2016). Identification of ACSL4 as a biomarker and contributor of ferroptosis. *Biochem. Biophys. Res. Commun.* 478 (3), 1338–1343. doi:10.1016/j.bbrc.2016.08.124

Yuan, H., Zhang, X., Huang, X., Lu, Y., Tang, W., Man, Y., et al. (2010). NADPH oxidase 2-derived reactive oxygen species mediate FFAs-induced dysfunction and apoptosis of β-cells via JNK, p38 MAPK and p53 pathways. *PLoS One* 5 (12), e15726. doi:10.1371/journal.pone.0015726

Zhang, D. D. (2006). Mechanistic studies of the Nrf2-Keap1 signaling pathway. *Drug Metab. Rev.* 38 (4), 769–789. doi:10.1080/03602530600971974

Zhou, Y., Zhang, E., Berggreen, C., Jing, X., Osmark, P., Lang, S., et al. (2012). Survival of pancreatic beta cells is partly controlled by a TCF7L2-p53-p53INP1-dependent pathway. *Hum. Mol. Genet.* 21 (1), 196–207. doi:10.1093/hmg/ddr454

Zhou, Y. (2020). The protective effects of cryptochlorogenic acid on β-cells function in diabetes *in vivo* and vitro via inhibition of ferroptosis. *Diabetes Metab. Syndr. Obes.* 13, 1921–1931. doi:10.2147/DMSO.S249382

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Mutant p53^{K120R} expression enables a partial capacity to modulate metabolism

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The *TP53* tumor suppressor gene is one of the most studied gene in virtue of its ability to prevent cancer development by regulating apoptosis, cell cycle arrest, DNA repair, autophagy and senescence. Furthermore, the modulation of metabolism by P53 is fundamental for tumor suppressor activity. Studies in mouse models showed that mice carrying *TP53* mutations affecting the acetylation in the DNA binding domain still retain the ability to transactivate genes involved in metabolism. Noteworthy, mice expressing the triple 3KR or the single K117R mutant do not show early on-set tumor development in contrast to *TP53*$^{-/-}$ mice. Interestingly, the mouse K117R mutation corresponds to the human tumor-derived K120R modification, which abrogates P53-dependent activation of apoptosis without affecting growth arrest. In this study, we investigated the property of the human P53 K120R mutant in the regulation of metabolism by analyzing the transcriptional specificity in yeast- and mammalian-based reporter assays, the metabolic phenotype associated to its expression in colon cancer HCT116$^{TP53-/-}$ cells and the induction of P53 targets and proteins involved in the antioxidant response. These properties were analyzed in comparison to wild type P53 protein, the human triple mutant corresponding to mouse 3KR and the cancer hot-spot R273H mutant. We confirm the selective functionality of P53 K120R mutant, which shows a transcriptional activity on cell cycle arrest but not on apoptotic targets. Interestingly, this mutant shows a partial transactivation activity on p53 response element belonging to the metabolic target TIGAR. Moreover, we observe a significant uncoupling between oxygen consumption and ATP production associated with higher lipid peroxidation level in all P53 mutants carrying cells with respect to wild type P53 expressing cells. Noteworthy, in the absence of a pro-oxidative challenge, cells expressing K120R mutant retain a partial capacity to modulate glucose metabolism, limiting lipid peroxidation with respect to the other P53 mutants carrying cells. Lastly, especially in presence of human 3KR mutant, a high expression of proteins involved in the antioxidant response is found. However, this response does not avoid the increased lipid peroxidation, confirming that only wild type P53 is able to completely counteract the oxidative stress and relative damages.

KEYWORDS

p53, energy metabolism, K120R mutation, antioxidant response, lipid peroxidation

Introduction

The *TP53* tumor suppressor gene, encoding the P53 tetrameric transcription factor, is the most commonly altered gene in human cancer, highlighting its key role in maintaining cellular homeostasis. The majority of *TP53* alterations found in tumors are missense mutations affecting six codons located in the central DNA binding domain (DBD) (i.e., 175, 245, 248, 249, 273, 282 referred as hot spots) that typically result in the complete loss of tumor suppressor functions. However, the spectrum of missense mutations is extremely broad along the *TP53* coding sequence, with more than 2,000 different amino-acid changes reported; indeed, *TP53* mutations can be classified not only as loss of function (LoF) but also as partial function, with altered specificity or gain of function (GoF), highlighting the functional heterogeneity of the encoded P53 mutant proteins (Leroy et al., 2013; Bisio et al., 2014; Monti et al., 2020b).

The *TP53* missense mutation, giving rise to the Lysine (K) to Arginine (R) substitution at codon 120 (here indicated as $p53^{K120R}$), is an example of a DNA contact alteration found in cancers able to maintain some DNA binding capacity in virtue of the positive charge preservation of the amino acid side chain, which allows a heterogenous transcriptional activity (Liu et al., 2019; Xia et al., 2022). Indeed, it has been reported that the $p53^{K120R}$ mutant is functionally active on cell cycle arrest target genes but not on apoptotic ones. This amino acid substitution, avoiding the acetylation of the K120 residue, results in the inability of the mutant protein to induce apoptosis (Sykes et al., 2006; Tang et al., 2006). Recently, a GoF activity for $p53^{K120R}$ was also described; in fact, $p53^{K120R}$ but not wild type P53 ($p53^{WT}$) is able to bind and activate the transcription of the pro-survival *TNFAIP8* gene, that is critical for escape from apoptosis in tumor cells (Monteith et al., 2016).

For a long time, apoptosis, cell cycle arrest, and senescence were believed to mediate the tumor suppressive functions of P53; recently, it was highlighted that other pathways play a role in P53 ability to function as a tumor suppressor. Among these, the involvement of $p53^{WT}$ in the aerobic energy metabolism modulation emerged, due to its ability to inhibit glucose transport, glycolysis, and fatty acid synthesis, but to promote lipid uptake, fatty acid oxidation, oxidative phosphorylation, and glutaminolysis (Lacroix et al., 2020; Liu and Gu, 2021). Furthermore, P53 has a crucial role in regulating ferroptosis, an iron-dependent form of non-apoptotic cell death (Liu and Gu, 2022; Xia et al., 2022).

Studies in mouse models showed that mice carrying K to R mutations at three acetylation sites in the central DBD of P53 (K117R; K161R; K162R encoding the so called $p53^{3KR}$) and comprising the K117 site that corresponds to K120 codon of human P53, have impaired P53 function in apoptosis, cell cycle arrest, and senescence. However, these mice are not prone to early tumor development as the *TP53* null mice (Li et al., 2012; Xia et al., 2022). Interestingly, the $p53^{3KR}$ mutant retains the ability as the $p53^{WT}$ protein to transactivate some P53 target genes involved in the regulation of metabolism, including *TIGAR* and *GLS2*, coding for proteins that play critical role in glycolysis and mitochondrial respiration (Li et al., 2012; Xia et al., 2022). In addition, the cystine/glutamate antiporter *SLC7A11* (named also *xCT*) was identified from microarray analysis as a P53 target repressed by mouse $p53^{3KR}$ mutant and $p53^{WT}$ (Jiang et al., 2015). Interestingly, *xCT* has been found to be involved in ferroptosis, where lipid peroxidation plays a central role (Yang and Stockwell, 2016; Xie et al., 2017; Koppula et al., 2021; Liu and Gu 2022; Xia et al., 2022).

Conversely, defective P53-mediated apoptosis but efficient cell cycle-arrest and senescence were observed in mouse carrying the K117R mutation (here indicated as $p53^{K117R}$), according to the inability of this mutant protein to induce the apoptotic targets *BBC3* (*PUMA*), *TNFRSF10B* (*KILLER*) and *PMAIP1* (*NOXA*) and the ability to induce the cell-cycle inhibitor *CDKN1A* (*P21*). As for $p53^{WT}$ mice, $p53^{K117R}$ animals did not develop early-onset tumors (Li et al., 2012; Liu et al., 2019; Liu and Gu 2022; Xia et al., 2022).

Although the functional specificity of the $p53^{K120R}$ protein described above has been extensively studied, its contribution to metabolism modulation remains to be explored. Based on this premises, we investigated the property of human $p53^{K120R}$ mutant, corresponding to mouse $p53^{K117R}$, in the regulation of metabolism by analyzing i) its transcriptional specificity in yeast- and mammalian-based reporter assays, ii) the metabolic phenotypes of human colon cancer $HCT116^{TP53-/-}$ cells expressing the mutant protein, and iii) the induction of P53 targets and proteins involved in the antioxidant response. The $p53^{K120R}$ features were analyzed in comparison not only to $p53^{WT}$ but also to the human P53 mutant resembling the triple mutant previously analyzed in mouse models. Furthermore, since the mutation giving rise to R273H DNA contact mutant protein (here indicated as $p53^{R273H}$) is one of the main hot spot mutation found in human tumors, generating a mutant P53 protein unable to transactivate all type of P53 response elements (REs) or promoters (Loss of function, LoF) (Monti et al., 2020b), the metabolic functionality of $p53^{R273H}$ was also studied.

Materials and methods

Yeast strains, expression vectors and reporter assay

The following yeast *S. cerevisiae* LUC1 reporter strains were used: yLFM-P21-5', yLFM-MDM2P2C, yLFM-BAX A + B,

yLFM-KILLER, yLFM-AIP1, yLFM-NOXA, and yLFM-TIGAR (Inga et al., 2002; Resnick and Inga, 2003). The strains are isogenic with the exception of the p53 Response Element (RE), a 20–22 bp nucleotide sequence that drives the expression of the firefly reporter gene (*LUC1*); the REs belong to apoptotic [*BAX*, *WDR1* (*AIP1*), *NOXA* and *KILLER*], cell cycle arrest [*CDKN1A* (*P21*)], P53 regulation (*MDM2*), and metabolic (*TIGAR*) targets. Yeast plasmids expressing the different *TP53* mutations were constructed exploiting *in vivo* yeast homologous recombination and all manipulations involving the reporter assay and yeast transformations were performed as previously described (Marengo et al., 2018; Monti et al., 2020a, 2019).

A pTSG-based vector was used in order to express P53 proteins ($p53^{WT}$, $p53^{K120R}$, $p53^{3KR}$, $p53^{R273H}$) under a galactose inducible expression (8 h, 0.128% Galactose); pRS314 was used as empty vector. Regarding the construction of the human P53 mutant that resembles the mouse $p53^{3KR}$ mutant, since the mouse position K162 is replaced in human by Q165, an acetyl-mimetic amino acid, we constructed the human triple P53 K120R + K164R + Q165R mutant and named it $p53^{3KR}$ for the analogy with the mouse mutant (Supplementary Figure S1).

Mammalian cells line, expression vectors and reporter assay

Human colon carcinoma $HCT116^{TP53-/-}$ cells were obtained from Dr. B. Vogelstein (The Johns Hopkins Kimmel Cancer Center, Baltimore, MD). Cells were grown in RPMI containing 10% foetal bovine serum, L-glutamine, penicillin-streptomycin antibiotic mixture (Euroclone, Milano, Italy), and maintained at 37 °C, in 5% CO2, at 100% humidity.

A pCIneo-based vector (Promega) was used to express P53 proteins ($p53^{WT}$, $p53^{K120R}$, $p53^{3KR}$, $p53^{R273H}$) in mammalian cells. Plasmids expressing P53 mutants were constructed as previously described (Marengo et al., 2018; Monti et al., 2020a). The pGL3-P21, pGL3-MDM2 and pGL3-BAX were available as reporter vectors; the pRL-SV40 plasmid was used as normalization vector. For reporter assays, $HCT116^{TP53-/-}$ cells were transfected in medium free serum with 200 ng of pCIneo-based P53 expression vector, 250 ng of reporter vector, 50 ng of normalization vector in presence of Mirus Bio™ TransIT™-LT1 Transfection Reagent (Reagent: DNA ratio 3:1). After 24 h, cells were collected and washed with cold phosphate buffer saline (PBS). Lysis was performed in 1X PLB buffer (Passive Lysis Buffer, Promega). Luciferase assays were conducted, as previously described (Marengo et al., 2018; Monti et al., 2020a). For all reporter assay (yeast- and mammalian-based) wild type and mutant P53 fold induction over empty vector (pRS314 or pCIneo, respectively) were used to calculate the transactivation ability (as percentage, %) of the P53 mutant protein with respect to $p53^{WT}$ (100%). For western blot and metabolic analysis, $HCT116^{TP53-/-}$ cells were transfected as reported above with 1 μg of pCIneo-based P53 expression vector. After 16 h of transfection, cells were treated with 100 μM hydrogen peroxide (H_2O_2) for further 6 h. The percentage of living cells following this treatment did not differ among cells carrying different P53 proteins (Supplementary Figure S2). The use of pTSG- and pCINeo-based vectors for all experiments guarantee an equal expression of wild type and mutant P53 proteins (3).

Western blotting analysis

Mammalian protein extracts were prepared as previously described (Marengo et al., 2018; Foggetti et al., 2019). 20–50 μg of mammalian cell lysates were resolved on 4–15% Mini protean TGX precast gels and transferred to nitrocellulose membranes by Trans-Blot Turbo Blotting System (Bio-Rad, Milano, Italy). Membranes were blocked with 2% non-fat dry milk in 0.1% Tween-20 in PBS for 1 h, then incubated at room temperature (1 h) or overnight at 4 °C with the appropriate primary antibody. The following antibodies were employed: anti-P53 (clone DO-1, Santa Cruz Biotechnology), anti-MDM2 (clone SPM14, sc-965 Santa Cruz), anti-P21 Waf1/Cip1 (DCS60 #2946 Cell Signaling Technology), anti-BAX (clone N-20, sc-493 Santa Cruz), anti-TIGAR (clone E-2, sc-166290 Santa Cruz), anti-G6PD (D5D2, #12263 Cell Signaling Technology), anti-GCLM (ab154017, Abcam), anti-GCLC (clone EP13475, ab190685 Abcam), anti-NRF2 (clone 21HCLC, #710574 Invitrogen), anti-xCT/SLC7A11 (Clone D2M7A, #12691 Cells Signaling Technology), anti-human β-Actin (clone AC-74, Sigma–Aldrich). The appropriate IgG-horseradish peroxidase-conjugated secondary antibodies (anti-mouse or anti-rabbit IgG HRP, A9044 and A9169 respectively, Sigma-Aldrich) were used. Detection was carried out with ECL FAST PICO (ECL-1002, Immunological Sciences, Roma, Italy). Chemiluminescence was analyzed by Alliance LD, UVITEC Cambridge (Cambridge, U.K.).

Oxygen consumption rate and ATP synthesis evaluation

Oxygen consumption rate (OCR) was evaluated in 100,000 cells for each experiment by a thermostatically controlled oxygraph apparatus equipped with an amperometric electrode (Unisense-Microrespiration, Unisense A/S). Cells were resuspended in PBS and permeabilized with 0.03% digitonin. To evaluate the OCR performed by Complexes I, III and IV pathway, 10 mM pyruvate +5 mM malate were added, while 20 mM succinate were used to activate the pathways composed by Complexes II, III and IV; after the addition of respiratory substrates, 0.1 mM ADP was added in each experiment. The respiratory rates were expressed as nmol O/min/10^6 cells (Cappelli et al., 2020).

To evaluate the mitochondrial ATP synthesis, the F_o-F_1 ATP synthase activity was measured in 100,000 cells incubated for 10 min at 37°C in a medium containing 10 mM Tris-HCl pH 7.4, 100 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 2.5 mM EDTA, and 5 mM $MgCl_2$, 0.6 mM ouabain, 0.3 mM P1, P5-Di (adenosine-5′) pentaphosphate, and 25 mg/ml ampicillin. The ATP synthesis was induced by the addition of 10 mM pyruvate +5 mM malate or 20 mM succinate, to stimulate the pathways composed by Complexes I, III and IV or Complexes II, III and IV, respectively. The reaction was monitored for 2 min, every 30 s, in a luminometer (GloMax® 20/20n Luminometer, Promega Italia), by the luciferin/luciferase chemiluminescent method (luciferin/luciferase ATP bioluminescence assay kit CLSII, Roche), with ATP standard solutions between 10^{-8} and 10^{-5} M. Data were expressed as nmol ATP produced/min/10^6 cells (Cappelli et al., 2020). To verify that OCR and ATP synthesis are dependent on OxPhos machinery, samples were treated with 1 μM Rotenone or 10 μM Antimycin A, specific inhibitors of Complex I and Complex III, respectively (Supplementary Figure S4).

By measuring the OCR and the ATP synthesis trough F_o-F_1 ATP synthase, the oxidative phosphorylation (OxPhos) efficiency was calculated as the ratio between the produced ATP concentration and the consumed oxygen amount (P/O ratio).

Glucose consumption, lactate release assay, and lactate dehydrogenase activity

To evaluate the glucose consumption, the glucose content in the growth medium was evaluated following the reduction of NADP at 340 nm, by the hexokinase (HK) and glucose six phosphate dehydrogenase (G6PD) coupling system. The assay medium contained 100 mM Tris-HCl pH 7.4, 2 mM ATP, 10 mM NADP, 2 mM MgCl2, 2 International Units (IU) of hexokinase, and 2 IU of glucose 6-phosphate dehydrogenase. Data were normalized on the cell number and expressed as mM consumed glucose/10^6 cells (Cappelli et al., 2017).

Lactate concentration was assayed spectrophotometrically in the growth medium, following the reduction of NAD+, at 340 nm. The assay medium contained 100 mM Tris-HCl pH 7.4, 5 mM NAD+, and 1 IU/ml of lactate dehydrogenase (LDH). Data were normalized on the cell number and expressed as mM released lactate/10^6 cells (Cappelli et al., 2017).

Once the glucose consumption and lactate release amounts have been obtained, the lactate fermentation yield was calculated by comparing the values of lactate produced with those of theoretical lactate calculated as twice the glucose consumed.

Lactate dehydrogenase activity was evaluated as a marker of lactate fermentation, following the NADH oxidation, at 340 nm. The reaction mixtures contained: 100 mM Tris-HCl pH 7.4, 0.2 mM NADH, and 5 mM pyruvate (Cappelli et al., 2020). For the assay 20 μg of cell homogenate, obtained by sonication of cells resuspended in PBS, were employed.

Evaluation of malondialdehyde content

Malondialdehyde (MDA) concentration was assayed as a lipid peroxidation marker, by the thiobarbituric acid reactive substances (TBARS) assay (Colla et al., 2016). 600 μl of TBARS solution was added to 50 μg of cell homogenate dissolved in 300 μl of Milli-Q water. The mix was incubated for 40 min at 100 °C. Afterwards, the sample was centrifuged at 14,000 rpm for 2 min, and the supernatant was analyzed spectrophotometrically at 532 nm.

Statistical analysis

T student test, ordinary One-way anova and Two-way anova followed by comparisons test were performed by using GraphPad Prism version 8.0. For Windows, GraphPad Software, San Diego, California, United States .

Results

The human $p53^{K120R}$, $p53^{3KR}$, and $p53^{R273H}$ mutants show different transactivation activity towards P53 targets

The functional activity of the human $p53^{K120R}$, $p53^{3KR}$ and $p53^{R273H}$ mutants was examined using a yeast-based functional assay. Our analyses confirm that $p53^{K120R}$ maintains high functionality on *P21* and *MDM2* REs but shows partial (*BAX* and *KILLER*) or poor (*AIP1*, *NOXA*) activity on the REs of apoptotic genes (Figure 1A). The human $p53^{3KR}$ mutant shows a similar or a lower functionality ($p53^{K120R}$ > $p53^{3KR}$) on the same REs. The $p53^{R273H}$ mutant, used as a control of LoF mutant, shows no transactivation activity in any yeast strain tested. The $p53^{K120R}$ and $p53^{3KR}$ mutants were also evaluated on the RE of the metabolic target *TIGAR*; they show a partial activity ($p53^{K120R}$ > $p53^{3KR}$) and not a complete loss of transactivation ability as displayed by $p53^{R273H}$ mutant (Figure 1A).

The transactivation specificity toward *P21*, *MDM2*, and *BAX* targets was confirmed with a mammalian cells-based reporter assay in colon cancer $HCT116^{TP53-/-}$ cells expressing mutant P53 proteins (Figure 1B). The $p53^{K120R}$ is able to transactivate the *P21* and *MDM2* promoters but less efficiently the *BAX* promoter. On the contrary, the $p53^{3KR}$ mutant loses 70–80% of its activity on all promoters, showing a behavior more similar to $p53^{R273H}$ mutant (Figure 1B).

In the same mammalian cells, the level of P21, MDM2, BAX, and TIGAR endogenous proteins was also assessed (Figure 2). In

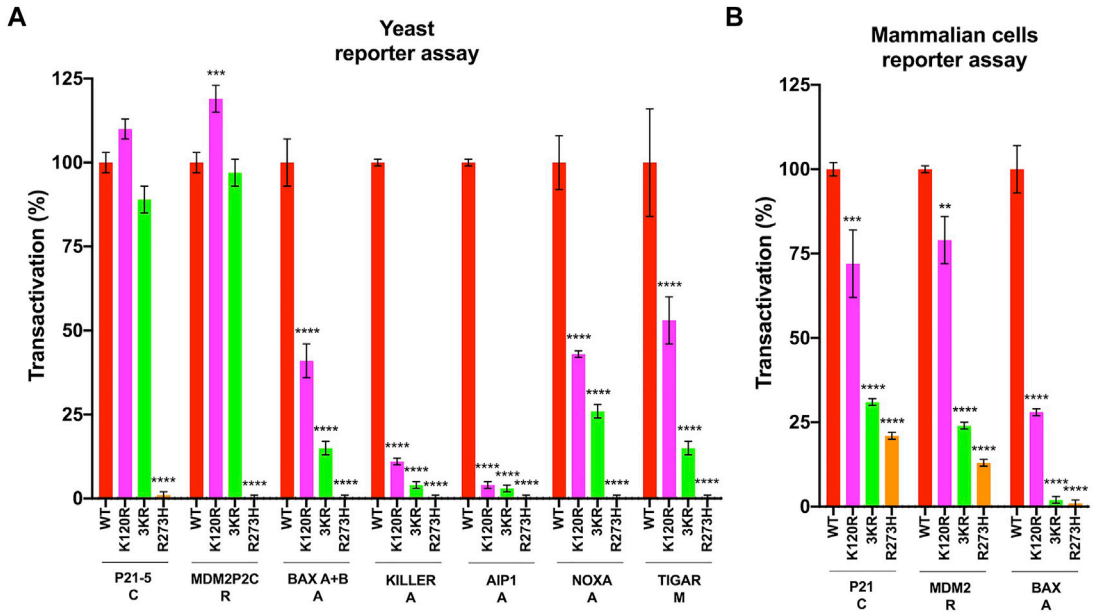

FIGURE 1
Transactivation activity of $p53^{WT}$, $p53^{3KR}$, $p53^{K120R}$, and $p53^{R273H}$ proteins. **(A)** $p53^{K120R}$, $p53^{3KR}$, $p53^{R273H}$ mutant proteins transactivation ability towards seven p53 REs with respect to $p53^{WT}$ in yLFM yeast strains. **(B)** $p53^{K120R}$, $p53^{3KR}$, $p53^{R273H}$ mutant proteins transactivation ability towards three p53 responsive promoters with respect to $p53^{WT}$ in $HCT116^{TP53-/-}$ cell line. Data of reporter assays are presented as mean ± standard deviation (SD) of two independent experiments with three biological replicates. The symbols **, *** and **** indicate significant differences for $p = 0.0015$, $p \leq 0.001$ and $p < 0.0001$, respectively, between cells expressing $p53^{WT}$ (WT) and cells expressing $p53^{K120R}$ (K120R), $p53^{3KR}$ (3KR) or $p53^{R273H}$ (R273H). Functional classification of the gene associated with the p53 REs or promoters is indicated as following: C, cell cycle arrest; R, P53 regulation; A, apoptosis; M, metabolism.

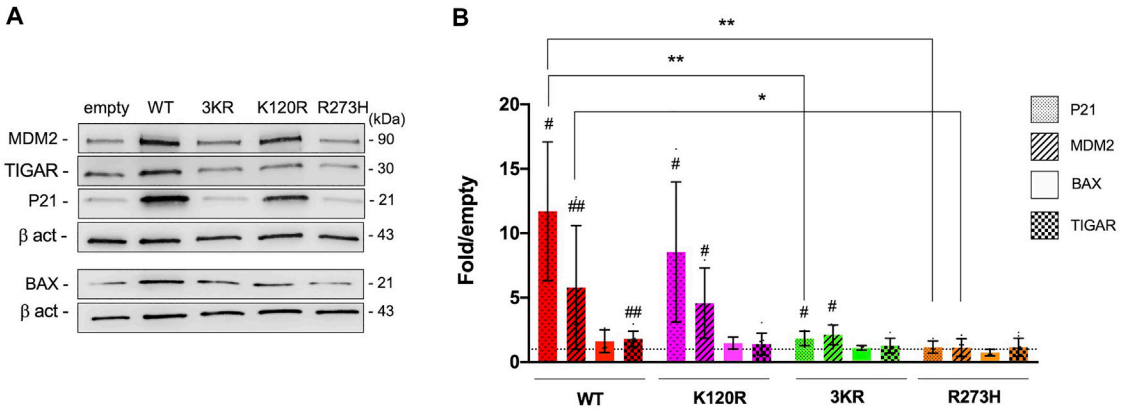

FIGURE 2
Modulation of endogenous P53 targets by $p53^{WT}$, $p53^{K120R}$, $p53^{3KR}$, and $p53^{R273H}$ in $HCT116^{TP53-/-}$ cells. **(A)** Representative western blots showing the level of MDM2, TIGAR, P21, BAX and Beta-actin (β-act) endogenous proteins in $HCT116^{TP53-/-}$ cells transiently expressing $p53^{WT}$, $p53^{3KR}$, $p53^{K120R}$, and $p53^{R273H}$. **(B)** Histogram represents the amount of P21, MDM2, BAX, and TIGAR proteins detected in $HCT116^{TP53-/-}$ transfected cells and normalized for β-act. Data are reported as mean ± SD and are obtained from at least three independent experiments. The levels of the different proteins are calculated as fold over the level of the same proteins found in cells transfected with the empty vector (#$p < 0.05$; ##$p < 0.005$; t Student); the dotted line corresponds to one-fold over empty. The symbols * and ** indicate significant differences of protein expression (with $p = 0.0130$ and $p \leq 0.0091$, respectively) between cells expressing $p53^{WT}$ (WT) and cells expressing $p53^{K120R}$ (K120R), $p53^{3KR}$ (3KR) or $p53^{R273H}$ (R273H). MDM2 induction by $p53^{3KR}$ results not quite significantly different ($p = 0.0563$) from the corresponding induction by $p53^{WT}$.

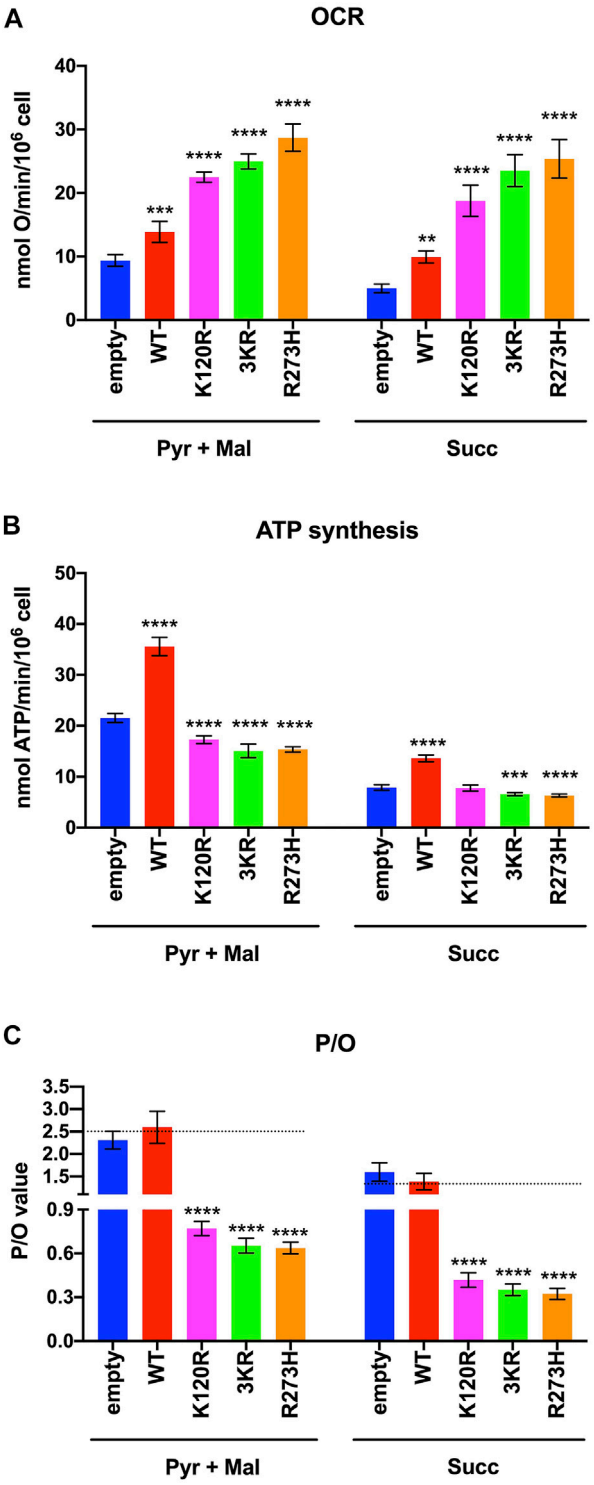

FIGURE 3
OxPhos function in HCT116$^{TP53-/-}$ cells expressing p53^{WT}, p53^{3KR}, p53^{K120R}, and p53^{R273H} proteins. **(A)** OCR in the presence of Pyruvate (Pyr) plus Malate (Mal) or Succinate (Succ). **(B)** ATP synthesis through the F_o-F_1 ATP synthase in the same condition as above. **(C)** OxPhos efficiency as P/O ratio in the same condition as above. The dotted lines indicate the expected P/O

(*Continued*)

keeping with the functional assays described above, both p53^{WT} and p53^{K120R} efficiently induce the endogenous P21 and MDM2 proteins (Figures 2A,B), whereas p53^{3KR} retains low ability with a behavior resembling the p53^{R273H} mutant. The induction of BAX is not significant in any conditions. Regarding TIGAR, the induction is significant only in cells expressing p53^{WT} (1.8-fold over empty), but not in cells expressing the other P53 mutant proteins.

All together these data confirm the selective functionality of p53^{K120R} mutant protein with respect to p53^{3KR} and p53^{R273H} mutants.

The human p53^{K120R}, p53^{3KR}, and p53^{R273H} mutants determine an altered metabolic phenotype

To evaluate the metabolic phenotype induced by the expression of P53 mutant proteins (p53^{K120R}, p53^{3KR}, and p53^{R273H}), the OxPhos activity and the lactate fermentation rate were investigated in HCT116$^{TP53-/-}$ cells transiently transfected as previously described.

The OxPhos function was assayed in terms of OCR and ATP synthesis, in the presence of the respiratory substrate pyruvate plus malate or succinate. As reported in Figure 3A, the OCR appears increasingly and significantly higher in p53^{WT}, p53^{K120R}, p53^{3KR}, and p53^{R273H} expressing cells compared to the cells transfected with the empty vector. Moreover, the expression of all type of P53 mutants causes at least a two-fold increase in OCR than the one measured in p53^{WT} carrying cells. Conversely, the ATP synthase activity significantly increases only in cells expressing the p53^{WT}, while in the presence of all P53 mutant proteins it is slightly lower or comparable to cells transfected with empty vector (Figure 3B).

The evaluation of the P/O ratio as a marker of OxPhos efficiency (Figure 3C) shows that only empty vector and p53^{WT} expressing cells have values similar to those reported when the oxygen consumption is entirely devoted to energy production (e.g., 2.5 with pyruvate plus malate or 1.5 with succinate, Hinkle, 2005). Conversely, cells expressing p53^{K120R}, p53^{3KR}, and p53^{R273H} display lower P/O values, suggesting the uncoupling between oxygen consumption and ATP production.

FIGURE 3 (Continued)
ratio levels based on the employed respiratory substrates. Each panel is representative of at least four independent experiments and data are reported as mean ± SD. The symbols **, *** and **** indicate significant differences for $p = 0.0048$, $p \leq 0.0009$ and $p < 0.0001$, respectively, between the control (empty) cells and those expressing p53^{WT} (WT), p53^{K120R} (K120R), p53^{3KR} (3KR) or p53^{R273H} (R273H), for each OxPhos stimulation condition (addition of pyruvate + malate or succinate).

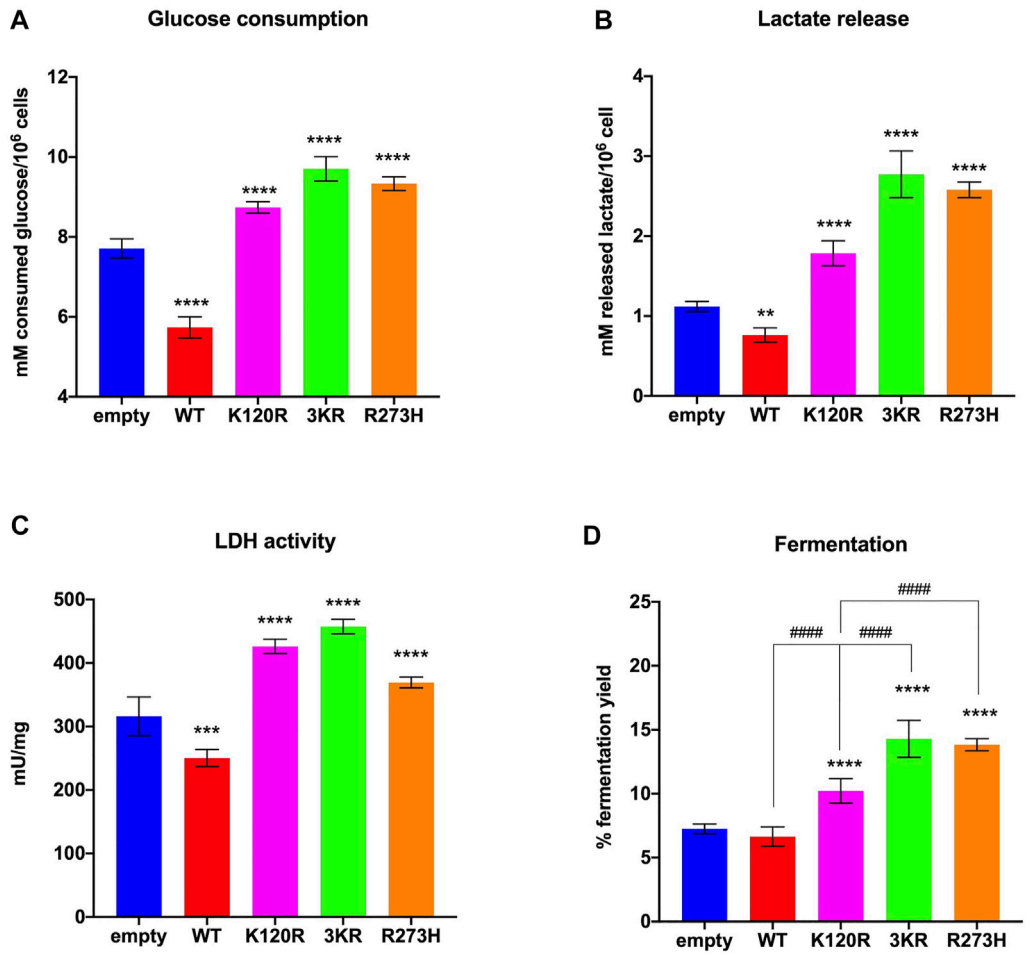

FIGURE 4
Anaerobic metabolism in HCT116$^{TP53-/-}$ cells expressing p53^{WT}, p53^{3KR}, p53^{K120R}, and p53^{R273H} proteins. **(A)** Glucose consumption. **(B)** Lactate release in the medium. **(C)** LDH activity. **(D)** Percentage of lactic acid fermentation yield. Each panel is representative of at least four independent experiments; data are reported as mean ± SD. The symbols **, ***, **** indicate significant differences for $p < 0.01$, 0.001, and 0.0001, respectively, between the control (empty) cells and those expressing p53^{WT} (WT), p53^{K120R} (K120R), p53^{3KR} (3KR) or p53^{R273H} (R273H); the symbol #### indicates significant differences for $p < 0.0001$ between p53^{K120R} and the indicated samples.

The impairment of the mitochondrial energy functions can lead to the anaerobic glycolysis increase, as an attempt to maintain a stable energy status and to recycle the reduced coenzymes (e.g., NADH), converting pyruvate to lactic acid by LDH activity (Prochownik and Wang, 2021; Ravera et al., 2021). Thus, the lactic acid fermentation extent was determined in the same cells and conditions by measuring the glucose consumption and the released lactate amount in the culture medium (i.e., lactic acid in solution), as well as the LDH activity.

In the presence of p53^{WT} glucose consumption, lactate release, and LDH activity are significantly lower than in cells transfected with the empty vector, while cells expressing p53^{K120R}, p53^{3KR}, and p53^{R273H} proteins show a significant increment (Figures 4A–C). These effects ultimately lead to a lower lactate fermentation yield in the presence of p53^{WT} (that is comparable to empty cells) with respect to P53 mutant proteins (Figure 4D) due to the corresponding enhancement of aerobic mitochondrial energy metabolism, as previously reported (Figure 3). Conversely, the increase in fermentation yield observed in the presence of the p53^{K120R}, p53^{3KR}, and p53^{R273H} mutant proteins indicates a metabolic switch from aerobic to anaerobic metabolism. Nevertheless, the observation that the p53^{K120R} mutant triggers a significant lower fermentation yield with respect to p53^{3KR} and p53^{R273H}, although significantly higher than p53^{WT} (Figure 4D), may suggest that this mutant still preserves some capacity to modulate glucose metabolism.

We also determined the level of glutaminase (GLS), glutamate dehydrogenase activity (GDH), as well as 3-hydroxyacyl-CoA dehydrogenase as markers of amino acid metabolism and beta-oxidation, respectively. Data reported in

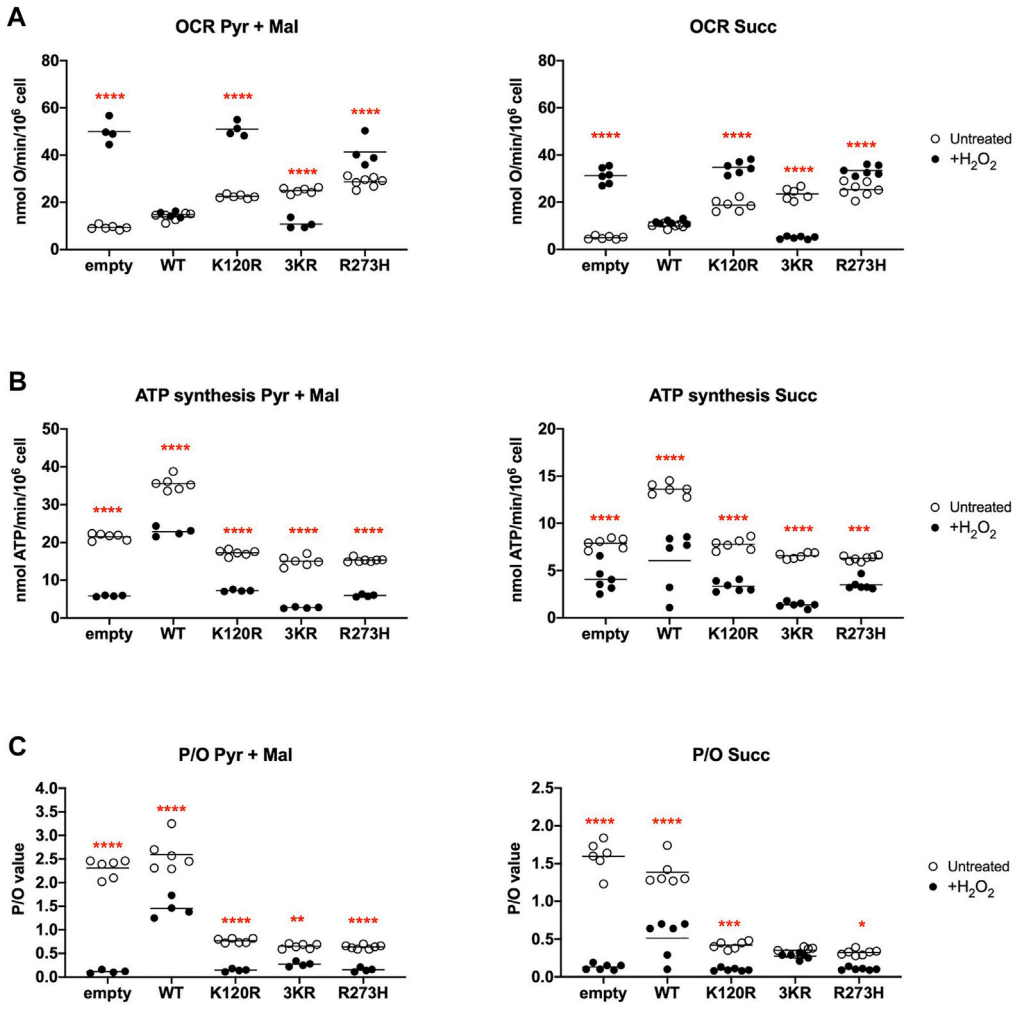

FIGURE 5
Comparison of OxPhos function in H_2O_2 treated HCT116$^{TP53-/-}$ cells with untreated cells, expressing p53^{WT}, p53^{3KR}, p53^{K120R}, and p53^{R273H} proteins. **(A)** OCR in untreated (empty dots) and 100 μM H_2O_2 treated (black dots) cells in the presence of Pyruvate (Pyr) plus Malate (Mal) or Succinate (Succ). **(B)** ATP synthesis through the F_o-F_1 ATP synthase in the same condition as above. **(C)** OxPhos efficiency as P/O ratio in the same condition as above. Each panel is representative of at least four independent experiments and data are reported as mean. The red symbols *, **, *** and **** indicate significant differences for $p = 0.0110$, $p = 0.0026$, $p \leq 0.0003$ and $p < 0.0001$, respectively, between the untreated and 100 μM H_2O_2 treated samples. In each panel, the empty dots represent the data from untreated samples depicted in Figure 3.

Supplementary Figure S5 show that the activities of the three analyzed enzymes are significantly increased in p53^{WT} expressing cells compared to the control, confirming the central role of P53 in the modulation of amino acid and lipid metabolisms related to OxPhos (Simabuco et al., 2018). Conversely, cells expressing P53 mutant proteins (p53^{K120R}, p53^{3KR}, and p53^{R273H}) are characterized by a lower activation of these two metabolic pathways with respect to p53^{WT}, despite maintaining a higher activity than the empty control. In detail, p53^{3KR} displays lower enzymatic activities compared to p53^{K120R} carrying cells, which, in turn, appear slightly but significantly lower than those observed in p53^{WT} cells. These results are in line with the decreasing efficiency of aerobic metabolism and the need to increase glucose consumption and anaerobic glycolysis in cells expressing these P53 mutant proteins since both the conversion of glutamine to alpha-ketoglutarate and beta-oxidation are two metabolisms that provide substrates for the Krebs cycle.

To evaluate the energy metabolism modulation during an oxidative insult, we used hydrogen peroxide (H_2O_2) as pro-oxidative stress. The addition of H_2O_2 determines an increase of OCR values independently from the substrate in empty vector, p53^{K120R}, and p53^{R273H} expressing cells compared to untreated cells but a decrease in cells with p53^{3KR} mutant (Figure 5A); only p53^{WT} expressing cells show a stable OCR. However, the described modulation of the oxygen consumption does not correspond to the ATP synthase activity, that shows a general

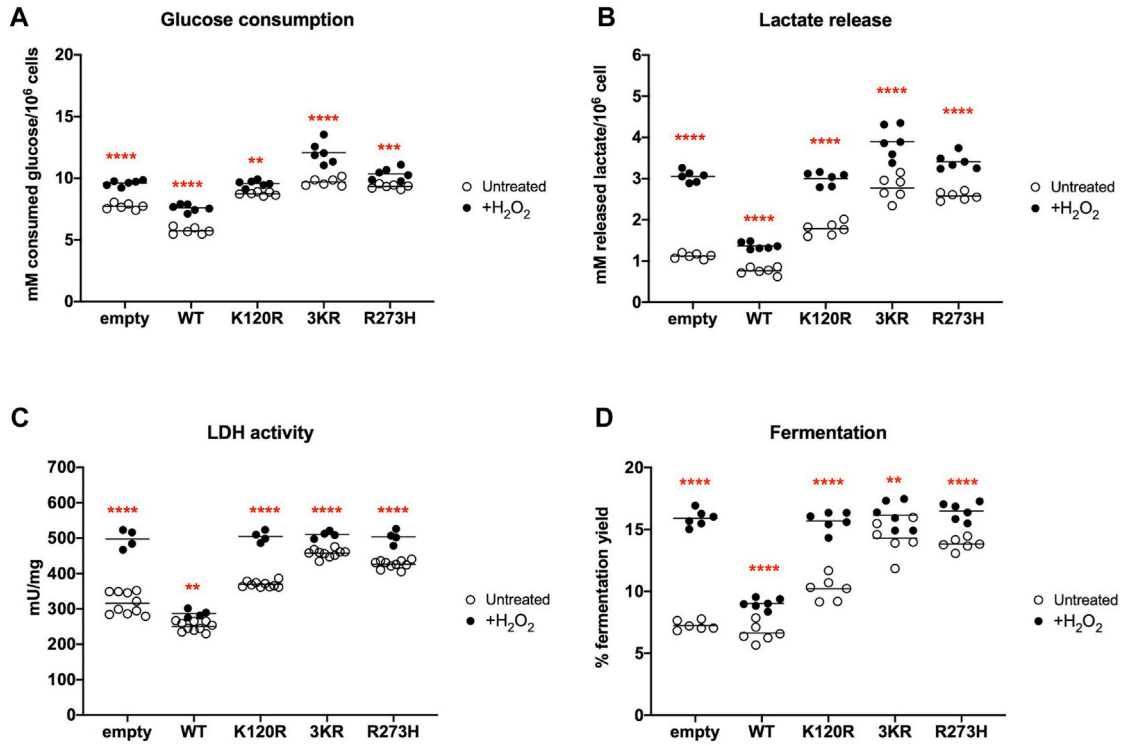

FIGURE 6
Comparison of anaerobic metabolism in H_2O_2 treated HCT116$^{TP53-/-}$ cells with untreated cells expressing p53^{WT}, p53^{3KR}, p53^{K120R}, and p53^{R273H} proteins. **(A)** Glucose consumption in untreated (empty dots) and 100 μM H_2O_2 treated (black dots) cells. **(B)** Lactate release in the medium in the same condition as above. **(C)** LDH activity in the same condition as above. **(D)** Percentage of lactic acid fermentation yield in the same condition as above. Each panel is representative of at least four independent experiments and data are reported as mean. The red symbols ** and **** indicate significant differences for $p \leq 0.0031$ and $p < 0.0001$, respectively, between the untreated and 100 μM H_2O_2 treated samples. In each panel, the empty dots represent the data from untreated samples depicted in Figure 4.

decrease in all samples (Figure 5B). However, these last results highlight again the highest capacity of p53^{WT} expressing cells to synthesize ATP, even under oxidative stress conditions. Consequently, a general decrement of OxPhos efficiency in H_2O_2 treated compared to untreated samples (Figure 5C) is evident as demonstrated by the P/O values, being this decrease less significant for p53^{3KR} expressing cells; again, the presence of p53^{WT} ensures a higher OxPhos efficiency with respect to the comparable values of P/O from samples expressing P53 mutant proteins.

This further uncoupling between the OCR and the ATP production leads to a general significant increase in glucose consumption, lactate release, LDH activity, and lactic acid fermentation yield in all H_2O_2 treated cells (Figures 6A–D). Notably, the p53^{3KR} cells seem to have almost reached the maximum of fermentation capacity in untreated conditions (Figure 6D). Following H_2O_2 treatment the presence of all three types of P53 mutant proteins causes a higher fermentation yield than the presence of the p53^{WT} protein, indicating a comparable altered metabolic phenotype in the presence of a pro-oxidative stress (Figure 6D).

The human p53^{K120R}, p53^{3KR} and p53^{R273H} mutants cause an increment of oxidative stress

The aerobic metabolism is associated with the production of ROS, which increases in uncoupled conditions (Cadenas and Davies, 2000; Turrens, 2003; Ravera et al., 2021). We measured the induction of ROS and the level of GSH in untreated HCT116$^{TP53-/-}$ cells transiently expressing p53^{WT}, p53^{3KR}, p53^{K120R} and p53^{R273H} (Supplementary Figure S6). As reported in Supplementary Figure S6A, cells expressing p53^{WT}, p53^{K120R}, p53^{3KR}, and p53^{R273H} displayed an increased ROS production compared to control cells, more evident in presence of mutant proteins than wild type P53 protein. However, while the oxidative stress in cell expressing p53^{WT} is associated with the increment of efficient OxPhos activity (Figure 3), the ROS production in P53 mutant proteins expressing cells depends on the uncoupling between the OCR and the ATP synthesis. The intracellular level of GSH, a marker of oxidative stress scavenger, was higher compared to the empty cells only in presence of wild type P53 protein (Supplementary Figure

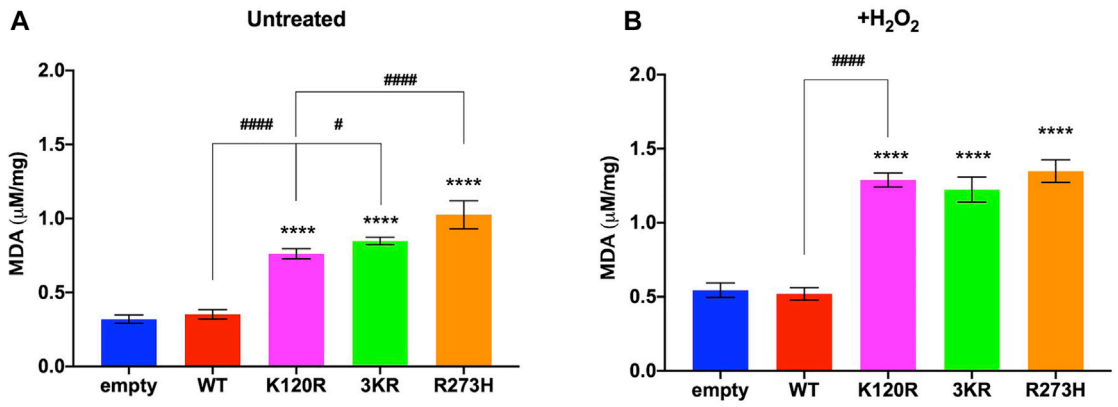

FIGURE 7
Evaluation of oxidative stress in untreated and H_2O_2 treated HCT$^{116TP53-/-}$ cells expressing p53^{WT}, p53^{3KR}, p53^{K120R}, and p53^{R273H} proteins. **(A)** MDA level in untreated cells. **(B)** MDA level in 100 μM H_2O_2 treated cells. The panels are representative of at least four independent experiments and data are reported as mean ± SD. The symbol **** indicates significant differences for $p < 0.0001$ between empty and the other samples; the symbols # and #### indicate significant differences for $p = 0.0445$ and $p < 0.0001$, respectively, between p53^{K120R} and the indicated samples.

S6B). Interestingly, the ROS/GSH ratio, a value that indicates the balance between oxidative stress and antioxidant defence, increased from the p53^{K120R} to p53^{R273H} cells, with the p53^{K120R} expressing cells having the lowest value (Supplementary Figure S6C). This indicate that among the P53 mutants, the p53^{K120R} possess the best antioxidant defence, although less efficient than the p53^{WT}.

Subsequently, the MDA concentration, a marker of lipid peroxidation, was evaluated to test whether the metabolic changes associated with the expression of p53^{K120R}, p53^{3KR}, and p53^{R273H} mutant proteins cause an increase of cellular oxidative stress compared to the presence of p53^{WT}.

In both untreated and H_2O_2 treated conditions, we observed an increase of MDA in P53 mutants but not in p53^{WT} expressing cells with respect to cells transfected with the empty vector (Figure 7A, B), although more pronounced following the pro-oxidative stress. This suggests that only the presence of p53^{WT} protein can counteract the oxidative stress to avoid basal and induced oxidative damages. Interestingly, in untreated conditions p53^{K120R} expressing cells show the lowest MDA accumulation among samples carrying mutant P53 proteins. These results are in keeping with the ROS/GSH ratio reported in Supplementary Figure S7 for untreated cells and confirm the lipid peroxidation as a reliable marker of the antioxidant defence capacity in this cell model.

To evaluate whether cells carrying p53^{K120R}, p53^{3KR}, and p53^{R273H} mutant proteins activated the antioxidant response to counteract the increment of oxidative stress associated to the metabolic changes, the expression of G6PD and xCT proteins was assessed (Figure 8). Specifically, G6PD is the first enzyme of the pentose phosphate pathway, providing the NADPH (reduced nicotinamide adenine dinucleotide phosphate) necessary for the GSSG (glutathione disulfide) reduction, and xCT is one of the principal transporters for cystine, a key component of GSH (Clemons et al., 2017; Yang et al., 2019). Expression of G6PD and xCT has been identified under P53 modulation with different mechanisms (Jiang et al., 2015, 2011; Xie et al., 2017). In addition, although not direct targets of P53, the level of GCLM and GCLC were measured since they are important player in the synthesis of GSH (Lu, 2013).

In untreated cells, only a significant induction of xCT expression in p53^{WT} expressing cells is measured (1.5-fold). No other significant P53-dependent modulations are evident with exception of a downregulation of GCLM level in p53^{K120R} carrying cells with respect to empty sample (Figures 8A,B). Following H_2O_2 treatment, a significant increase in xCT expression is associated to every condition (p53^{WT}, p53^{K120R}, p53^{3KR} and p53^{R273H}). Moreover, while p53^{WT} cells are characterized also by an upregulation of GCLC, p53^{3KR} cells show a significant induction of all analyzed proteins (Figures 8A,B). Lastly, when treated cells are compared to untreated, p53^{WT} and p53^{3KR} expressing cells show a significant increment of GCLM and xCT, respectively (Figures 8A,B).

The xCT increase is likely independent from change in level of NRF2 transcription factor, a known modulator of xCT, since its expression, although slightly increases following H_2O_2 treatment, does not significantly change in any transfection and treatment conditions (Supplementary Figure S7).

Lastly, the level of GPX4, known to be a marker of ferroptosis, was also measured to specifically measure a possible modulation of its level in P53^{WT}, P53^{K120R}, P53^{3KR} and P53^{R273H} expressing cells before and after treatment with H_2O_2. As reported in Supplementary Figure S8, in these treatment conditions the modulation of GPX4 was comparable in P53^{K120R} and P53^{WT} carrying cells. Slightly lower, although not significant, level of GPX4 was found in P53^{3KR} expressing cells.

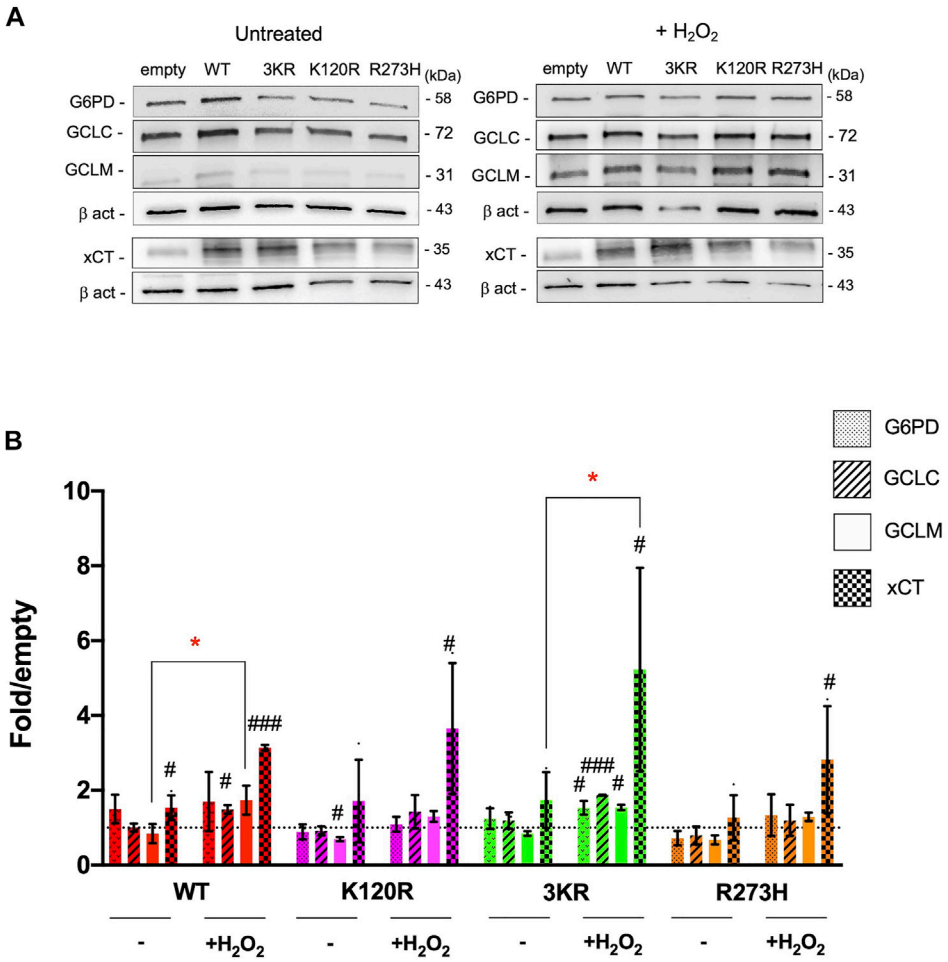

FIGURE 8
Modulation of proteins involved in the antioxidant response by $p53^{WT}$, $p53^{3KR}$, $p53^{K120R}$, and $p53^{R273H}$ proteins in untreated and H_2O_2 treated $HCT116^{TP53-/-}$ cells. **(A)** Representative western blots showing the level of G6PD, GCLC, GCLM, xCT and Beta-actin (β-act) endogenous proteins in untreated and 100 μM H_2O_2 treated $HCT116^{TP53-/-}$ cells transiently expressing $p53^{WT}$, $p53^{K120R}$, $p53^{3KR}$, and $p53^{R273H}$. **(B)** Histograms representing the amount of G6PD, GCLC, GCLM, and xCT proteins detected in $HCT116^{TP53-/-}$ transfected cells in untreated or treated samples and normalized for β-act. Data were obtained after chemiluminescence analysis of western blots from at least two independent experiments and are reported as mean ± SD. The levels of the different proteins were calculated as fold over the level of the same proteins found in cells transfected with the empty vector (#$p < 0.05$; ##$p < 0.005$; ###$p < 0.0005$; t Student); the dotted line corresponds to one-fold over empty. The red symbol * indicates a significant difference for $p \leq 0.0295$ between the H_2O_2 treated and the indicated untreated samples.

All together these results could indicate that, especially in the $p53^{3KR}$ expressing cells, an antioxidant response is activated with the tentative to balance the increment of oxidative stress. However, this response is not sufficient to avoid the lipid peroxidation as indicated by the previously observed MDA accumulation.

Discussion

In this paper, we investigated the property of human $p53^{K120R}$ mutant protein, corresponding to the $p53^{K117R}$ in mouse, in the regulation of metabolism by analyzing its transcriptional ability in yeast and mammalian reporter assays, the metabolic phenotype in $HCT116^{TP53-/-}$ cells expressing the mutant protein and the corresponding induction of P53 targets and proteins involved in the antioxidant response, before and after a pro-oxidative challenge (i.e., H_2O_2). In addition, the human P53 triple mutant ($p53^{3KR}$), that resembles the mouse mutant carrying Lysine to Arginine missense mutations at the three acetylation sites of the DBD (Li et al., 2012; Liu et al., 2019; Xia et al., 2022), and the well-known LoF and GoF $p53^{R273H}$ mutant, were studied. The properties of P53 mutants were compared to $p53^{WT}$, whose contribution in metabolism regulation has been recently demonstrated (Lacroix et al., 2020; Liu and Gu, 2022, 2021)

In yeast and mammalian reporter assays $p53^{K120R}$ protein is able to transactivate the *P21* and *MDM2* REs (yeast) or promoters (mammalian cells) but poorly or partially *BAX*, *KILLER*, *AIP1*, *NOXA* targets, confirming its transcriptional specificity (Figure 1); indeed, $p53^{K120R}$ has been previously characterized for its ability to induce cell cycle arrest targets and for its deficiency in activation of apoptosis genes (Sykes et al., 2006; Tang et al., 2006). The $p53^{3KR}$ mutant loses transactivation activity with respect to $p53^{K120R}$, but shows a behavior more similar to the $p53^{R273H}$ mutant in HCT116$^{TP53-/-}$ cells, where it decreases its activity on all promoters. The results obtained in reporter assays are in keeping with the expression of the same endogenous P53 target proteins (P21, MDM2, BAX) in HCT116$^{TP53-/-}$ cells transfected with $p53^{WT}$, $p53^{K120R}$, $p53^{3KR}$ and $p53^{R273H}$ proteins (Figure 2). Moreover, $p53^{K120R}$ is not able to induce the endogenous *TIGAR*; however, since only a 1.8-fold increase of TIGAR protein is detected in HCT116 cells by $p53^{WT}$ (Figure 2) and approximately a 50% of the wild type transcriptional activity is maintained by $p53^{K120R}$ on *TIGAR* RE in the functional yeast assay (Figure 1), we might speculate that the induction of TIGAR protein in human cells expressing the $p53^{K120R}$ mutant is undetectable.

The induction of TIGAR by $p53^{WT}$ can contribute to reduce the rate of glycolysis and to regulate ROS at cellular level (Bensaad et al., 2006). Consequently, the partial ability of $p53^{K120R}$ mutant to activate the *TIGAR* RE, as observed in the yeast functional assay, could have an impact on glucose metabolism measured in HCT116$^{TP53-/-}$ transfected cells. Our results show that although all mutant P53 proteins carrying cells switch from aerobic to anaerobic metabolism (Figures 3, 4), the $p53^{K120R}$ mutant triggers a lower percentage of fermentation with respect to $p53^{3KR}$ and $p53^{R273H}$. This suggests that $p53^{K120R}$ still conserves some capacity to modulate glucose metabolism and limit the fermentation yield, although such peculiar features are lost following the treatment with H_2O_2, as highlighted by the similar behavior of all P53 mutant proteins in pro-oxidant conditions (Figures 5, 6).

MDA level is known as a marker of oxidative stress, which increases when mitochondria are in uncoupled conditions (Cadenas and Davies, 2000; Turrens, 2003; Ravera et al., 2021). Interestingly, $p53^{K120R}$ expressing cells show the lowest MDA accumulation within samples carrying P53 mutant proteins in untreated conditions (Figure 7), suggesting again a partial capacity by $p53^{K120R}$ to counteract the oxidative stress to avoid oxidative damages. As expected, $p53^{WT}$ cells are characterized by a lower level of MDA with respect to $p53^{K120R}$, $p53^{3KR}$, and $p53^{R273H}$ cells both in untreated and treated conditions (Figure 7).

The increment of cellular oxidative stress in presence of all type of P53 mutant proteins is evident despite an increase in expression of proteins involved in antioxidant response (i.e., G6PD, GCLC, GCLM and xCT) (Figure 8), suggesting that cells expressing P53 mutant proteins, especially the $p53^{3KR}$, are able to sense the oxidant environment without being able to really counteract the oxidative stress, differently from $p53^{WT}$ cells. In $p53^{3KR}$ cells, the activation of an antioxidant response can also be inferred by the significant increase of G6PD, GCLC, GCLM and xCT not detected in other P53 mutant environment (Figure 8). Thus, the antioxidant response can be activated in $p53^{3KR}$ carrying cells, but it does not go to completion.

We evaluated the activity of the $p53^{K120R}$ mutant also in comparison to the human triple $p53^{3KR}$ mutant. Li and others, by using a mouse model found that the mouse $p53^{3KR}$ protein failed to activate P53 target genes of cell cycle arrest or apoptosis, but it retained the ability to regulate metabolism as $p53^{WT}$ by repressing *xCT*, coding for the main transporter for cystine (Jiang et al., 2015; Liu et al., 2019; Liu and Gu, 2022, 2021; Xia et al., 2022). Our data, while confirming the ability of $p53^{K120R}$ and the inability of $p53^{3KR}$ mutant to induce P21 and MDM2 at protein level (Figure 2), show a significant induction of xCT protein level by $p53^{K120R}$ and $p53^{3KR}$ after the oxidative challenge (Figure 8).

The NRF2 transcription factor is one of the main regulators of the genes involved in the antioxidant response, including *xCT*. In breast cancer cells, the NRF2-dependent transcription of *xCT* can be inhibited by a direct interaction of NRF2 with mutant P53 proteins (Lisek et al., 2018); as a consequence, breast tumor cells with a P53 mutant protein may present low levels of *xCT* expression, resulting in low GSH and high ROS levels (Liu et al., 2017). In the HCT116$^{TP53-/-}$ cellular background it was shown that also the wild type P53 protein suppresses the transcription of *xCT* by NRF2 (Faraonio et al., 2006). Taking these observations into consideration, we argue that in our experimental system neither wild type nor mutant P53 proteins interfere with NRF2, thus determining high levels of xCT protein. Moreover, NRF2 protein level did not change in any transfection and treatment conditions (Supplementary Figure S7), suggesting a NRF2-independent regulation of xCT in these experimental conditions.

Interestingly, we observe an induction of xCT in presence of $p53^{WT}$ protein both in untreated and H_2O_2 treated cells. Although the repressing activity of P53 on SLC7A11 expression has been reported in U2OS and MCF7 human cell lines (Jiang et al., 2015), in HCT116$^{TP53-/-}$ colon cancer cells P53 stimulates, instead of repressing, SLC7A11 expression. Our observation finds a confirmation in a recent paper by Xie et al. (2017), where $p53^{WT}$ protein mediate xCT expression in HCT116$^{TP53-/-}$ cells. In these cells, P53 wild type by interacting with the dipeptidyl-peptidase-4 (DDP4) protein, involved in lipid peroxidation, favors its nuclear localization; inside the nucleus it was supposed that DDP4 acts as P53 transcription cofactor, stimulating xCT expression (Xie et al., 2017). Since we observed an induction of xCT following H_2O_2 in HCT116$^{TP53-/-}$ cells with different P53 backgrounds, we can hypothesize an involvement of this

pathway in the modulation of the antioxidant response in HCT116 transfected cells. Thus, the activation of SLC7A11 by P53 can be considered tissue/cell line-dependent and not in contradiction with results obtained in other cell lines. This observation is in keeping with data reported in Eriksson et al., 2017 where cancer cell type specific metabolic functions of mutant P53 have been postulated (Eriksson et al., 2017). It has to be underlined that, even though we reported an increase of SLC7A11 protein following H_2O_2, the antioxidant response in mutant P53 carrying cells remains defective. Thus, our results indicate that, especially in the $p53^{3KR}$ cells, an antioxidant response is activated with the tentative to balance the increment of oxidative stress, but this response is not sufficient to avoid the lipid peroxidation, as indicated by the MDA load.

The effect of expression of different types of P53 mutant proteins on glycolysis and mitochondrial metabolism has been previously addressed (Eriksson et al., 2017). By analyzing the metabolic features of HCT116 and H1299 cells transfected with different *TP53* hot spot mutations, metabolic heterogeneity was observed based on the type and the stability of mutant P53 proteins; in general, it was observed that the OxPhos activity resulted altered in presence of most P53 mutant proteins compared to the control cells, while the ECAR (Extra Cellular Acidification Rate), a surrogate measure of lactate export, appeared significantly enhanced, suggesting a metabolic switch to the anaerobic energy metabolism. Specifically, the inducible expression of $p53^{R237H}$ caused the increment of both glycolytic capacity and OCR, in agreement with our data.

In conclusion, our results underline that not all P53 mutant proteins can be considered metabolically equivalent. We demonstrated that cells carrying $p53^{K120R}$ are characterized by a metabolic phenotype that is intermediate between $p53^{WT}$ and $p53^{R273H}$ expressing cells, indicating a partial activity by the peculiar *TP53* K120R cancer mutation in modulation of cellular metabolism. On the other hand, $p53^{R273H}$ cells showed the most evident metabolic change, which could compromise the cellular environment in agreement with its LoF and GoF activities. Such diversity can rely not only on P53 transcriptional specificity, as emerging from our results, but also from the interconnection with the cellular environment in which the P53 mutant protein can operate.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material further inquiries can be directed to the corresponding author.

Author contributions

GF, PM, and PMo conceived and designed the study. PMo, PM , AS, SR, GF GFo, IV, and PD performed the experiments. SR and PD supervised the metabolic experiments. PMo, PM, and SR cured the figures. GF, PM, PMo, and SR wrote the original draft. GF, PM, PMo, and SR revised and edited the manuscript. PMo and PM supervised the whole manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

Bensaad, K., Tsuruta, A., Selak, M. A., Vidal, M. N. C., Nakano, K., Bartrons, R., et al. (2006). TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* 126, 107–120. doi:10.1016/j.cell.2006.05.036

Bisio, A., Ciribilli, Y., Fronza, G., Inga, A., and Monti, P. (2014). *TP53* mutants in the tower of babel of cancer progression. *Hum. Mutat.* 35, 689–701. doi:10.1002/humu.22514

Cadenas, E., and Davies, K. J. A. (2000). Mitochondrial free radical generation, oxidative stress, and aging11This article is dedicated to the memory of our dear friend, colleague, and mentor Lars Ernster (1920-1998), in gratitude for all he gave to us. *Free Radic. Biol. Med.* 29, 222–230. doi:10.1016/S0891-5849(00)00317-8

Cappelli, E., Cuccarolo, P., Stroppiana, G., Miano, M., Bottega, R., Cossu, V., et al. (2017). Defects in mitochondrial energetic function compels Fanconi Anaemia cells to glycolytic metabolism. *Biochimica Biophysica Acta (BBA) - Mol. Basis Dis.* 1863, 1214–1221. doi:10.1016/j.bbadis.2017.03.008

Cappelli, E., Degan, P., Bruno, S., Pierri, F., Miano, M., Raggi, F., et al. (2020). The passage from bone marrow niche to bloodstream triggers the metabolic impairment in Fanconi Anemia mononuclear cells. *Redox Biol.* 36, 101618. doi:10.1016/j.redox.2020.101618

Clemons, N. J., Liu, D. S., Duong, C. P., and Phillips, W. A. (2017). Inhibiting system xC− and glutathione biosynthesis - a potential Achilles' heel in mutant-p53 cancers. *Mol. Cell. Oncol.* 4, e1344757. doi:10.1080/23723556.2017.1344757

Colla, R., Izzotti, A., De Ciucis, C., Fenoglio, D., Ravera, S., Speciale, A., et al. (2016). Glutathione-mediated antioxidant response and aerobic metabolism: Two crucial factors involved in determining the multi-drug resistance of high-risk neuroblastoma. *Oncotarget* 7, 70715–70737. doi:10.18632/oncotarget.12209

Eriksson, M., Ambroise, G., Ouchida, A. T., Lima Queiroz, A., Smith, D., Gimenez-Cassina, A., et al. (2017). Effect of mutant p53 proteins on glycolysis and mitochondrial metabolism. *Mol. Cell Biol.* 37, 003288–e417. doi:10.1128/MCB.00328-17

Faraonio, R., Vergara, P., Di Marzo, D., Pierantoni, M. G., Napolitano, M., Russo, T., et al. (2006). p53 suppresses the Nrf2-dependent transcription of antioxidant response genes. *J. Biol. Chem.* 281, 39776–39784. doi:10.1074/jbc.M605707200

Foggetti, G., Ottaggio, L., Russo, D., Mazzitelli, C., Monti, P., Degan, P., et al. (2019). Autophagy induced by SAHA affects mutant P53 degradation and cancer cell survival. *Biosci. Rep.* 39. doi:10.1042/BSR20181345

Hinkle, P. C. (2005). P/O ratios of mitochondrial oxidative phosphorylation. *Biochimica Biophysica Acta (BBA) - Bioenergetics* 1706, 1–11. doi:10.1016/j.bbabio.2004.09.004

Inga, A., Storici, F., Darden, T. A., and Resnick, M. A. (2002). Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. *Mol. Cell Biol.* 22, 8612–8625. doi:10.1128/MCB.22.24.8612-8625.2002

Jiang, L., Kon, N., Li, T., Wang, S.-J., Su, T., Hibshoosh, H., et al. (2015). Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520, 57–62. doi:10.1038/nature14344

Jiang, P., Du, W., Wang, X., Mancuso, A., Gao, X., Wu, M., et al. (2011). p53 regulates biosynthesis through direct inactivation of glucose-6-phosphate dehydrogenase. *Nat. Cell Biol.* 13, 310–316. doi:10.1038/ncb2172

Koppula, P., Zhuang, L., and Gan, B. (2021). Cystine transporter slc7a11/xCT in cancer: Ferroptosis, nutrient dependency, and cancer therapy. *Protein Cell* 12, 599–620. doi:10.1007/s13238-020-00789-5

Lacroix, M., Riscal, R., Arena, G., Linares, L. K., and Le Cam, L. (2020). Metabolic functions of the tumor suppressor p53: Implications in normal physiology, metabolic disorders, and cancer. *Mol. Metab.* 33, 2–22. doi:10.1016/j.molmet.2019.10.002

Leroy, B., Fournier, J. L., Ishioka, C., Monti, P., Inga, A., Fronza, G., et al. (2013). The *TP53* website: An integrative resource centre for the *TP53* mutation database and *TP53* mutant analysis. *Nucleic Acids Res.* 41, D962–D969. doi:10.1093/nar/gks1033

Li, T., Kon, N., Jiang, L., Tan, M., Ludwig, T., Zhao, Y., et al. (2012). Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. *Cell* 149, 1269–1283. doi:10.1016/j.cell.2012.04.026

Lisek, K., Campaner, E., Ciani, Y., Walerych, D., and Del Sal, G. (2018). Mutant p53 tunes the NRF2-dependent antioxidant response to support survival of cancer cells. *Oncotarget* 9, 20508–20523. doi:10.18632/oncotarget.24974

Liu, D. S., Duong, C. P., Haupt, S., Montgomery, K. G., House, C. M., Azar, W. J., et al. (2017). Inhibiting the system xC−/glutathione axis selectively targets cancers with mutant-p53 accumulation. *Nat. Commun.* 8, 14844. doi:10.1038/ncomms14844

Liu, Y., and Gu, W. (2022). p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell Death Differ.* 29, 895–910. doi:10.1038/s41418-022-00943-y

Liu, Y., and Gu, W. (2021). The complexity of p53-mediated metabolic regulation in tumor suppression. *Seminars Cancer Biol.* Epub ahead of print doi:10.1016/j.semcancer.2021.03.010

Liu, Y., Tavana, O., and Gu, W. (2019). p53 modifications: exquisite decorations of the powerful guardian. *J. Mol. Cell Biol.* 11, 564–577. doi:10.1093/jmcb/mjz060

Lu, S. C. (2013). Glutathione synthesis. *Biochimica Biophysica Acta (BBA) - General Subj.* 1830, 3143–3153. doi:10.1016/j.bbagen.2012.09.008

Marengo, B., Monti, P., Miele, M., Menichini, P., Ottaggio, L., Foggetti, G., et al. (2018). Etoposide-resistance in a neuroblastoma model cell line is associated with 13q14.3 mono-allelic deletion and miRNA-15a/16-1 down-regulation. *Sci. Rep.* 8, 13762. doi:10.1038/s41598-018-32195-7

Monteith, J. A., Mellert, H., Sammons, M. A., Kuswanto, L. A., Sykes, S. M., Resnick-Silverman, L., et al. (2016). A rare DNA contact mutation in cancer confers p53 gain-of-function and tumor cell survival via TNFAIP8 induction. *Mol. Oncol.* 10, 1207–1220. doi:10.1016/j.molonc.2016.05.007

Monti, P., Ciribilli, Y., Foggetti, G., Menichini, P., Bisio, A., Cappato, S., et al. (2019). P63 modulates the expression of the WDFY2 gene which is implicated in cancer regulation and limb development. *Biosci. Rep.* 39, BSR20192114. doi:10.1042/BSR20192114

Monti, P., Lionetti, M., De Luca, G., Menichini, P., Recchia, A. G., Matis, S., et al. (2020a). Time to first treatment and P53 dysfunction in chronic lymphocytic leukaemia: Results of the O-CLL1 study in early stage patients. *Sci. Rep.* 10, 18427. doi:10.1038/s41598-020-75364-3

Monti, P., Menichini, P., Speciale, A., Cutrona, G., Fais, F., Taiana, E., et al. (2020b). Heterogeneity of *TP53* mutations and P53 protein residual function in cancer: Does it matter? *Front. Oncol.* 10, 593383. doi:10.3389/fonc.2020.593383

Prochownik, E. V., and Wang, H. (2021). The metabolic fates of pyruvate in normal and neoplastic cells. *Cells* 10, 762. doi:10.3390/cells10040762

Ravera, S., Vigliarolo, T., Bruno, S., Morandi, F., Marimpietri, D., Sabatini, F., et al. (2021). Identification of biochemical and molecular markers of early aging in childhood cancer survivors. *Cancers* 13, 5214. doi:10.3390/cancers13205214

Resnick, M. A., and Inga, A. (2003). Functional mutants of the sequence-specific transcription factor p53 and implications for master genes of diversity. *Proc. Natl. Acad. Sci. U.S.A.* 100, 9934–9939. doi:10.1073/pnas.1633803100

Simabuco, F. M., Morale, M. G., Pavan, I. C. B., Morelli, A. P., Silva, F. R., and Tamura, R. E. (2018). p53 and metabolism: from mechanism to therapeutics. *Oncotarget* 9, 23780–23823. doi:10.18632/oncotarget.25267

Sykes, S. M., Mellert, H. S., Holbert, M. A., Li, K., Marmorstein, R., Lane, W. S., et al. (2006). Acetylation of the p53 DNA-binding domain regulates apoptosis induction. *Mol. Cell* 24, 841–851. doi:10.1016/j.molcel.2006.11.026

Tang, Y., Luo, J., Zhang, W., and Gu, W. (2006). Tip60-Dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol. Cell* 24, 827–839. doi:10.1016/j.molcel.2006.11.021

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *J. Physiology* 552, 335–344. doi:10.1113/jphysiol.2003.049478

Xia, Z., Kon, N., Gu, A. P., Tavana, O., and Gu, W. (2022). Deciphering the acetylation code of p53 in transcription regulation and tumor suppression. *Oncogene* 41, 3039–3050. doi:10.1038/s41388-022-02331-9

Xie, Y., Zhu, S., Song, X., Sun, X., Fan, Y., Liu, J., et al. (2017). The tumor suppressor p53 limits ferroptosis by blocking DPP4 activity. *Cell Rep.* 20, 1692–1704. doi:10.1016/j.celrep.2017.07.055

Yang, W., Wu, Y., Yen, C., Liu, H. Y., Hwang, T. L., Stern, A., et al. (2019). The redox role of G6PD in cell growth, cell death, and cancer. *Cells* 8, 1055. doi:10.3390/cells8091055

Yang, W. S., and Stockwell, B. R. (2016). Ferroptosis: Death by lipid peroxidation. *Trends Cell Biol.* 26, 165–176. doi:10.1016/j.tcb.2015.10.014

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Ferroptosis in lymphoma: Emerging mechanisms and a novel therapeutic approach

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Unlike apoptosis, necroptosis, autophagy, and pyroptosis, ferroptosis represents a new type of cell death, which is characterized by iron-dependent lipid peroxidation. This process relies largely on the metabolite reactive oxygen species (ROS), phospholipids containing polyunsaturated fatty acids (PUFA-PL), transition metal iron, intra-, and intercellular signaling events, and environmental stress that regulate cellular metabolism and ROS levels. Recent studies show that ferroptosis plays an important role in tumorigenesis, tumor development, and the treatment of hematological malignancies, including lymphoma. Despite the constant emergence of new drugs, the differences in morphological features, immunophenotypes, biological patterns, rates of onset, and response to treatment in lymphoma pose major therapeutic challenges. Since lymphoma is associated with ferroptosis and shows sensitivity towards it, targeting the potential regulatory factors may regulate lymphoma progression. This has emerged as a research hotspot. This review summarizes the current knowledge on ferroptosis induction and resistance mechanisms, their roles and mechanistic details of ferroptosis in lymphoma suppression and immunity, and finally the treatment strategies for lymphoma by targeting ferroptosis.

KEYWORDS

ferroptosis, iron metabolism, lipid Metabolism, reactive oxygen species (ROS), lymphoma, therapeutic applications, ferroptosis inducer cell death

1 Introduction

Cell death, including apoptosis (Nagata, 2018), NETosis (Segura et al., 2020), necroptosis (Guo et al., 2019), autophagy (Bonam et al., 2018), pyroptosis (Fu et al., 2017), and cuproptosis (Tsvetkov et al., 2022), is an important physiological process that maintains the integrity by regulating organismal metabolism and removing excess or damaged cells.

Ferroptosis, unlike other forms, is a new type of programmed cell death that shows iron-dependence (Li et al., 2020). Several tumor suppressors, including p53 and BAP1, promote ferroptosis by inhibiting cystine uptake, confirming that ferroptosis is a natural barrier against tumor development (Toyokuni et al., 2017; Zhang et al., 2018). Moreover, tumor cells are more prone to ferroptosis due to their unique metabolic program and high load of reactive oxygen species (ROS) (Chen et al., 2020a), rendering ferroptosis inducers as potential targets of cancer therapy, especially against some of the most drug-resistant and aggressive tumors (Hangauer et al., 2017; Viswanathan et al., 2017). Non-Hodgkin's lymphomas are clinically heterogeneous, and different subtypes show varying prognoses; even in the same type, some cases are curable with combination therapy, while for the remaining, treatment is difficult and patients exhibit poor prognosis (Chapuy et al., 2018). Diffuse large B cell lymphoma (DLBCL), adult T-cell leukemia/lymphoma (ATLL), and Burkitt's lymphoma (BL) are particularly sensitive to cell death by ferroptosis as shown in recent studies (Yang et al., 2014; Mancuso et al., 2021; Chen et al., 2022). Thus, treatment with ferroptosis inducers may be a promising therapeutic strategy for lymphoma in the foreseeable future.

2 Mechanisms underlying ferroptosis

Ferroptosis is driven by increased cellular iron load and is characterized by the loss in membrane integrity and changes in mitochondrial ultrastructure, including reduced mitochondrial volume, increased mitochondrial bilayer membrane density, decreased mitochondrial cristae, and ruptured mitochondrial membrane (Doll et al., 2017; Du et al., 2022; Zhou et al., 2022). Ferroptosis can be induced through the interaction of iron regulation, lipid metabolism, and ROS biology, and research in these fields is expected to deepen the mechanistic understanding, biological significance, and clinical therapeutic relevance of ferroptosis (Table 1). However, to date, our understanding of ferroptosis remains incomplete, and the molecular mechanisms that trigger ferroptosis and its selective regulation under certain circumstances, are largely unclear (Davidson and Wood, 2020).

2.1 Role of iron metabolism in ferroptosis

Fe2+ first binds to transferrin (TF) in the intestine and is subsequently absorbed by intestinal mucosal cells, wherein Fe2+ is oxidized to Fe3+ by ceruloplasmin and other ferroxidases (Cronin et al., 2019). Fe^{3+} enters the capillaries and then binds to TF, which in turn binds to the TF receptor 1 (TfR1) and is endocytosed into cells (Pandrangi et al., 2022). Fe^{3+} is then reduced to Fe2+ by the metalloreductase, six-transmembrane epithelial antigen of the prostate (STEAP3), and subsequently, Fe^{2+} is released into the labile iron pool (LIP) *via* divalent metal transporter one or stored as ferritin, the iron-storage protein (Li et al., 2020). Excessive Fe2+ is oxidized to Fe^{3+} by the cellular iron exporter, ferroportin (FPN) (Li et al., 2021). There are two major

TABLE 1 Inducers and inhibitors of ferroptosis.

	Drugs or pathways	Mechanisms
Inducer	ATM, ferritinophagy	Regulate ferritin abundance
	FINO2	Oxidize ferrous iron and lipidome, inactivate GPX4
	FIN56	Degrade GPX4 and deplete antioxidant CoQ10
	RSL3, DPI7	inhibit GPX4
	HO-1	Supplement iron
	Erastin	Inhibit system Xc- and degrade GPX4
	CDO1	Deplete cysteine
	P53 protein	Supress SLC7A11
	MDR1	Cause efflux of GSH
Inhibitor	Ferroportin, MVBs	Deplete labile iron
	HSPB1	Inhibit TRF1
	FSP1/CoQ10/NADPH	Inhibit phospholipid peroxidation
	DHODH	Reduce mithchondrial CoQ10
	GCH1/BH4	Prevetn lipid peroxdation, deplete PUFA-PL
	Transsulfuration way	Produce cysteine from methionine
	mTOR	Increase GPX4 synthesiss
	P53-P21 pathway	Slow depletion of intracellular GSH and reduce accumulation of ROS

ATM, serine/threonine kinase; *RSL3,* RAS-selective-lethal-3; *HO-1*, Heme oxygenase-1; *CDO1,* cysteine dioxygenase1; *MDR1,* multidrug resistance gene; *MVBs*, multivesicular bodies; *HSPB1,* Heat shock protein beta-1; *DHODH*, dihydroorotate dehydrogenase; *GCH/BH4*, GTP cyclohydrolase 1/tetrahydrobiopterin; *mTOR*, mechanistic target of the rapamycin.

mechanisms by which iron ions regulate ferroptosis. First, Fe^{2+} can mediate the Fenton reaction to generate excess ROS, which subsequently reacts with polyunsaturated fatty acids (PUFAs) to promote lipid peroxidation, ultimately leading to ferroptosis (Shah et al., 2018). Second, some enzymes that mediate the formation of lipid hydroperoxides for the Fenton reaction require Fe^{2+} as a cofactor, including arachidonate lipoxygenases (ALOXs) (Li et al., 2021). The regulation of the iron-storage protein, ferritin, *via* ferritinophagy (Stockwell, 2022) and the serine/threonine kinase mutated in Ataxia-Telangiectasia (ATM) (Chen et al., 2020a) control the size of the LIP, which promotes labile iron accumulation and regulates cellular sensitivity to ferroptosis (Chen et al., 2020a; Stockwell, 2022). Ferroptosis-inducer-1,2-dioxolane (FINO2) induces ferroptosis by oxidizing Fe^{2+} to Fe^{3+} through the Fenton reaction to generate alkoxyl radical which initiates lipid peroxidation. Moreover, FINO2 can indirectly inactivate GPX4 by depleting glutathione (GSH) (Abrams et al., 2016). Fe^{2+} from the LIP binds to GSH(Patel et al., 2019) and its subsequent depletion promotes the availability of labile iron, which further mobilizes Fe^{2+} for the Fenton reaction, thereby promoting lipid peroxidation and ferroptosis (Patel et al., 2019). Heme oxygenase-1 (HO-1) can induce ferroptosis by promoting the accumulation of intracellular ferrous ions (Jing et al., 2022). Ferroportin and prominin-2-mediated ferritin-containing multivesicular bodies can deplete the LIP and induce resistance to ferroptosis (Brown et al., 2019). Overexpression of heat shock protein beta-1 can inhibit ferroptosis by downregulating TfR1-mediated iron uptake by stabilizing the F-actin cytoskeleton (Ren et al., 2020). Consequently, silencing the TFRC gene encoding TfR1 protein can inhibit ferroptosis (Song et al., 2021).

2.2 Lipid metabolism in ferroptosis

Ferroptosis is mainly driven by the peroxidation of specific membrane lipids. PUFAs are highly susceptible to ROS-induced peroxidation and are crucial to ferroptosis (Conrad et al., 2018). Free PUFAs cannot induce ferroptosis. After incorporation into membrane lipids, including phospholipids (PLs), the excessive accumulation of oxidized (PUFA-PLs) can contribute to ferroptosis (Xu et al., 2021). Lysophosphatidylcholine acyltransferase 3 and acyl-coenzyme A synthetase long-chain family member 4 (ACSL4) are involved in activating and incorporating PUFAs into PLs (Brown et al., 2019). Other ACSL enzymes, including ACSL1, are required to exert the pro-ferroptosis activity of conjugated linolenic acids (Beatty et al., 2021). Moreover, monounsaturated fatty acids (MUFAs), including oleic acid and palmitoleic acid, are not susceptible to peroxidation due to a lack of a bisallyl moiety (Magtanong et al., 2019). In turn, they require ACSL3 to displace the PUFA component from PUFA-PLs, thus exerting anti-ferroptosis effects (Magtanong et al., 2019). Activation of the liver kinase B1-AMP-activated protein kinase axis also plays a protective role against ferroptosis by limiting PUFA biosynthesis by inhibiting the phosphorylation of acetyl-CoA carboxylase (ACC) (Lee et al., 2020).

2.3 ROS accumulation in ferroptosis

Generation of ROS is a result of Fenton reactions and its accumulation can induce ferroptosis (Tang et al., 2021). The cytoplasmic and mitochondrial cystine/cysteine/GSH/GPX4 axis is a central regulator to protect cells from ferroptosis (Jiang et al., 2021). System Xc-is a heterodimeric 12-pass transmembrane cystine/glutamate antiporter comprising SLC3A2 and SLC7A11 subunits, which import cystine that is reduced to cysteine in the cell (Sato et al., 2018). Cysteine and glutamate are substrates for the biosynthesis of GSH, and GPX4 is a selenoprotein acting as a GSH-dependent peroxidase against lipid peroxidation by reducing the toxic lipid peroxide PL-PUFA-OOH to non-toxic PUFA-PL-OH (Ursini and Maiorino, 2020).

Substances that induce ferroptosis through the cystine/cysteine/GSH/GPX4 axis can be divided into four categories. Erastin is the prototype ferroptosis inducer that reduces cellular cystine uptake by directly inhibiting system Xc- and promotes the degradation of GPX4 by enhancing chaperone-mediated autophagy (Wu et al., 2019). Rat sarcoma-selective-lethal-3 (RSL3) and 5,6-dihydro-2H-pyrano [3,2-g]indolizine-7 can induce ferroptosis by directly inhibiting the activity of GPX4 (7). Ferroptosis-Inducer-56 (FIN56) can induce ferroptosis either by promoting GPX4 degradation or depleting endogenous antioxidant coenzyme Q10 (COQ10) (Liang et al., 2019). FINO2, as discussed above, can also initiate ferroptosis by indirectly inhibiting GPX4 (42). Other new molecules that induce ferroptosis have been recently discovered. The multidrug resistance gene, MDR1, increases cellular sensitivity to ferroptosis through GSH efflux (Cao et al., 2019), and the iron metalloenzyme, cysteine dioxygenase1, drives cell susceptibility to ferroptosis by depleting cysteine, which in turn reduces GSH (Hao et al., 2017).

GPX4 plays an important role in maintaining intracellular redox homeostasis and is the primary enzyme that inhibits ferroptosis. However, three other GPX4-independent systems also play a role in suppressing ferroptosis. The ferroptosis suppressor protein 1 (FSP1)/CoQ10/nicotinamide adenine dinucleotide phosphate (NADPH) pathway regulates ferroptosis independent of GPX4 and GSH. FSP1 catalyzes the formation of reduced CoQ10 in an NADPH-dependent manner, thereby reducing lipid free radicals and ultimately inhibiting lipid peroxidation and ferroptosis (Bersuker et al., 2019). Dihydroorotate dehydrogenase inhibits mitochondrial lipid peroxidation and ferroptosis by reducing mitochondrial

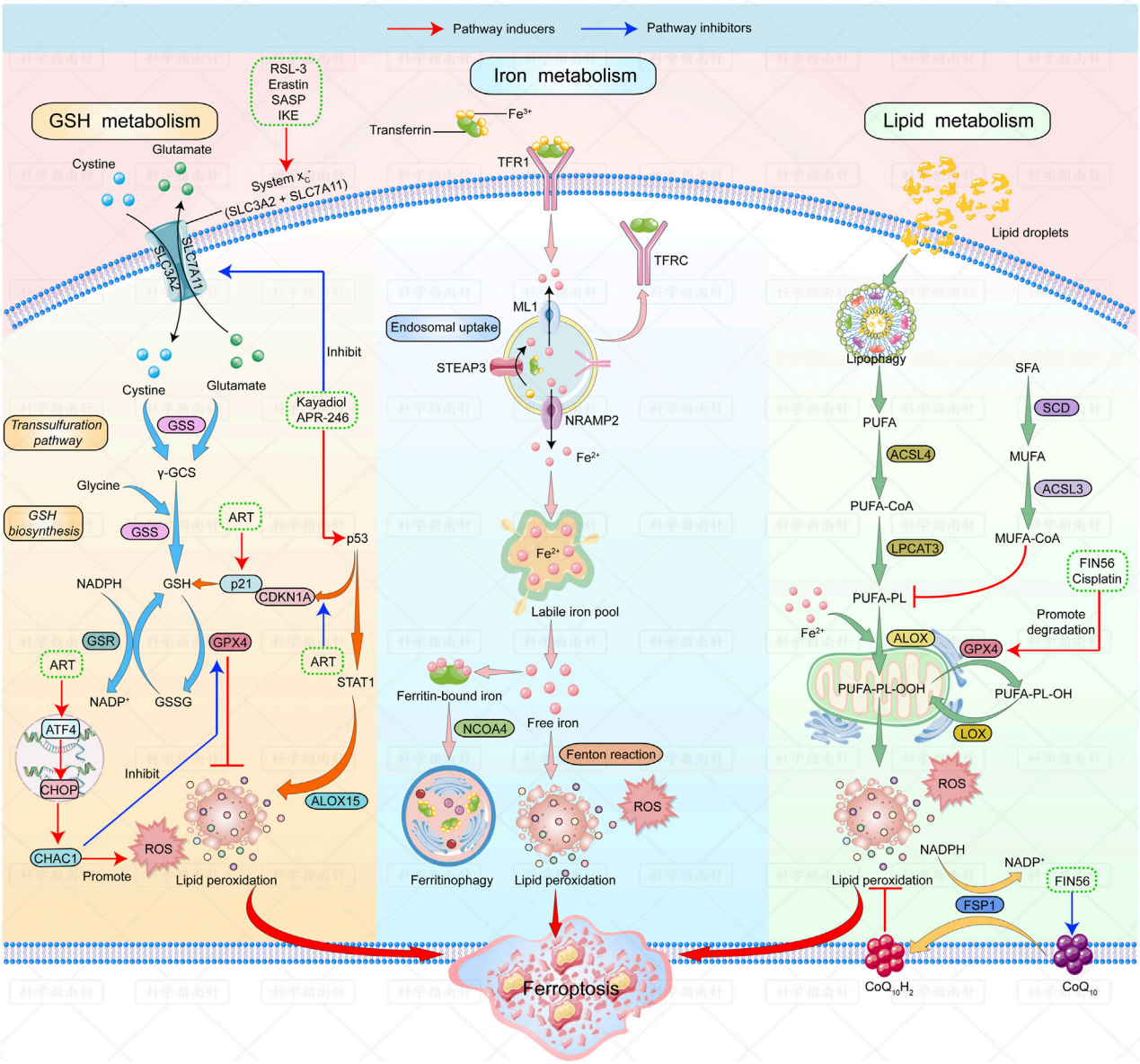

FIGURE 1
| Mechanisms of ferroptosis and ferroptosis inducers in lymphoma. The figure shows the regulatory pathways of ferroptosis can be divided into three categories. The first one is regulated by GSH/GPX4 pathway. The second one is the iron metabolism pathway that regulates the labile iron pool. The third category is the lipid metabolism pathways. Ferroptosis inducers that have been studied in lymphoma are listed: RSL-3, erastin, SASP and IKE can induce ferroptosis by inhibiting system Xc-; Kayadiol and APR-246 induce ferroptosis by both inhibiting system Xc- and promoting P53-P21 pathway; ART could induce ferroptosis by activating the ATF4-CHOP-CHAC1 pathway or by increasing p21 expression. Fin56 induces ferroptosis by promoting degradation of GPX4 and depleting endogenous antioxidant coenzyme Q10. Abbreviations: RSL-3, RAS-selective-lethal-3; SASP, safasalazine; IKE, Imidazole-ketone-erastin; ART, Artesunate.

CoQ10 to CoQH2 (Mao et al., 2021). The GTP cyclohydrolase 1/tetrahydrobiopterin axis also inhibits ferroptosis by reducing CoQ10 against lipid peroxidation and depleting PUFA-PLs (Kraft et al., 2020). Furthermore, several negative regulators of ferroptosis are known. The antioxidant transcription factor, NRF2, suppresses ferroptosis by regulating the expression of genes related to GSH biosynthesis, NADPH regeneration, and iron homeostasis (Song and Long, 2020). Trans-sulfuration produces cystine from methionine, providing more substrates for GSH and inhibiting ferroptosis (Zhang et al., 2022). Activation of the recombination activating gene (Rag)- the mechanistic target of rapamycin complex 1 (mTORC1)-eukaryotic initiation factor 4E (eIF4E)-binding proteins (4EBPs) signaling axis induces resistance to ferroptosis by

increasing GPX4 synthesis (Zhang et al., 2021) and the phosphatidylinositol 3-kinase-alpha serine/threonine-protein kinase-the mechanistic target of rapamycin pathway can suppress ferroptosis by increasing sterol regulatory element-binding protein-mediated lipogenesis (Yi et al., 2020).

2.4 p53 in ferroptosis

p53 can inhibit the expression of SLC7A11 at the transcriptional level and subsequently reduce the biosynthesis of GSH, thus inducing ferroptosis and suppressing tumor growth (Hu et al., 2022). In 2019, Gu Wei *et al.* demonstrated that the p53-SLC7A11 axis can also promote ferroptosis in a GSH-independent manner (Liu and Gu, 2022). ALOX12, a lipoxygenase, oxidizes PUFA and induces cellular ferroptosis. p53 induces ALOX12 activity by repressing SLC7A11 expression as the latter can directly bind to the former, thereby limiting its functions (Liu and Gu, 2022). p53 induces SAT1 expression, thus promoting the function of ALOX15 and inducing ferroptosis (Liu and Gu, 2022). Recently, a new target gene of p53, independent phospholipase A2beta, was found to suppress ferroptosis by cleaving peroxidized lipids and releasing PUFAs from membrane PLs (Chen et al., 2021a). However, p53 also inhibits ferroptosis under certain circumstances, a process requiring the involvement of P21, a p53 transcriptional target that can inhibit cell cycle progression, thereby converting part of the raw materials necessary for the synthesis of nucleic acids to those required for NADPH and GSH and inhibiting ferroptosis (Tarangelo et al., 2018). Therefore, p53 may regulate ferroptosis through distinct mechanisms which need further investigation.

3 Potential role of ferroptosis in lymphoma

DLBCL is the most common type of adult non-Hodgkin's lymphoma (NHL), accounting for nearly 30% of the cases of adult NHL wordwide (Beham-Schmid, 2017). DLBCL cell lines are susceptible to ferroptosis induced by system Xc-inhibitors because of their inability to use the trans-sulfuration pathway to convert methionine to cysteine (Gout et al., 2001; Sun et al., 2014). Ferroptosis slows down tumor growth in a DLBCL xenograft model (Deng et al., 2019). Yuko Kinowaki et al. (2018) assessed the expression of GPX4 by immunohistochemistry and found that the GPX4-positive group showed poor overall survival relative to the GPX4-negative group, indicating that GPX4 overexpression is an independent prognostic predictor of adverse prognosis in DLBCL. Moreover, the activation of ferroptosis participates in p53-mediated cell cycle arrest and cell death in B-cell lymphomas (Li et al., 2016). Hydroxyacyl-CoA dehydrogenase (HADHA) is involved in fatty acid beta-oxidation (FAO) and is overexpressed in high-grade lymphoma. Its overexpression indicates a poor prognosis in DLBCL(62). Recently, some studies have attempted to identify ferroptosis-related genes in DLBCL and elucidate a method to predict the prognosis of these patients, along with novel treatment strategies. Huan Chen et al. (2021b) performed a systematic study and identified a ferroptosis-related gene signature comprising 8 genes that could be used to divide DLBCL patients into high- and low-risk groups. Moreover, Junmei Weng *et al.* built a risk score model related to ferroptosis with 11 genes and revealed that the high-risk score group showed resistance to ibrutinib treatment, while ferroptosis inducer, acetaminophen, inhibited the expression of the high-risk genes in the DLBCL cell lines (Weng et al., 2022). Julie Devin et al. (2022) built the iron score-related model to identify patients with DLBCL showing poor prognosis who might benefit from treatment targeted to maintaining iron homeostasis. All these studies suggest that therapeutic drugs targeting ferroptosis might contribute to alleviating DLBCL.

Despite limited information, other types of lymphoma that have been studied in the light of ferroptosis, include adult T-cell leukemia/lymphoma, BL, and natural killer/T cell lymphoma (NKTCL) (Du et al., 2022). These lymphomas are highly invasive and treatment options are limited especially in patients older than 60 years. Ferroptosis inducers, especially artesunate, alleviate these types of lymphoma through different mechanisms (Wang et al., 2019; Ishikawa et al., 2020; He et al., 2022), thus providing perspectives for the development of novel therapeutic strategies. However, unlike DLBCL, basic research for elucidating the mechanism of ferroptosis on tumorigenesis of these lymphomas is lacking.

4 Therapeutic applications

Several ferroptosis inducers against lymphoma have shown good outcomes in animal models. A summary of ferroptosis inducers against lymphomas is shown in Figure 1.

4.1 Drugs targeting system Xc-

GSH metabolic pathways are classical targets of ferroptosis inducers. Erastin and its analog, RSL3, and sulfasalazine (SASP), have been widely applied as classical ferroptosis-inducing agents by targeting system Xc- and GPX4 (Xiao et al., 2015; Zhang et al., 2020). Imidazole-ketone-erastin (IKE), an improved erastin analog, can inhibit system Xc-at low concentrations (Ye et al., 2020). Zhang et al. (2019) demonstrated that IKE prevented tumor progression by inhibiting system Xc-in a DLBCL xenograft model through GSH depletion and lipid peroxidation. Erastin and RSL3-termed ferroptosis inducers can inhibit tumor growth in

two DLBCL cell lines (Yang et al., 2014). In rats treated with sulfasalazine (SASP), a system Xc-inhibitor, the transplanted lymphoma growth shows marked reduction (Gout et al., 2003). As lymphoid cells cannot synthesize cysteine and since their growth is dependent on cysteine uptake from the microenvironment, SASP exerts inhibitory effects of cysteine secretion by somatic cells, causing cysteine starvation in lymphoma cells and inducing ferroptosis *in vivo* (Gout et al., 2001).

Since GSH metabolism is well understood, it is easy to speculate that the above drugs should be among the most promising ferroptosis inducers but more *in vivo* experiments are needed to validate their efficacy and safety in the future.

4.2 Drugs targeting p53-mediated ferroptosis

Drugs targeting the p53-mediated ferroptosis pathway, including eprenetapopt (or APR-246) and kayadiol, are new research hotspots. APR-246 reactivates the transcriptional activity of the mutant p53 by promoting its binding to target genes, thereby showing efficacy in p53-mutated tumors (Cluzeau et al., 2021; Mishra et al., 2022). APR-246 reactivates the transcriptional activity of mutant p53 by promoting their binding to its target genes and is effective in p53-mutated tumors (Cluzeau et al., 2021; Mishra et al., 2022). APR-246 can induce tumor protein p53 TP53-mutation-mediated cell death in DLBCL through ferroptosis by p53-dependent ferritinophagy (Hong et al., 2022). APR-246 can also induce ferroptosis in a p53-independent manner by binding to GSH or inhibiting antioxidant enzymes in acute myeloid leukemia but this has not been validated in lymphomas yet (Birsen et al., 2022). Thus, APR-246 may be a promising new therapeutic drug for DLBCL patients.

Kayadiol, a diterpenoid, shows a strong inhibitory effect on extranodal NKTCL cells. Kayadiol treatment triggers significant ferroptosis events through the p53-mediated pathway, including ROS accumulation and GSH depletion and reducing the expressions of SLC7A11 and GPX4. Moreover, Kayadiol also promotes the phosphorylation of p53, thus upregulating its protein expression. Hence, Kayadiol can be used as an alternative for NK/T cell lymphoma treatment, especially in cases of failure of initial therapeutic strategies (He et al., 2022).

Ferroptosis inducers involved in the p53-mediated pathway have been recently developed, and APR-246 has been studied in different types of hematologic malignancies, showing good results in clinical trials (Gout et al., 2003). It is expected that drugs targeting the p53 pathway may soon enter clinical trials for lymphoma.

4.3 Other ferroptosis inducers

Other types of drugs, including artesunate (ART), the FAO inhibitor, and dimethyl fumarate, can promote ferroptosis through different mechanisms. ART, a widely used antimalarial compound exerting cytotoxicity, induces ferroptosis in different types of lymphomas (Wang et al., 2019; Ishikawa et al., 2020; Chen et al., 2021c). By inducing lysosomal degradation of ferritin, ART increases intracellular labile iron and ROS levels, rendering cells sensitive to ferroptosis (Li, 2012; Chen et al., 2020b). Moreover, ART can inhibit the activation of signal transducer and activator of transcription 3 (STAT3), thereby downregulating the expression of GPX4, finally leading to ferroptosis (Chen et al., 2021c). Ning Wang et al. (2019) showed that ART could induce ferroptosis by activating the transcription factor 4-CEBP-homologous protein-cation transport regulator-like protein 1 (ATF4-CHOP-CHAC1) pathway, thus inhibiting GPX4 expression in BL cell lines. As mentioned above, the upregulation of fatty acid beta-oxidation is observed in high-grade lymphoma (Yamamoto et al., 2020). Inhibition of HADHA may disrupt the balance between saturated and unsaturated fatty acids that are subsequently supplied to PLs, in turn promoting the accumulation of polyunsaturated fatty acids in the cell membrane along with ferroptosis (Zhou et al., 2012a; Zhou et al., 2012b). Treatment with the FAO inhibitor, ranolazine, increases cell death in DLBCL (Sekine et al., 2022). Finally, dimethyl fumarate (DMF) potently and rapidly depletes GSH by inducing the succination of cysteine residues and induces lipid peroxidation, subsequently inducing ferroptosis, particularly in DLBCL (Schmitt et al., 2021).

With the continued and improved understanding of the mechanisms underlying ferroptosis, targeting different pathways to induce ferroptosis in lymphomas is emerging as a promising new option that has been validated in animal and *in vitro* experiments. Follow-up studies should focus on the effects and safety of these drugs in humans.

5 Conclusion and perspectives

Ferroptosis is an iron-dependent form of programmed cell death, a new direction for future research. It is regulated by several cellular metabolic pathways such as redox homeostasis, iron metabolism, mitochondrial activity, lipid, and amino acid metabolism, and ROS accumulation, as described above. Novel pathways such as p53-mediated signaling also play a complicated role in regulating of ferroptosis. Presumably, the p53-p21 pathway is activated to protect cells from damage when the external stimuli are small. On the contrary, other p53-mediated pathways are initiated to destroy cells *via* ferroptosis as stimuli exceed the threshold. Several non-classical regulatory pathways associated with ferroptosis are emerging such as mitochondrial

VDACs (Skonieczna et al., 2017) and FSP1(45)-induced ferroptosis. Moreover, other kinds of programmed cell death such as autophagy can cause changes in ferritin levels, subsequently increasing the labile iron pool and inducing ferroptosis (Hou et al., 2016). Therefore, crosstalk between ferroptosis and other cell death modes exists but the exact underlying mechanisms remain elusive.

Ferroptosis was proposed for the first time in 2012, and since, it has emerged as an attractive target in tumor biology and cancer therapy. Certain types of tumors are more prone to ferroptosis due to their unique metabolic characteristics and high load of ROS, making ferroptosis a promising candidate for targeted therapy. The sensitivity of lymphoma to ferroptosis can be increased by drugs that regulate intracellular ROS production, iron metabolism, GPX4 levels, and other molecules. As most evidence has been obtained from animal models, there remains a long way toward the clinical application of ferroptosis inducers in lymphoma treatment. More research is needed in the future to uncover all the underlying mechanisms of ferroptosis in lymphoma.

The figure shows the regulatory pathways involved in ferroptosis. There are three main types of mechanisms involved in regulating ferroptosis: first, the regulation by the GSH/GPX4 pathway. The second is the iron metabolism pathway that regulates the labile iron pool. The third includes pathways related to lipid metabolism that affect lipid regulation. Ferroptosis inducers studied in lymphoma are as follows: RSL-3, Erastin, SASP, and IKE can induce ferroptosis by inhibiting system Xc-; Kayadiol and APR-246 induce ferroptosis by both inhibiting system Xc- and promoting the p53-p21 pathway; ART can induce ferroptosis by activating the ATF4-CHOP-CHAC1 pathway or by increasing the expression of P21. FIN56 induces ferroptosis by accelerating GPX4 degradation and depleting the endogenous antioxidant coenzyme, Q10.

Author contributions

YW and XH contributed to the conception and design of the work. QZ and TL drafted the manuscript. QQ prepared the figure. YW and XH substantively revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abrams, R. P., Carroll, W. L., and Woerpel, K. A. (2016). Five-membered ring peroxide selectively initiates ferroptosis in cancer cells. *ACS Chem. Biol.* 11 (5), 1305–1312. doi:10.1021/acschembio.5b00900

Beatty, A., Singh, T., Tyurina, Y. Y., Tyurin, V. A., Samovich, S., Nicolas, E., et al. (2021). Ferroptotic cell death triggered by conjugated linolenic acids is mediated by ACSL1. *Nat. Commun.* 12 (1), 2244. doi:10.1038/s41467-021-22471-y

Beham-Schmid, C. (2017). Aggressive lymphoma 2016: Revision of the WHO classification. *Memo* 10 (4), 248–254. doi:10.1007/s12254-017-0367-8

Bersuker, K., Hendricks, J. M., Li, Z., Magtanong, L., Ford, B., Tang, P. H., et al. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 575 (7784), 688–692. doi:10.1038/s41586-019-1705-2

Birsen, R., Larrue, C., Decroocq, J., Johnson, N., Guiraud, N., Gotanegre, M., et al. (2022). APR-246 induces early cell death by ferroptosis in acute myeloid leukemia. *Haematologica* 107 (2), 403–416. doi:10.3324/haematol.2020.259531

Bonam, S. R., Wang, F., and Muller, S. (2018). Autophagy: A new concept in autoimmunity regulation and a novel therapeutic option. *J. Autoimmun.* 94, 16–32. doi:10.1016/j.jaut.2018.08.009

Brown, C. W., Amante, J. J., Chhoy, P., Elaimy, A. L., Liu, H., Zhu, L. J., et al. (2019). Prominin2 drives ferroptosis resistance by stimulating iron export. *Dev. Cell* 51 (5), 575–586. e4. doi:10.1016/j.devcel.2019.10.007

Cao, J. Y., Poddar, A., Magtanong, L., Lumb, J. H., Mileur, T. R., Reid, M. A., et al. (2019). A genome-wide haploid genetic screen identifies regulators of glutathione abundance and ferroptosis sensitivity. *Cell Rep.* 26 (6), 1544–1556. e8. doi:10.1016/j.celrep.2019.01.043

Chapuy, B., Stewart, C., Dunford, A. J., Kim, J., Kamburov, A., Redd, R. A., et al. (2018). Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat. Med.* 24 (5), 679–690. doi:10.1038/s41591-018-0016-8

Chen, D., Chu, B., Yang, X., Liu, Z., Jin, Y., Kon, N., et al. (2021). iPLA2β-mediated lipid detoxification controls p53-driven ferroptosis independent of GPX4. *Nat. Commun.* 12 (1), 3644. doi:10.1038/s41467-021-23902-6

Chen, G. Q., Benthani, F. A., Wu, J., Liang, D., Bian, Z. X., and Jiang, X. (2020). Artemisinin compounds sensitize cancer cells to ferroptosis by regulating iron homeostasis. *Cell Death Differ.* 27 (1), 242–254. doi:10.1038/s41418-019-0352-3

Chen, H., He, Y., Pan, T., Zeng, R., Li, Y., Chen, S., et al. (2021). Ferroptosis-related gene signature: A new method for personalized risk assessment in patients with diffuse large B-cell lymphoma. *Pharmgenomics. Pers. Med.* 14, 609–619. doi:10.2147/PGPM.S309846

Chen, P. H., Wu, J., Ding, C. C., Lin, C. C., Pan, S., Bossa, N., et al. (2020). Kinome screen of ferroptosis reveals a novel role of ATM in regulating iron metabolism. *Cell Death Differ.* 27 (3), 1008–1022. doi:10.1038/s41418-019-0393-7

Chen, Y., Wang, F., Wu, P., Gong, S., Gao, J., Tao, H., et al. (2021). Artesunate induces apoptosis, autophagy and ferroptosis in diffuse large B cell lymphoma cells by impairing STAT3 signaling. *Cell. Signal.* 88, 110167. doi:10.1016/j.cellsig.2021.110167

Chen, Z., Jiang, J., Fu, N., and Chen, L. (2022). Targetting ferroptosis for blood cell-related diseases. *J. Drug Target.* 30 (3), 244–258. doi:10.1080/1061186X.2021.1971237

Cluzeau, T., Sebert, M., Rahmé, R., Cuzzubbo, S., Lehmann-Che, J., Madelaine, I., et al. (2021). Eprenetapopt Plus Azacitidine in TP53-Mutated Myelodysplastic Syndromes and Acute Myeloid Leukemia: A Phase II Study by the Groupe Francophone des Myélodysplasies (GFM). *J. Clin. Oncol.* 39 (14), 1575–1583. doi:10.1200/JCO.20.02342

Conrad, M., Kagan, V. E., Bayir, H., Pagnussat, G. C., Head, B., Traber, M. G., et al. (2018). Regulation of lipid peroxidation and ferroptosis in diverse species. *Genes Dev.* 32 (9-10), 602–619. doi:10.1101/gad.314674.118

Cronin, S. J. F., Woolf, C. J., Weiss, G., and Penninger, J. M. (2019). The role of iron regulation in immunometabolism and immune-related disease. *Front. Mol. Biosci.* 6, 116. doi:10.3389/fmolb.2019.00116

Davidson, A. J., and Wood, W. (2020). Igniting the spread of ferroptotic cell death. *Nat. Cell Biol.* 22 (9), 1027–1029. doi:10.1038/s41556-020-0570-4

Deng, R., Zhang, J., and Chen, J. (2019). lncRNA SNHG1 negatively regulates miRNA-101-3p to enhance the expression of ROCK1 and promote cell proliferation, migration and invasion in osteosarcoma. *Int. J. Mol. Med.* 43 (3), 1157–1166. doi:10.3892/ijmm.2018.4039

Devin, J., Cañeque, T., Lin, Y. L., Mondoulet, L., Veyrune, J. L., Abouladze, M., et al. (2022). Targeting cellular iron homeostasis with ironomycin in diffuse large B-cell lymphoma. *Cancer Res.* 82 (6), 998–1012. doi:10.1158/0008-5472.CAN-21-0218

Doll, S., Proneth, B., Tyurina, Y. Y., Panzilius, E., Kobayashi, S., Ingold, I., et al. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat. Chem. Biol.* 13 (1), 91–98. doi:10.1038/nchembio.2239

Du, G., Zhang, Q., Huang, X., and Wang, Y. (2022). Molecular mechanism of ferroptosis and its role in the occurrence and treatment of diabetes. *Front. Genet.* 13, 1018829. doi:10.3389/fgene.2022.1018829

Fu, R., Guo, C., Wang, S., Huang, Y., Jin, O., Hu, H., et al. (2017). Podocyte activation of NLRP3 inflammasomes contributes to the development of proteinuria in lupus nephritis. *Arthritis Rheumatol.* 69 (8), 1636–1646. doi:10.1002/art.40155

Gout, P. W., Buckley, A. R., Simms, C. R., and Bruchovsky, N. (2001). Sulfasalazine, a potent suppressor of lymphoma growth by inhibition of the x(c)- cystine transporter: A new action for an old drug. *Leukemia* 15 (10), 1633–1640. doi:10.1038/sj.leu.2402238

Gout, P. W., Simms, C. R., and Robertson, M. C. (2003). *In vitro* studies on the lymphoma growth-inhibitory activity of sulfasalazine. *Anticancer. Drugs* 14 (1), 21–29. doi:10.1097/00001813-200301000-00004

Guo, C., Fu, R., Zhou, M., Wang, S., Huang, Y., Hu, H., et al. (2019). Pathogenesis of lupus nephritis: RIP3 dependent necroptosis and NLRP3 inflammasome activation. *J. Autoimmun.* 103, 102286. doi:10.1016/j.jaut.2019.05.014

Hangauer, M. J., Viswanathan, V. S., Ryan, M. J., Bole, D., Eaton, J. K., Matov, A., et al. (2017). Drug-tolerant persister cancer cells are vulnerable to GPX4 inhibition. *Nature* 551 (7679), 247–250. doi:10.1038/nature24297

Hao, S., Yu, J., He, W., Huang, Q., Zhao, Y., Liang, B., et al. (2017). Cysteine dioxygenase 1 mediates erastin-induced ferroptosis in human gastric cancer cells. *Neoplasia (New York, NY)* 19 (12), 1022–1032. doi:10.1016/j.neo.2017.10.005

He, C., Wang, C., Liu, H., and Shan, B. (2022). Kayadiol exerted anticancer effects through p53-mediated ferroptosis in NKTCL cells. *BMC cancer* 22 (1), 724. doi:10.1186/s12885-022-09825-5

Hong, Y., Ren, T., Wang, X., Liu, X., Fei, Y., Meng, S., et al. (2022). APR-246 triggers ferritinophagy and ferroptosis of diffuse large B-cell lymphoma cells with distinct TP53 mutations. *Leukemia* 36, 2269–2280. doi:10.1038/s41375-022-01634-w

Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M. T., Zeh, H. J., 3rd, et al. (2016). Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 12 (8), 1425–1428. doi:10.1080/15548627.2016.1187366

Hu, J., Zhang, R., Chang, Q., Ji, M., Zhang, H., Geng, R., et al. (2022). p53: A regulator of ferroptosis induced by galectin-1 derived peptide 3 in MH7A cells. *Front. Genet.* 13, 920273. doi:10.3389/fgene.2022.920273

Ishikawa, C., Senba, M., and Mori, N. (2020). Evaluation of artesunate for the treatment of adult T-cell leukemia/lymphoma. *Eur. J. Pharmacol.* 872, 172953. doi:10.1016/j.ejphar.2020.172953

Jiang, X., Stockwell, B. R., and Conrad, M. (2021). Ferroptosis: Mechanisms, biology and role in disease. *Nat. Rev. Mol. Cell Biol.* 22 (4), 266–282. doi:10.1038/s41580-020-00324-8

Jing, S., Lu, Y., Zhang, J., Ren, Y., Mo, Y., Liu, D., et al. (2022). Levistilide a induces ferroptosis by activating the Nrf2/HO-1 signaling pathway in breast cancer cells. *Drug Des. devel. Ther.* 16, 2981–2993. doi:10.2147/DDDT.S374328

Kinowaki, Y., Kurata, M., Ishibashi, S., Ikeda, M., Tatsuzawa, A., Yamamoto, M., et al. (2018). Glutathione peroxidase 4 overexpression inhibits ROS-induced cell death in diffuse large B-cell lymphoma. *Lab. Invest.* 98 (5), 609–619. doi:10.1038/s41374-017-0008-1

Kraft, V. A. N., Bezjian, C. T., Pfeiffer, S., Ringelstetter, L., Müller, C., Zandkarimi, F., et al. (2020). GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. *ACS Cent. Sci.* 6 (1), 41–53. doi:10.1021/acscentsci.9b01063

Lee, H., Zandkarimi, F., Zhang, Y., Meena, J. K., Kim, J., Zhuang, L., et al. (2020). Energy-stress-mediated AMPK activation inhibits ferroptosis. *Nat. Cell Biol.* 22 (2), 225–234. doi:10.1038/s41556-020-0461-8

Li, J., Cao, F., Yin, H. L., Huang, Z. J., Lin, Z. T., Mao, N., et al. (2020). Ferroptosis: Past, present and future. *Cell Death Dis.* 11 (2), 88. doi:10.1038/s41419-020-2298-2

Li, T., Liu, X., Jiang, L., Manfredi, J., Zha, S., and Gu, W. (2016). Loss of p53-mediated cell-cycle arrest, senescence and apoptosis promotes genomic instability and premature aging. *Oncotarget* 7 (11), 11838–11849. doi:10.18632/oncotarget.7864

Li, Y. (2012). Qinghaosu (artemisinin): Chemistry and pharmacology. *Acta Pharmacol. Sin.* 33 (9), 1141–1146. doi:10.1038/aps.2012.104

Li, Y., Zeng, X., Lu, D., Yin, M., Shan, M., and Gao, Y. (2021). Erastin induces ferroptosis via ferroportin-mediated iron accumulation in endometriosis. *Hum. Reprod.* 36 (4), 951–964. doi:10.1093/humrep/deaa363

Liang, C., Zhang, X., Yang, M., and Dong, X. (2019). Recent progress in ferroptosis inducers for cancer therapy. *Adv. Mat.* 31 (51), e1904197. doi:10.1002/adma.201904197

Liu, Y., and Gu, W. (2022). p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell Death Differ.* 29 (5), 895–910. doi:10.1038/s41418-022-00943-y

Magtanong, L., Ko, P. J., To, M., Cao, J. Y., Forcina, G. C., Tarangelo, A., et al. (2019). Exogenous monounsaturated fatty acids promote a ferroptosis-resistant cell state. *Cell Chem. Biol.* 26 (3), 420–432. e9. doi:10.1016/j.chembiol.2018.11.016

Mancuso, R. I., Foglio, M. A., and Olalla Saad, S. T. (2021). Artemisinin-type drugs for the treatment of hematological malignancies. *Cancer Chemother. Pharmacol.* 87 (1), 1–22. doi:10.1007/s00280-020-04170-5

Mao, C., Liu, X., Zhang, Y., Lei, G., Yan, Y., Lee, H., et al. (2021). DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature* 593 (7860), 586–590. doi:10.1038/s41586-021-03539-7

Mishra, A., Tamari, R., DeZern, A. E., Byrne, M. T., Gooptu, M., Chen, Y. B., et al. (2022). Eprenetapopt plus azacitidine after allogeneic hematopoietic stem-cell transplantation for TP53-mutant acute myeloid leukemia and myelodysplastic syndromes. *J. Clin. Oncol.*, Jco2200181. doi:10.1200/JCO.22.00181

Nagata, S. (2018). Apoptosis and clearance of apoptotic cells. *Annu. Rev. Immunol.* 36, 489–517. doi:10.1146/annurev-immunol-042617-053010

Pandrangi, S. L., Chittineedi, P., Chikati, R., Lingareddy, J. R., Nagoor, M., and Ponnada, S. K. (2022). Role of dietary iron revisited: In metabolism, ferroptosis and pathophysiology of cancer. *Am. J. Cancer Res.* 12 (3), 974–985.

Patel, S. J., Frey, A. G., Palenchar, D. J., Achar, S., Bullough, K. Z., Vashisht, A., et al. (2019). A PCBP1-BolA2 chaperone complex delivers iron for cytosolic [2Fe-2S] cluster assembly. *Nat. Chem. Biol.* 15 (9), 872–881. doi:10.1038/s41589-019-0330-6

Ren, W., Zhao, W., Cao, L., and Huang, J. (2020). Involvement of the actin machinery in programmed cell death. *Front. Cell Dev. Biol.* 8, 634849. doi:10.3389/fcell.2020.634849

Sato, M., Kusumi, R., Hamashima, S., Kobayashi, S., Sasaki, S., Komiyama, Y., et al. (2018). The ferroptosis inducer erastin irreversibly inhibits system x(c)- and synergizes with cisplatin to increase cisplatin's cytotoxicity in cancer cells. *Sci. Rep.* 8 (1), 968. doi:10.1038/s41598-018-19213-4

Schmitt, A., Xu, W., Bucher, P., Grimm, M., Konantz, M., Horn, H., et al. (2021). Dimethyl fumarate induces ferroptosis and impairs NF-κB/STAT3 signaling in DLBCL. *Blood* 138 (10), 871–884. doi:10.1182/blood.2020009404

Segura, B. T., Bernstein, B. S., McDonnell, T., Wincup, C., Giles, I., Ripoll, V. M., et al. (2020). Damage accrual and mortality over long-term follow-up in 300 patients with systemic lupus erythematosus in a multi-ethnic British cohort. *Rheumatol. Oxf. Engl.* 59 (3), 524–533. doi:10.1093/rheumatology/kez292

Sekine, Y., Yamamoto, K., Kurata, M., Honda, A., Onishi, I., Kinowaki, Y., et al. (2022). HADHB, a fatty acid beta-oxidation enzyme, is a potential prognostic predictor in malignant lymphoma. *Pathology* 54 (3), 286–293. doi:10.1016/j.pathol.2021.06.119

Shah, R., Shchepinov, M. S., and Pratt, D. A. (2018). Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. *ACS Cent. Sci.* 4 (3), 387–396. doi:10.1021/acscentsci.7b00589

Skonieczna, M., Cieslar-Pobuda, A., Saenko, Y., Foksinski, M., Olinski, R., Rzeszowska-Wolny, J., et al. (2017). The impact of DIDS-induced inhibition of voltage-dependent anion channels (VDAC) on cellular response of lymphoblastoid cells to ionizing radiation. *Med. Chem.* 13 (5), 477–483. doi:10.2174/1573406413666170421102353

Song, J., Liu, T., Yin, Y., Zhao, W., Lin, Z., Yin, Y., et al. (2021). The deubiquitinase OTUD1 enhances iron transport and potentiates host antitumor immunity. *EMBO Rep.* 22 (2), e51162. doi:10.15252/embr.202051162

Song, X., and Long, D. (2020). Nrf2 and ferroptosis: A new research direction for neurodegenerative diseases. *Front. Neurosci.* 14, 267. doi:10.3389/fnins.2020.00267

Stockwell, B. R. (2022). Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. *Cell* 185 (14), 2401–2421. doi:10.1016/j.cell.2022.06.003

Sun, T., Zhang, Y. S., Pang, B., Hyun, D. C., Yang, M., and Xia, Y. (2014). Engineered nanoparticles for drug delivery in cancer therapy. *Angew. Chem. Int. Ed. Engl.* 53 (46), 12320–12364. doi:10.1002/anie.201403036

Tang, D., Chen, X., Kang, R., and Kroemer, G. (2021). Ferroptosis: Molecular mechanisms and health implications. *Cell Res.* 31 (2), 107–125. doi:10.1038/s41422-020-00441-1

Tarangelo, A., Magtanong, L., Bieging-Rolett, K. T., Li, Y., Ye, J., Attardi, L. D., et al. (2018). p53 suppresses metabolic stress-induced ferroptosis in cancer cells. *Cell Rep.* 22 (3), 569–575. doi:10.1016/j.celrep.2017.12.077

Toyokuni, S., Ito, F., Yamashita, K., Okazaki, Y., and Akatsuka, S. (2017). Iron and thiol redox signaling in cancer: An exquisite balance to escape ferroptosis. *Free Radic. Biol. Med.* 108, 610–626. doi:10.1016/j.freeradbiomed.2017.04.024

Tsvetkov, P., Coy, S., Petrova, B., Dreishpoon, M., Verma, A., Abdusamad, M., et al. (2022). Copper induces cell death by targeting lipoylated TCA cycle proteins. *Sci. (New York, NY)* 375 (6586), 1254–1261. doi:10.1126/science.abf0529

Ursini, F., and Maiorino, M. (2020). Lipid peroxidation and ferroptosis: The role of GSH and GPx4. *Free Radic. Biol. Med.* 152, 175–185. doi:10.1016/j.freeradbiomed.2020.02.027

Viswanathan, V. S., Ryan, M. J., Dhruv, H. D., Gill, S., Eichhoff, O. M., Seashore-Ludlow, B., et al. (2017). Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* 547 (7664), 453–457. doi:10.1038/nature23007

Wang, N., Zeng, G. Z., Yin, J. L., and Bian, Z. X. (2019). Artesunate activates the ATF4-CHOP-CHAC1 pathway and affects ferroptosis in Burkitt's Lymphoma. *Biochem. Biophys. Res. Commun.* 519 (3), 533–539. doi:10.1016/j.bbrc.2019.09.023

Weng, J., Chen, L., Liu, H., Yang, X. P., and Huang, L. (2022). Ferroptosis markers predict the survival, immune infiltration, and ibrutinib resistance of diffuse large B cell lymphoma. *Inflammation* 45 (3), 1146–1161. doi:10.1007/s10753-021-01609-6

Wu, Z., Geng, Y., Lu, X., Shi, Y., Wu, G., Zhang, M., et al. (2019). Chaperone-mediated autophagy is involved in the execution of ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* 116 (8), 2996–3005. doi:10.1073/pnas.1819728116

Xiao, Y., Yu, F., Pang, L., Zhao, H., Liu, L., Zhang, G., et al. (2015). MeSiC: A model-based method for estimating 5 mC levels at single-CpG resolution from MeDIP-seq. *Sci. Rep.* 5, 14699. doi:10.1038/srep14699

Xu, C., Liu, Z., and Xiao, J. (2021). Ferroptosis: A double-edged sword in gastrointestinal disease. *Int. J. Mol. Sci.* 22 (22), 12403. doi:10.3390/ijms222212403

Yamamoto, K., Abe, S., Honda, A., Hashimoto, J., Aizawa, Y., Ishibashi, S., et al. (2020). Fatty acid beta oxidation enzyme HADHA is a novel potential therapeutic target in malignant lymphoma. *Lab. Invest.* 100 (3), 353–362. doi:10.1038/s41374-019-0318-6

Yang, W. S., SriRamaratnam, R., Welsch, M. E., Shimada, K., Skouta, R., Viswanathan, V. S., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156 (1-2), 317–331. doi:10.1016/j.cell.2013.12.010

Ye, L. F., Chaudhary, K. R., Zandkarimi, F., Harken, A. D., Kinslow, C. J., Upadhyayula, P. S., et al. (2020). Radiation-induced lipid peroxidation triggers ferroptosis and synergizes with ferroptosis inducers. *ACS Chem. Biol.* 15 (2), 469–484. doi:10.1021/acschembio.9b00939

Yi, J., Zhu, J., Wu, J., Thompson, C. B., and Jiang, X. (2020). Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 117 (49), 31189–31197. doi:10.1073/pnas.2017152117

Zhang, H. F., Klein Geltink, R. I., Parker, S. J., and Sorensen, P. H. (2022). Transsulfuration, minor player or crucial for cysteine homeostasis in cancer. *Trends Cell Biol.* 32 (9), 800–814. doi:10.1016/j.tcb.2022.02.009

Zhang, Y., Shi, J., Liu, X., Feng, L., Gong, Z., Koppula, P., et al. (2018). BAP1 links metabolic regulation of ferroptosis to tumour suppression. *Nat. Cell Biol.* 20 (10), 1181–1192. doi:10.1038/s41556-018-0178-0

Zhang, Y., Swanda, R. V., Nie, L., Liu, X., Wang, C., Lee, H., et al. (2021). mTORC1 couples cyst(e)ine availability with GPX4 protein synthesis and ferroptosis regulation. *Nat. Commun.* 12 (1), 1589. doi:10.1038/s41467-021-21841-w

Zhang, Y., Tan, H., Daniels, J. D., Zandkarimi, F., Liu, H., Brown, L. M., et al. (2019). Imidazole ketone erastin induces ferroptosis and slows tumor growth in a mouse lymphoma model. *Cell Chem. Biol.* 26 (5), 623–633. doi:10.1016/j.chembiol.2019.01.008

Zhang, Y., Zhang, Y., Hu, J., Zhang, J., Guo, F., Zhou, M., et al. (2020). scTPA: a web tool for single-cell transcriptome analysis of pathway activation signatures. *Bioinformatics* 36 (14), 4217–4219. doi:10.1093/bioinformatics/btaa532

Zhou, Q., Yang, L., Li, T., Wang, K., Huang, X., Shi, J., et al. (2022). Mechanisms and inhibitors of ferroptosis in psoriasis. *Front. Mol. Biosci.* 9, 1019447. doi:10.3389/fmolb.2022.1019447

Zhou, Z., Zhou, J., and Du, Y. (2012). Estrogen receptor alpha interacts with mitochondrial protein HADHB and affects beta-oxidation activity. *Mol. Cell. Proteomics* 11 (7), M111.011056. doi:10.1074/mcp.M111.011056

Zhou, Z., Zhou, J., and Du, Y. (2012). Estrogen receptor beta interacts and colocalizes with HADHB in mitochondria. *Biochem. Biophys. Res. Commun.* 427 (2), 305–308. doi:10.1016/j.bbrc.2012.09.047

Glossary

ART Artesunate

ACSL3 Acyl-coenzyme A synthetase long-chain family member 3

ACSL4 Acyl-coenzyme A synthetase long-chain family member 4

ALOX Arachidonate lipoxygenase

ALOX15 Arachidonate lipoxygenase 15

ATF4-CHOP-CHAC1 Activating transcription factor 4-CEBP-homologous protein-cation transport regulator-like protein 1

CoQ10 Coenzyme Q10

CoQ10H2 Reduced coenzyme Q10

FIN56 Ferroptosis-inducer-56

FSP1 Ferroptosis suppressor protein 1

GPX4 Glutathione peroxidase 4

GSH Glutathione

GSR Glutathione reductase

GSS Glutathione synthetase

GSSG Oxidized glutathione

IKE Imidazole-ketone-erastin

LIP Labile iron pool

LOX Lipoxygenase

LPCAT3 Lysophosphatidylcholine acyltransferase 3

ML1 Mucolipin 1

MUFA Monounsaturated fatty acids

MUFA-COA Monounsaturated fatty acids-coenzyme A

NADPH Nicotinamide adenine dinucleotide phosphate (NADPH)

NCOA4 Nuclear receptor activator 4

NRAMP2 Natural resistance-associated macrophage protein 2

PUFA Polyunsaturated fatty acids

PUFA-PL Polyunsaturated fatty acids of phospholipids

γ-GCS γ-glutamyl-cysteine synthetase

RSL-3 Rat sarcoma-selective-lethal-3

ROS Reactive oxygen species;

STAT1 Signal transducer and activator of transcription 1

SASP Safasalazine

SCD Stearoyl-CoA desaturase

SFA Saturated fatty acids

STEAP3 Six-transmembrane epithelial antigen of prostate 3

TFR1 Transferrin receptor 1

TFRC Transferrin receptor

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P53 protein and the diseases in central nervous system

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P53 protein is the product of *P53* gene, which is a well acknowledged tumor suppressor gene. The function of P53 and the relevant mechanisms of anti-neoplasm have raised the interest of researchers since many years ago. It is demonstrated that P53 is a basic cell cycle regulator and a strong inhibitor for versatile cancers in humans. However, most research focuses on other organs and systems instead of the central nervous system (CNS). In fact, in recent years, more and more studies have been suggesting that P53 plays a significant role in multiple CNS tumors and other diseases and disorders such as cerebral stroke and neurodegenerative diseases. In this work, we mainly reviewed the P53's relationship with CNS tumors, cerebral stroke and neurodegenerative diseases, together with the relevant mechanisms, aiming to summarize the research achievements and providing new insight to the future study on diseases in CNS.

KEYWORDS

P53, cerebral stroke, neurodegenerative diseases, gliomas, apoptosis, ferroptosis, neuroinflammation

1 Introduction

The *P53* gene, with the full name of *Tp53* gene, is a well acknowledged tumor suppressor gene and thus thoroughly and repeatedly studied in numerous cancer types all over the human body and even in other diverse mammals. Its protein product, with the molecular weight of 53 kDa, therefore got its name. The expression and structure of P53 is relatively constant and conservative in mammals and other organisms. For the past decades, researchers have proven that it is an important cell cycle checker and a cornerstone to impact almost all cancers in humans. This study reviewed the current knowledge we have acquired from P53 and its relationship with CNS diseases including gliomas, cerebral stroke and neurodegenerative diseases. In addition, the relevant mechanisms involving the regulation of apoptosis, ferroptosis and inflammation were reviewed and discussed.

Glioblastoma is the most common primary malignant and aggressive brain tumors in humans. The treatment for it, unfortunately, no progress can be seen till recent years. Whether it is surgical resection or combined with radiotherapy and chemotherapy, there has been no significant improvement in the prognosis of high grade or recurrent gliomas. It is well known that P53 can regulate apoptosis, thus theoretically it is able to inhibit the

proliferation of gliomas (Hernández Borrero and El-Deiry, 2021). Though less reported, we believe that P53 may have the potential to play a role in the treatment of gliomas.

Cerebral stroke is clinically divided into two types: ischemic stroke and hemorrhagic stroke. The former has the highest incidence, and is due to the brain blood insufficiency or cut-off, resulting in brain tissue damage and massive neuronal deaths. Although hemorrhagic stroke has lower incidence compared to the ischemic one, it is with higher mortality and severer clinical outcome (Zhang et al., 2022). In stroke, reducing the death of neurons is a treatment plan that can improve the prognosis of patients (Zhang et al., 2022). By regulating the expression of P53 to increase the survival of neuronal cells, it might be used as a molecular-targeted treatment after stroke.

Neurodegenerative diseases are a cluster of diseases involving CNS and can be characterized by sensorimotor function impairment, memory loss and dementia, among which Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) are most common in clinical practice. The shared pathological changes in such diseases are atrophy of cerebral lobes, loss of neurons and synapses and accumulation of abnormal proteins in neurons or brain parenchyma such as amyloid beta (Aβ), tau protein and Lewy bodies. Neurodegenerative diseases are now considered to be incurable and the mechanisms behind are still unknown. However, *via* countless research, it is revealed that apoptosis, ferroptosis and neuroinflammation may play a fundamental role in them.

2 P53 structure

2.1 Wild-type P53 structure

The P53 protein structure includes five main parts: the reverse activation domain, the proline-rich domain, the DNA binding domain, the tetramer domain and the regulatory domain (Harris, 1996a).

The protein P53 has been proven to have transcription activation function that may be located at the amino terminal residues 1–42 (Lin et al., 1994). The reverse activation domain may be related to the tumor inhibitory function of P53, because the mutated P53 protein of many tumor patients have lost both transcription activation activity and tumor suppressor activity (Raycroft et al., 1990). This domain also can mediate the interaction between P53 and some basic transcription factors, like TATA-binding protein (TBP) (Liu et al., 1993).

The proline-rich domain has proved to have little to do with transcriptional activation, but the absence of this domain has weakened the ability of P53 to inhibit the growth of tumor cells *in vitro* (Walker and Levine, 1996). P53 proline-rich domain plays a key role in inhibiting signal transmission downstream of P53 protein and can be associated with signaling pathways transduction. Research shows that the domain participates in growth suppression cell signal transmission (Walker and Levine, 1996).

The P53 protein is a tetrameric transcription factor. Oligomerization appears to be essential for the tumor suppressing activity of P53 because oligomerization-deficient P53 mutants cannot suppress the growth of carcinoma cell lines (Jeffrey et al., 1995; Kato et al., 2003). The DNA binding domain (DBD) is key point of P53 for functioning. Experiments show *in vitro* that it is composed of the basis of two decamers RRRCWWGYYY (n) RRRCWWGYYY (R = purine, C = cytosine, W = adenine or thymine, G = guanine, Y = pyrimidine, and n means 0–13 bases) (Jordan et al., 2008; Hernández Borrero and El-Deiry, 2021). Through this domain, P53 acts as a transcription factor in a sequence-specific way by identifying the P53 reaction element (el-Deiry et al., 1992). Another area is the regulatory domain (RD), which can combine with PUMA and MDM2 to produce corresponding changes (Figure 1).

2.2 Common structure mutation of P53

In P53 mutants, missense mutations are one of the most common mutation types, which are divided into DNA contact mutants and structural mutants. The first type of mutant, such as the P53-R273H mutant, can be replaced with residues in direct contact with DNA (Hainaut and Pfeifer, 2016). This kind of mutant may not shake the structure of P53. However, it may destruct the heat-stability. The mutant is characterized by the core unfolding area compared to the wild type. Another type of mutation is amino acid substitution that can destroy the core folding area, such as the P53-R175H mutation, which will lead to changes in the structure of P53. In humans, the replacement of codons 175 and 273 is one of the most common mutations (Bykov et al., 2018). In previous experiments, it has suggested that the mutations of R175H and R273H subverted the ability to bind zinc ions, and the function of P53 can be restored after treatment with zinc ion metallochaperones (Garufi et al., 2013). The mutated P53 will combine with wild P53 to make it inactive and no longer have tumor inhibitory function (Hernández Borrero and El-Deiry, 2021) (Figure 1).

3 Regulation of P53

As is known, the common post-translation modifications (PTM) include: phosphorylation, acetylation, ubiquitinization, methylation, demethylation and glycosylation (Hernández Borrero and El-Deiry, 2021). These modifications play an important role in stabilizing and activiting P53 (Brooks and Gu, 2003).

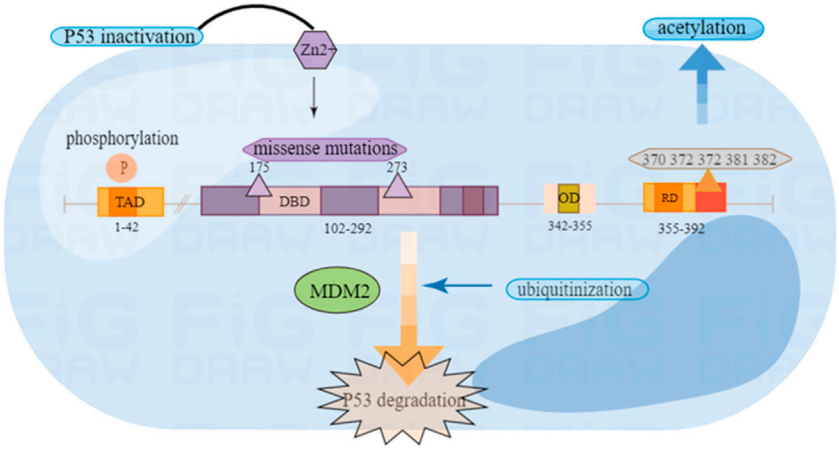

FIGURE 1
Wild type P53 structure and common mutation sites.

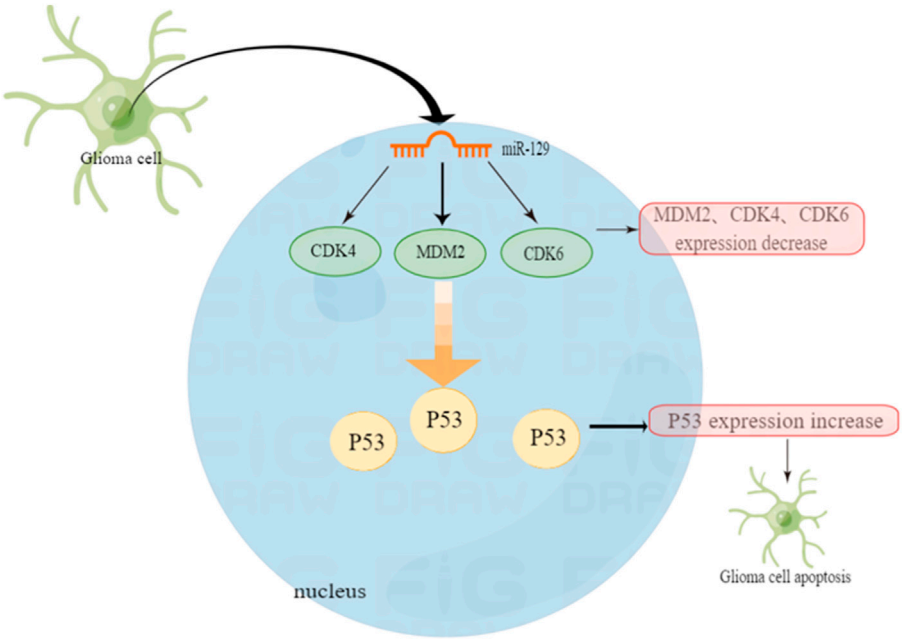

FIGURE 2
P53 function in glioblastoma and the regulation mechanism of non-coding micro RNA.

3.1 Phosphorylation

Phospholation is a modification of the P53 protein after translation, which can be phosphorylated on multiple amino acids in the N-terminal transcriptional activation area of the protein (Nakamizo et al., 2008). Post-translational modification of P53 through phosphorylation is considered to be an important mechanism to regulate the stability and function of P53. Moreover, the phosphorylation of P53 plays an important role in regulating activities such as DNA binding (Ashcroft et al., 1999). Most importantly, the phosphorylation of P53 plays an important role in regulating its activity. For

example, it can actively regulate the transcriptional activity of many transcription factors, including c-jun, CREB and NF-IL6/LAP, and regulate the stability of other proteins such as c-jun and NF-κB. Several serine residues of P53 protein in amino and carboxyl end regions are phosphorylated by some cytokinases (Unger et al., 1999). Phosphorylation and acetylation of P53 can interrupt negative regulatory factors (Chao et al., 2006).

The experimental results show that the simulated structural phosphorylation P53 constructor (Ad-P53-18D20D) at Thr18 and Ser20 can induce G1 stagnation of normal cell strains after exogenous non-phosphorylation P53 (Ad-P53) administration, indicating that the phosphorylation of P53 at Thr18 and Ser20 is enough to induce P53-mediated glioma cell apoptosis (Nakamizo et al., 2008). Experiments have also shown that Ser18 and 23 phosphorylation is required for P53-dependent apoptosis and tumor suppression in mice (Chao et al., 2006). Therefore, the regulation of phosphorylation after P53 translation may offer a potential treatment strategy in the treatment of tumors.

3.2 Ubiquitinization

Ubiquitin is a polypeptide composed of 72 amino acids. It can modify the translated proteins, and the most common function of ubiquitin modification is to target the degradation of substrate protein through the proteasome. The covalent connection process of ubiquitin is called ubiquitinization (Pickart, 2001). MDM2 is a ubiquitin ligase and exhibits the negative regulating effect on P53 (Watson and Irwin, 2006). Both MDM2 and MDMX can negatively regulate the transcription activity and stability of P53. MDM2 is the target of P53 transcription. As a ubiquitin ligase, P53 is ubiquitinized by MDMX, which is a kind of PTM that damages the function of P53 (Joerger and Fersht, 2016; Kwon et al., 2017). Therefore, blocking the interaction between P53 and MDM2 can stabilize it, leading to the cessation of the cell cycle and preventing the further development of the tumor. It also provides strategies for molecular treatment of tumors (Vassilev et al., 2004). MDM2 is functioning in three pathways, as the negative regulator of P53. The first is to combine P53 with its activation area to inhibit its transcription ability (Linke et al., 2008). The second is to participate in the nuclear output of P53. Third, it promotes the degradation of P53 as a ubiquitin ligase (Michael and Oren, 2003). Some scholars used small-molecule inhibitor to suppress the MDM2-P53 complex and proved that in mouse, P53 could be activated and tumor growth be suppressed (Vassilev et al., 2004; Zhao et al., 2015). Experiments show that MDM2 antagonists promote cell growth inhibition and apoptosis, which brings potential value to the treatment of tumors (Tortora et al., 2000).

3.3 Acetylation

Acetylation can modify the lysine (K) residue at C-terminal of P53, which is an important modification site after the transcription of P53. The C-terminal regulation domain of six lysine residues of P53 (K370 K372 K373 K381 K382 K386) can be targeted by MDM2. Acetylated modification can cause the transcription activity of P53 to be activated and increase its stability. CBP/p300 (p300 is the auxiliary activator required for P53-dependent Waf1/Cip1 transcription activation) is a transcriptional co-activator protein, which can interact with P53 to regulate the early cell cycle and trigger apoptosis of genetically intoxicated cells. So as to prevent the further development of the tumor (Lee et al., 1998; Grossman et al., 2003). After DNA damage, P53 can also be protected from degradation through the acetylation targeting on carboxyl terminal. This acetylation is conducive to cell survival because it promotes the expression of cell cycle stagnation target genes controlled by P53, such as the cell cyclin-dependent kinase inhibitor 1A (CDKN1A, also known as P21) (Knights et al., 2006; Kruse and Gu, 2008). Moreover, there are K120 and K164 in the binding domain of P53 DNA, which are the most common mutation regions of P53 in solid malignant tumors. For example, in glioblastoma, K164 has a mutation, indicating that acetylation of P53 plays a significant role in tumor inhibition (Zhang et al., 2015; He et al., 2017).

4 Regulation target of P53

4.1 PUMA and P53

P53 upregulated modulator of apoptosis (PUMA) was identified as a transcription target for P53 (Nakano and Vousden, 2001). It is highly conservative between humans and mice. The genetic structure of PUMA in mice and humans is also similar (Han et al., 2001). This protein belongs to the BH3-Only subgroup of Bcl-2 protein family. The BH3 domain of PUMA is necessary for its interaction with Bcl2-like proteins (Naik et al., 2007). The BH3 domain of PUMA forms a bikinetic alpha-helical structure, which binds directly to the anti-apoptotic Bcl2 family (Day et al., 2008). The C-terminal part of PUMA contains a hydrophobic domain to guide its mitochondrial location. BH3 domain and mitochondrial localization are essential for PUMA's ability to induce apoptosis or inhibit cell survival (Yu and Zhang, 2008). PUMA is usually expressed at a low level, but once stressed, its expression will be immediately induced (Yu et al., 2001). Bioinformatic analysis revealed the gene promoters, exons and introns of PUMA. In the transcription factors of PUMA, P53 plays a significant role, and its function is explored most thoroughly for now.

PUMA is a member of the Bcl-2 family that only relies on BH3 (BH3). It is an important mediator of P53-dependent and

independent apoptosis. It transmits death signals to mitochondria, where it indirectly acts on Bcl2 family members by removing the inhibition exerted by anti-apoptosis members. Bax and/or Bak. It directly binds and antagonizes all known members of the Bcl2 family who are anti-apoptotic, thus inducing mitochondrial dysfunction and caspase activation. Therefore, PUMA can be activated to inhibit tumor growth by restoring the apoptosis of cancer cells (Yu and Zhang, 2008). In this process, the promoter of PUMA binds to P53 to promote the modification of core histones. For example, as is mentioned above about the post-translational acetylation, P53 acetylates the core histones, leading to the opening and transcriptional activation of the chromatin structure. After P53 activates PUMA, it initiates cell apoptosis, thus preventing tumor cells from growing (Kim et al., 2019). To sum up, after DNA damage occurs, nuclear P53 will immediately induce PUMA production, thus protmoting apoptosis.

Zhang et al. reported in their study that the role of miR-221/222 in the regulation of apoptosis was confirmed. MiR-221/222 gene knockout (KO) can cause mitochondrial membrane potential changes and caspase-mediated apoptosis. In addition, they also proved that the apoptotic protein PUMA is negatively regulated by miR-221/222 (Zhang et al., 2010). In glioblastoma, experiments show that miR-221/222 regulates the mitochondrial pathway by directly targeting PUMA to induce cell survival (Zhang et al., 2010).

4.2 P21 and P53

Similar to PUMA, CDKN1A (P21) is also one of the downstream factors regulated by P53. It is one of the important target genes for P53 to induce cell cycle stagnation. *Cdkn1a* gene encodes P21W AF1 protein. AF1 protein is a cyclin-dependent kinase inhibitor that can directly interact and inhibit the cyclin-dependent kinase (CDK) complex, causing cell cycle stagnation (el-Deiry et al., 1993; Harper et al., 1993). Through the action of CDK, P21 inhibits the phosphorylation of Rb, and then Rb binds to E2F to prevent the transcription required for the progression of the cell cycle. P21 can interact with multiple CDK complexes expressed in the cell cycle, resulting in the cell cycle stagnation in different phases. Specifically, the interaction between P21 and Cyclin E/CDK2 and Cyclin D/CDK4 promotes the binding of Rb to E2F, leading to the cease in G1 phase (Nakanishi et al., 1999; Stewart et al., 1999). On the other hand, the binding of P21 to Cyclin B/CDK1 leads to G2/M cell cycle stagnation (Dash and El-Deiry, 2005). Therefore, as a downstream factor of P53, P21 can also participate in the regulation of the cell cycle.

5 P53 and gliomas

Tumors arising from glial cells (gliomas) make up the most common group of primary brain tumors (Friedmann-Morvinski et al., 2012). Despite the use of multiple therapies in combination regarding surgery, radiotherapy and/or chemotherapy, the survival of patients with high grade or recurrent gliomas remains poor; the median survival of patients with glioblastoma is less than a year (Phillips et al., 2006; Ma et al., 2021). In the latest WHO glioma classification, adult-type diffuse gliomas are divided into the following categories: 1) Astrocytoma, IDH-mutant; 2) Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted; 3) Glioblastoma, IDH-wild type (Louis et al., 2021). To improve therapeutic approaches for patients with gliomas and to understand glioma biology better, current research has focused on molecular and genetic alterations associated with the development and progression of gliomas (Ma et al., 2021).

The *P53* gene, which resides on chromosome 17p13.1 and encodes the P53 protein, is the most frequent target for mutations in human cancers, with more than half of all tumors exhibiting a mutation at this locus (Vogelstein et al., 2000; Freed-Pastor and Prives, 2012). Loss of *P53* transcriptional activity, mutations in *P53* gene or inhibition on P53 signaling, are major contributing factors to malignant transformation (Jain and Barton, 2018). P53 participates in many cellular functions including cell cycle control, DNA repair, cell differentiation, genomic plasticity, and programmed cell death (Hollstein et al., 1991; Harris, 1996b). The prime function of wild type P53 is the ability to promote the stagnation of the cell cycle and apoptosis (Kastenhuber and Lowe, 2017). Therefore, the activation of P53 can prevent and eliminate DNA-damaged cells to prevent the accumulation of oncogene mutations to ward off cancer (Livingstone et al., 1992). P53 also functions to modulate the downstream signaling pathway or modify the translated protein to regulate the apoptosis of tumor cells and cell cycle (Brady et al., 2011).

The glioma is the most common primary brain tumors in neurosurgery, especially glioblastoma (GBM) which is with the highest grade. It usually has a fully structured *P53* gene. Therefore, the proliferation of GBM and resistance to treatment may be related to the loss of P53 function (Lou et al., 2020). In the experiment, cyclic RNA CDR1as is widely expressed in the brain of mammals, which can decrease with the increase of glioma grade and can predict the total survival period of patients with glioma. Moreover, CDR1as can bind to and stabilize P53 by preventing ubiquitination. CDR1as interacts directly with the P53 DBD domain necessary for MDM2 binding, thus undermining the formation of the P53/MDM2 complex (Lou et al., 2020), preventing MDM2 from negatively regulating P53. The study also proved that the enhanced expression of CDR1as significantly inhibits cell proliferation, while the down-regulation of CDR1as promotes cloning (Lou et al., 2020).

Not only cyclic RNA CDR1as can inhibit cell proliferation by changing the relationship between MDM2 and P53. In one experiment, miRNA-129 inhibits glioma cell growth by targeting CDK4, CDK6, and MDM2. The experimental results show that over-expression of miR-129 can reduce the expression of CDK4 genes in HEK293 cells by 58.9% and CDK6 by 35.7%.

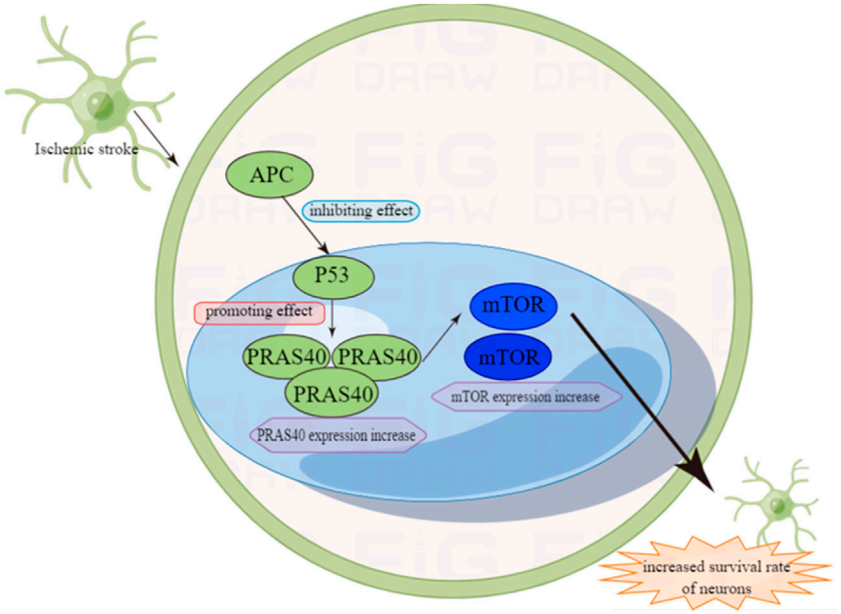

FIGURE 3
The role of P53 in ischemic stroke.

The expression of MDM2 has also been reduced by 49%. Therefore, we can say that miR-129 significantly targets and inhibits the expression of CDK4, CDK6 and MDM2. Additionally, miR-129 also inhibits cell proliferation by affecting MDM2 (Moradimotlagh et al., 2020).

In another experiment, it was also confirmed that miR-29a can raise the level of P53 and induce apoptosis dependent on P53 function (Park et al., 2009). Chen et al. reported that miR-29a negatively regulates the expression of MDM2 by directly targeting MDM2 in glioma cells (Chen et al., 2021). As a negative regulator of the *P53* gene, over-expression of miR-29 can precisely inhibit MDM2. That is to say, it can enhance the stable expression of P53, thus facilitating apoptosis and cell cycle stagnation in tumor cells (Moradimotlagh et al., 2020). The various MDM2 inhibitors mentioned above have shown inhibitory effects on MDM2 and P53, laying the foundation for future treatment of glioma and improvement of its prognosis. For example, the MDM2 inhibitor RG7112, the first one to be admitted to clinical trials, although not yet extensively carried out, it provides a certain basis for molecular targeted treatment of gliomas (Figure 2).

6 P53 and cerebral stroke

6.1 P53 and ischemic stroke

Ischemic stroke is the main cause of morbidity and death, and the result depends on the extent on the number of neuronal deaths. Cerebral ischemia and hypoxia activate P53 to provide targets for the treatment of stroke (Arumugam et al., 2018). Many studies have shown that P53 is an apoptosis-promoting factor, which expresses an increase in cell apoptotic injury after cerebral ischemia. In addition, in different stroke models, the loss of P53 or the application of P53 inhibitors may potentially reduce the volume of cerebral infarction (Hong et al., 2010). Not only that, more and more experiments have shown that P53 is related to the death of neurons in animal experimental models of cerebral ischemia or hypoxia (Banasiak and Haddad, 1998; Leker et al., 2004; Endo et al., 2006; Yonekura et al., 2006; Damisah et al., 2020). P53-mediated apoptosis is a common cell death mechanism that can be triggered by oxidative stress or DNA damage (Amundson et al., 1998). It is activated in the cerebral ischemia area and promotes the apoptosis of neurons. The lack of P53 or the application of its inhibitors can significantly reduce brain damage. P53-mediated apoptosis of nerve cells occurs through a variety of molecular mechanisms, such as Notch signaling pathway (Hong et al., 2010; Arumugam et al., 2018). Notch1 is a membrane receptor that regulates the proliferation, differentiation and transition of cells in a series of tissues (Lathia et al., 2008). In the developing brain, Notch signals participate in the preservation of nerve precursor cells in an unduplicated state, partly by inhibiting neurogenesis. Notch signals also affect synaptic plasticity and learning memory in the adult brain (Alberi et al., 2013; Sargin et al., 2013). As shown in a study, active Notch inhibits the growth of B-cell, interrupts the cell cycle, and induces apoptosis (Morimura et al., 2000). An

active form of Notch1 can raise the level of nuclear P53 to promote the transcription of apoptosis genes (Yang et al., 2004). Notch1 signaling pathways and four important interaction pathways (NF-κB, P53, HIF-1α and PIN1) are aggregated on a conservative DNA-related nucleopolyprotein complex that control the expression of genes that determine the fate of neurons. In mice experiments, mice with Notch inhibitors showed reduced damage to brain cells and improved functional results. Therefore, inhibiting Notch may prevent P53-mediated apoptosis and improve the activity of neurons (Arumugam et al., 2018).

Similarly, in the model of simulating cerebral ischemia, mouse focal cerebral ischemia, global cerebral ischemia (GCI) and transient frontal-temporal ischemia, it is found that the mRNA and protein of P53 in the ischemic area are elevated (van Lookeren Campagne and Gill, 1998; Watanabe et al., 1999; Hong et al., 2010). Research also shows that not only does the expression of P53 increase in ischemic areas, but also in astrocytes and neuron cells (Banasiak and Haddad, 1998). In ischemic stroke, the damage of cerebral endothelial cells is caused by ischemia. In order to prove the role of activated protein C (APC) in stroke, a hypoxic cerebral endothelial cell (CEC) injury model is constructed (Cheng et al., 2003). APC is a systemic anticoagulant and anti-inflammatory factor which can reduce organ damage. Experiments show that APC directly prevents the apoptosis of hypoxic human endothelial cells by suppressing P53 at transcriptional level, normalizing the Bax/Bcl2 ratio that promotes apoptosis and reducing the Caspase-3 signal transduction (Cheng et al., 2003).

P53 can also regulate ischemic stroke through the P53/PRAS40/mTOR pathway. The mTOR pathway is involved in a variety of physiological processes, including cell metabolism, growth, differentiation, development and cell survival (Liu and Sabatini, 2020). In addition, it is also involved in the protection of cerebral ischemia (Xie et al., 2018). In previous studies, it has been proven that the activation of mTOR can reduce stroke-related neurons (Xie et al., 2014). In the P53/PRAS40/mTOR pathway, one of the important components of the mTOR complex is the proline-rich Akt and substrate PRAS40, which is at downstream of Akt, and the phosphorylated PRAS40 (pPRAS40) can activate the mTOR pathway (Kovacina et al., 2003; Wiza et al., 2014). Previous studies have reported the negative feedback relationship between P53 and PRAS40, indicating that P53 can be inhibited by its downstream factor PRAS40. Therefore, it can connect P53 with PRAS40 and mTOR and play an important role in ischemic stroke (Xiong et al., 2014). The brain damage of mice with gene *PRAS40* knockout is more serious than normal mice after ischemic stroke. The over-expression of pPRAS40 can reduce brain ischemia/reperfusion (I/R) damage and autophagy by activating mTOR. Not only that, studies have suggested that there is a negative feedback relationship between P53 and PRAS40, and P53 can be inhibited by its downstream factor PRAS40 (Havel et al., 2015; Zhao et al., 2021). In three groups of mice experiments: *P53* KO mice (*P53*−/−), heterozygous (*P53*+/−) mice, and WT mice (*P53*+/+), the results showed that both P53 KO and heterozygous groups had an improved neurological function and reduced area of cerebral infarction, and P53 KO group showed a better protective effect (Havel et al., 2015). This study indicates that I/R damage to neurons *in vivo* and *in vitro* can be reduced by inhibiting the P53/PRAS40/mTOR pathway (Figure 3).

6.2 P53 and hemorrhagic stroke

P53 can regulate hemorrhagic stroke by indirectly regulating ferroptosis. Ferroptosis is a non-apoptotic programed cell death which has been discovered in recent years (Zhang et al., 2021). Being different from apoptosis, ferroptosis is triggered by iron overload intracellularly, and characterized by specific pathological changes such as unique mitochondrial shrinking under transmission electronic microscope (TEM) (Alim et al., 2019). It has been noted that P53 has a strong relationship with ferroptosis especially in cerebral stroke (Jiang et al., 2015).

In ferroptosis, the three most important components are lipids, iron and ROS. Metabolic disorder of one of the three may lead to ferroptosis. P53 participates in the metabolic regulation of the three, therefore we have good rationale to infer that P53 can regulate ferroptosis (Liu and Gu, 2021).

Ferroptosis can be driven by fatal lipid peroxidation, which may be caused by cell metabolism and redox imbalances (Jiang et al., 2021). In 1950s, studies showed that cysteine deficiency may cause cell death, and endogenously synthesized cysteine may resist cell death (Coltorti et al., 1956; Eagle et al., 1961). Cysteine is a speed limiting factor in synthesis of glutathione (GSH), which can pass through neutral amino acid transporter or oxidized by cysteine/glutamate reverse transporter (a transmembrane protein complex containing SLC7A11 and SLC3A2 subunits, known as system xc−) (Bannai and Kitamura, 1980; Sato et al., 1999) to absorbed in environment or synthesized using the sulfurization pathway of methionine and glucose (Meister, 1995). GSH is a cofactor of many enzymes, including glutathione peroxidase (GPX4) and glutathione-S-transferase (Meister, 1995).

It has been reported that GSH and GPX4 can protect cells from various oxidative stress (Liu and Gu, 2021). GPX4 is a selenium protein that can reduce oxidized lipids. In the experiment from Conrad et al., the mouse model that knocked out the gene *Gpx4* showed that the deletion of the *Gpx4* gene would lead to the death of non-apoptotic cells

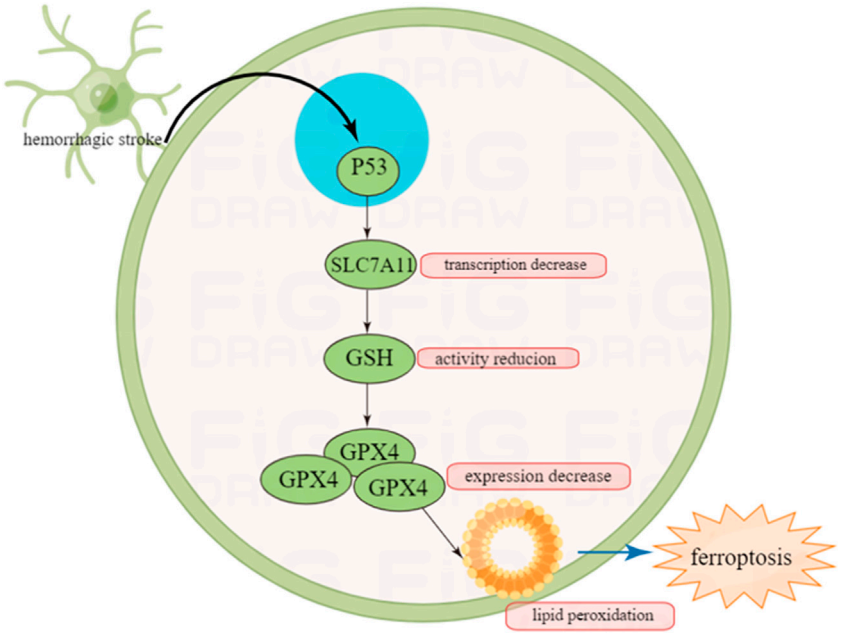

FIGURE 4
Inhibition of P53 on ferroptosis after hemorrhagic stroke and this may provide new insight in the treatment of cerebral stroke in the future.

realted to lipid peroxidation in mouse embryonic fibroblasts (Seiler et al., 2008). Therefore, inhibiting the lipid peroxidation of cells can reduce ferroptosis.

A study in 2015 demonstrated that P53 can promote ferroptosis by inhibiting the ability of cysteine to enter target cells (Jiang et al., 2015). In terms of mechanism, P53 can prevent cysteine from further generating GSH by inhibiting the transcription of subunit SLC7A11 of the cysteine/glutamate reverse transporter core. GSH is an antioxidant utilized by GPX4 to inhibit ferroptosis. Therefore, the inhibition of SLC7A11 by P53 can reduce intracellular GSH levels and promote cell ferroptosis (Yang et al., 2014).

It is well know that after intracerebral hemorrhage (ICH), one of the mechanisms causing brain cell death is *via* ferroptosis (Karuppagounder et al., 2016). Once ICH occurs, an increase in lipid peroxidation can be observed in neuronal cells (Karuppagounder et al., 2018). In mice experiments, the level of GPX4 in neurons is selectively manipulated to verify whether improving GPX4 has a protective effect on neurons after ICH. The results show that in the first 2 weeks of ICH, increased expression of GPX4 can reduce apoptosis, and have a protective effect on neurons (Alim et al., 2019).

To sum up, P53 may indirectly regulate the prognosis of intracranial hemorrhage by indirectly regulating ferroptosis, which also provides a certain basis for the treatment of hemorrhagic stroke (Figure 4).

7 P53 and neurodegenerative diseases

7.1 P53 and Alzheimer's disease

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease (Olivier et al., 2010). Its clinical characteristics are progressive memory disorders, personality and behavioral changes (Qi et al., 2019). Its pathology is characterized by three main histological lesions, the senile plaques, the neurofibrillary tangles and atrophy of cerebral cortex. While senile plaques are extracellular deposits mainly due to the aggregation of a set of various hydrophobic peptides under the term of amyloid-β (Aβ) peptides (Checler and Alves da Costa, 2014). Aβ protein may cause neurotoxicity *via* inflammatory responses, especially small and soluble Aβ oligomers. The main pathological characteristics of AD are diffuse atrophy of the cerebral cortex and massive synaptic loss and excessive phosphorylated tau protein aggregates in cells (Qi et al., 2019), which is considered to be associated with many neurodegenerative diseases, and changes in tau protein are believed to be the result of downstream Aβ protein toxicity in the amylosis hypothesis (Dickson et al., 2013).

Due to the lack of timely access to anatomical tissue and various technical limitations, the death of nerve cells from apoptosis has always been controversial, but there is also some evidence that cell death in the brain of AD patients occurs to some extent through apoptosis (Kusiak et al., 1996). Some observations show that the decomposition and metabolism derived from β-Amyloid precursor

protein (β-APP) can indeed trigger apoptosis in cell and animal models (Matsui et al., 2006). β-APP-derived fragments can also adjust P53. In order to identify P53 as a marker of AD, the experiment compared the impact of AD on the brain and the P53-like immune response in the control group. Through measurement, it was found that the expression of P53 was significantly enhanced, not only in glial cells, but also in many neuronal cells. This shows that P53 may lead to cell death in the AD brain (Ohyagi et al., 2005). Parallel increase in P53 and cell death can be observed in AD-affected brains to suggest that P53 plays a role in cell cycle stagnation, DNA repair and apoptosis (Bourdon et al., 2003; Aylon and Oren, 2011). Therefore, enhancing the expression of P53 may be the cause of an increase in cell death observed in the AD brain. P53 can also control the expression of proteins involved in AD pathology, indirectly affecting the course of the disease, rather than the classical target proteins.

P53 is also an important regulator for the expression of miRNAs. Some miRNAs, especially miRNAs from the miR-34 family, have been identified as direct transcription targets for P53. The expression of P53 horizontally induces the expression of miR-34 after transcription by binding to the DROSHA complex (Rokavec et al., 2014). In particular, miR-34a has the highest expression in the brain. As was reported, miR-34a could regulate the differentiation of neurons and the growth of axons. In the brain tissue of AD patients, it was found that its expression was up-regulated. John R. Dickson's study found that changes in the expression of miR-34a can regulate the expression of tau in the pathogenesis of AD. The possible mechanism is that when the protein of tau aggregates, the up-regulation of miR-34a may be used as a compensation mechanism to reduce the expression of tua. This maight offer the opportunity to improve the prognosis of AD patients (Dickson et al., 2013). The miR-34a also regulates the expression of several synaptic proteins in cortical neurons (Jazvinšćak Jembrek et al., 2018). Oxidative stress is one of the conditions under which AD occurs. Under the condition of oxidative stress, P53 will react (Jazvinšćak Jembrek et al., 2018). Generally speaking, in mild oxidative damage, P53 can antioxidate to promote cell survival, while in severe oxidative stress, P53 can promote oxidative activity and lead to cell death (Jazvinšćak Jembrek et al., 2018). In human AD, the increase in the expression of P53 is directly proportional to the accumulation of Aβ protein in cells. Oxidative stress is closely related to mitochondrial dysfunction, and P53 can protect mitochondrial function by promoting the production of new mitochondria (Jazvinšćak Jembrek et al., 2018).

7.2 P53 and Parkinson's disease

Parkinson's disease (PD) is a motor-related disease characterized by the loss of dopamine energy neurons in the dense part of the grey matter (Pariyar et al., 2017). Oxidative stress and mitochondrial dysfunction are the main pathological motivations of PD. Slow motion, stiffness, tremor and abnormal posture are clinical manifestations (Talebi et al., 2021). The treatment of PD is drug therapy and surgical operation, mainly for symptoms. In autopsy studies, the expression of P53 and its target gene Bax in the tissues of PD patients increased (Qi et al., 2016). Recent literature shows that P53 is involved in the occurrence of familial PD through Parkin-mediated transcription regulation. Experiments show that the toxicity of α-synaptic nucleoprotein associated with P53 is shown in the PD model *in vitro* and *in vivo* (Duplan et al., 2016). P53 is also involved in the regulation of DJ-1, Park7's mRNA and protein expression. P53 is a feasible missing link between hereditary and sporadic PD (Venderova and Park, 2012).

Parkin is a ubiquitin E3 ligase, a transcription inhibitor of P53. Exogenous *Parkin* gene deletion leads to increased *P53* gene expression (da Costa et al., 2009). Phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1/NOXA) balances the P53-dependent apoptosis associated with mitochondrial injures (Talebi et al., 2021). P53 can bind to Bcl2 to induce apoptosis by releasing Bcl2-associated protein X/Bcl2 homologous antagonist (Bax/Bak) from MPTP (Mattson and Magnus, 2006). The over-expression of P53 weakens the calcium ion transfer of mitochondria and provocates mitochondrial disturbance (Moon and Paek, 2015). Therefore, mitochondrial dysfunction caused by excessive expression of P53 is an important factor in PD progression. Moreover, the interaction of P53/Bclxl destroys the mitochondrial membrane potential and participates in the pathogenesis of PD.

8 Discussion and perspective

As a classic oncogene, P53 has guiding significance in tumor treatment and CNS diseases. In molecular biology, it provides a theoretical basis for treatment. For example, in glioblastoma, surgical operation plus radiotherapy and chemotherapy do not significantly improve the 5-year survival rate; the regulation of tumor cell death by P53 can be used as a potential strategy to improve the outcome of glioblastoma. Research on P53 has not been interrupted, and it has also been found that more and more molecules are regulated or controlled by P53, and *vice versa*. This has laid a solid foundation for the future development of drugs and molecular targeted treatment. However, there are relatively few studies of P53 in cerebral ischemic and hemorrhage stroke, particularly hemorrhagic stroke. After hemorrhagic stroke, how to reduce the apoptosis of neurons and glial cells through the regulation of P53 is an important subject in the

future. Regulation of the expression of P53 to treat cerebral hemorrhagic stroke may become a potential hotspot.

Author contributions

LL, QL, and GM established the work and wrote the manuscript; TL revised the manuscript; JD and WL supervised and coordinated the study and are shared correspondence.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Alberi, L., Hoey, S. E., Scotti, A. L., and Marathe, S. (2013). Notch signaling in the brain: In good and bad times. *Ageing Res. Rev.* 12 (3), 801–814. doi:10.1016/j.arr.2013.03.004

Alim, I., Caulfield, J. T., Chen, Y., Swarup, V., Geschwind, D. H., Ivanova, E., et al. (2019). Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. *Cell* 177 (5), 1262–1279. doi:10.1016/j.cell.2019.03.032

Amundson, S. A., Myers, T. G., and Fornace, A. J., Jr. (1998). Roles for p53 in growth arrest and apoptosis: Putting on the brakes after genotoxic stress. *Oncogene* 17 (25), 3287–3299. doi:10.1038/sj.onc.1202576

Arumugam, T. V., Baik, S. H., Balaganapathy, P., Sobey, C. G., Mattson, M. P., and Jo, D. G. (2018). Notch signaling and neuronal death in stroke. *Prog. Neurobiol.* 165-167, 103–116. doi:10.1016/j.pneurobio.2018.03.002

Ashcroft, M., Kubbutat, M. H., and Vousden, K. H. (1999). Regulation of p53 function and stability by phosphorylation. *Mol. Cell Biol.* 19 (3), 1751–1758. doi:10.1128/mcb.19.3.1751

Aylon, Y., and Oren, M. (2011). p53: guardian of ploidy. *Mol. Oncol.* 5 (4), 315–323. doi:10.1016/j.molonc.2011.07.007

Banasiak, K. J., and Haddad, G. G. (1998). Hypoxia-induced apoptosis: Effect of hypoxic severity and role of p53 in neuronal cell death. *Brain Res.* 797 (2), 295–304. doi:10.1016/s0006-8993(98)00286-8

Bannai, S., and Kitamura, E. (1980). Transport interaction of L-cystine and L-glutamate in human diploid fibroblasts in culture. *J. Biol. Chem.* 255 (6), 2372–2376. doi:10.1016/s0021-9258(19)85901-x

Bourdon, J. C., Laurenzi, V. D., Melino, G., and Lane, D. (2003). p53: 25 years of research and more questions to answer. *Cell Death Differ.* 10 (4), 397–399. doi:10.1038/sj.cdd.4401243

Brady, C. A., Jiang, D., Mello, S. S., Johnson, T. M., Jarvis, L. A., Kozak, M. M., et al. (2011). Distinct p53 transcriptional programs dictate acute DNA-damage responses and tumor suppression. *Cell* 145 (4), 571–583. doi:10.1016/j.cell.2011.03.035

Brooks, C. L., and Gu, W. (2003). Ubiquitination, phosphorylation and acetylation: The molecular basis for p53 regulation. *Curr. Opin. Cell Biol.* 15 (2), 164–171. doi:10.1016/s0955-0674(03)00003-6

Bykov, V. J. N., Eriksson, S. E., Bianchi, J., and Wiman, K. G. (2018). Targeting mutant p53 for efficient cancer therapy. *Nat. Rev. Cancer* 18 (2), 89–102. doi:10.1038/nrc.2017.109

Chao, C., Herr, D., Chun, J., and Xu, Y. (2006). Ser18 and 23 phosphorylation is required for p53-dependent apoptosis and tumor suppression. *Embo J.* 25 (11), 2615–2622. doi:10.1038/sj.emboj.7601167

Checler, F., and Alves da Costa, C. (2014). p53 in neurodegenerative diseases and brain cancers. *Pharmacol. Ther.* 142 (1), 99–113. doi:10.1016/j.pharmthera.2013.11.009

Chen, Q., Wang, W., Chen, S., Chen, X., and Lin, Y. (2021). miR-29a sensitizes the response of glioma cells to temozolomide by modulating the P53/MDM2 feedback loop. *Cell Mol. Biol. Lett.* 26 (1), 21. doi:10.1186/s11658-021-00266-9

Cheng, T., Liu, D., Griffin, J. H., Fernandez, J. A., Castellino, F., Rosen, E. D., et al. (2003). Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat. Med.* 9 (3), 338–342. doi:10.1038/nm826

Coltorti, M., De Ritis, F., and Giusti, G. (1956). Enzymatic mechanisms of transsulfuration in biology and clinical practice. *G. Clin. Med.* 37 (3), 285–323.

da Costa, C. A., Sunyach, C., Giaime, E., West, A., Corti, O., Brice, A., et al. (2009). Transcriptional repression of p53 by parkin and impairment by mutations associated with autosomal recessive juvenile Parkinson's disease. *Nat. Cell Biol.* 11 (11), 1370–1375. doi:10.1038/ncb1981

Damisah, E. C., Hill, R. A., Rai, A., Chen, F., Rothlin, C. V., Ghosh, S., et al. (2020). Astrocytes and microglia play orchestrated roles and respect phagocytic territories during neuronal corpse removal *in vivo*. *Sci. Adv.* 6 (26), eaba3239. doi:10.1126/sciadv.aba3239

Dash, B. C., and El-Deiry, W. S. (2005). Phosphorylation of P21 in G2/M promotes cyclin B-Cdc2 kinase activity. *Mol. Cell Biol.* 25 (8), 3364–3387. doi:10.1128/MCB.25.8.3364-3387.2005

Day, C. L., Smits, C., Fan, F. C., Lee, E. F., Fairlie, W. D., and Hinds, M. G. (2008). Structure of the BH3 domains from the p53-inducible BH3-only proteins Noxa and Puma in complex with Mcl-1. *J. Mol. Biol.* 380 (5), 958–971. doi:10.1016/j.jmb.2008.05.071

Dickson, J. R., Kruse, C., Montagna, D. R., Finsen, B., and Wolfe, M. S. (2013). Alternative polyadenylation and miR-34 family members regulate tau expression. *J. Neurochem.* 127 (6), 739–749. doi:10.1111/jnc.12437

Duplan, E., Giordano, C., Checler, F., and Alves da Costa, C. (2016). Direct α-synuclein promoter transactivation by the tumor suppressor p53. *Mol. Neurodegener.* 11, 13. doi:10.1186/s13024-016-0079-2

Eagle, H., Piez, K. A., and Oyama, V. I. (1961). The biosynthesis of cystine in human cell cultures. *J. Biol. Chem.* 236, 1425–1428. doi:10.1016/s0021-9258(18)64190-0

el-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W., and Vogelstein, B. (1992). Definition of a consensus binding site for p53. *Nat. Genet.* 1 (1), 45–49. doi:10.1038/ng0492-45

el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., PaRsons, R., Trent, J. M., et al. (1993). WAF1, a potential mediator of p53 tumor suppression. *Cell* 75 (4), 817–825. doi:10.1016/0092-8674(93)90500-p

Endo, H., Kamada, H., Nito, C., Nishi, T., and Chan, P. H. (2006). Mitochondrial translocation of p53 mediates release of cytochrome c and hippocampal CA1 neuronal death after transient global cerebral ischemia in rats. *J. Neurosci.* 26 (30), 7974–7983. doi:10.1523/JNEUROSCI.0897-06.2006

Freed-Pastor, W. A., and Prives, C. (2012). Mutant p53: One name, many proteins. *Genes Dev.* 26 (12), 1268–1286. doi:10.1101/gad.190678.112

Friedmann-Morvinski, D., Bushong, E. A., Ke, E., Soda, Y., Marumoto, T., Singer, O., et al. (2012). Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. *Science* 338 (6110), 1080–1084. doi:10.1126/science.1226929

Garufi, A., Trisciuoglio, D., Porru, M., Leonetti, C., Stoppacciaro, A., D'Orazi, V., et al. (2013). A fluorescent curcumin-based Zn(II)-complex reactivates mutant (R175H and R273H) p53 in cancer cells. *J. Exp. Clin. Cancer Res.* 32 (1), 72. doi:10.1186/1756-9966-32-72

Grossman, S. R., Deato, M. E., Brignone, C., Chan, H. M., Kung, A. L., Tagami, H., et al. (2003). Polyubiquitination of p53 by a ubiquitin ligase activity of p300. *Science* 300 (5617), 342–344. doi:10.1126/science.1080386

Hainaut, P., and Pfeifer, G. P. (2016). Somatic TP53 mutations in the era of genome sequencing. *Cold Spring Harb. Perspect. Med.* 6 (11), a026179. doi:10.1101/cshperspect.a026179

Han, J., Flemington, C., Houghton, A. B., Gu, Z., Zambetti, G. P., Lutz, R. J., et al. (2001). Expression of bbc3, a pro-apoptotic BH3-only gene, is regulated by diverse cell death and survival signals. *Proc. Natl. Acad. Sci. U. S. A.* 98 (20), 11318–11323. doi:10.1073/pnas.201208798

Harper, J. W., Adami, G. R., WeiN.Keyomarsi, K., and Elledge, S. J. (1993). The P21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 75 (4), 805–816. doi:10.1016/0092-8674(93)90499-g

Harris, C. C. (1996). p53 tumor suppressor gene: at the crossroads of molecular carcinogenesis, molecular epidemiology, and cancer risk assessment. *Environ. Health Perspect.* 104 (3), 435–439. doi:10.1289/ehp.96104s3435

Harris, C. C. (1996). Structure and function of the p53 tumor suppressor gene: Clues for rational cancer therapeutic strategies. *J. Natl. Cancer Inst.* 88 (20), 1442–1455. doi:10.1093/jnci/88.20.1442

Havel, J. J., Li, Z., Cheng, D., Peng, J., and Fu, H. (2015). Nuclear PRAS40 couples the Akt/mTORC1 signaling axis to the RPL11-HDM2-p53 nucleolar stress response pathway. *Oncogene* 34 (12), 1487–1498. doi:10.1038/onc.2014.91

He, Y., Gao, M., Cao, Y., Tang, H., Liu, S., and Tao, Y. (2017). Nuclear localization of metabolic enzymes in immunity and metastasis. *Biochim. Biophys. Acta Rev. Cancer* 1868 (2), 359–371. doi:10.1016/j.bbcan.2017.07.002

Hernández Borrero, L. J., and El-Deiry, W. S. (2021). Tumor suppressor p53: Biology, signaling pathways, and therapeutic targeting. *Biochim. Biophys. Acta Rev. Cancer* 1876 (1), 188556. doi:10.1016/j.bbcan.2021.188556

Hollstein, M., SiDransky, D., Vogelstein, B., and Harris, C. C. (1991). p53 mutations in human cancers. *Science* 253 (5015), 49–53. doi:10.1126/science.1905840

Hong, L. Z., Zhao, X. Y., and Zhang, H. L. (2010). p53-mediated neuronal cell death in ischemic brain injury. *Neurosci. Bull.* 26 (3), 232–240. doi:10.1007/s12264-010-1111-0

Jain, A. K., and Barton, M. C. (2018). p53: emerging roles in stem cells, development and beyond. *Development* 145 (8), dev158360. doi:10.1242/dev.158360

Jazvinšćak Jembrek, M., Slade, N., Hof, P. R., and Simic, G. (2018). The interactions of p53 with tau and Aß as potential therapeutic targets for Alzheimer's disease. *Prog. Neurobiol.* 168, 104–127. doi:10.1016/j.pneurobio.2018.05.001

Jeffrey, P. D., Gorina, S., and Pavletich, N. P. (1995). Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. *Science* 267 (5203), 1498–1502. doi:10.1126/science.7878469

Jiang, L., Kon, N., Li, T., Wang, S. J., Su, T., Hibshoosh, H., et al. (2015). Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520 (7545), 57–62. doi:10.1038/nature14344

Jiang, X., Stockwell, B. R., and Conrad, M. (2021). Ferroptosis: Mechanisms, biology and role in disease. *Nat. Rev. Mol. Cell Biol.* 22 (4), 266–282. doi:10.1038/s41580-020-00324-8

Joerger, A. C., and Fersht, A. R. (2016). The p53 pathway: Origins, inactivation in cancer, and emerging therapeutic approaches. *Annu. Rev. Biochem.* 85, 375–404. doi:10.1146/annurev-biochem-060815-014710

Jordan, J. J., Menendez, D., Inga, A., Noureddine, M., Nourredine, M., Bell, D. A., et al. (2008). Noncanonical DNA motifs as transactivation targets by wild type and mutant p53. *PLoS Genet.* 4 (6), e1000104. doi:10.1371/journal.pgen.1000104

Karuppagounder, S. S., Alim, I., Khim, S. J., Bourassa, M. W., Sleiman, S. F., John, R., et al. (2016). Therapeutic targeting of oxygen-sensing prolyl hydroxylases abrogates ATF4-dependent neuronal death and improves outcomes after brain hemorrhage in several rodent models. *Sci. Transl. Med.* 8 (328), 328ra29. doi:10.1126/scitranslmed.aac6008

Karuppagounder, S. S., Alin, L., Chen, Y., Brand, D., Bourassa, M. W., Dietrich, K., et al. (2018). N-acetylcysteine targets 5 lipoxygenase-derived, toxic lipids and can synergize with prostaglandin E(2) to inhibit ferroptosis and improve outcomes following hemorrhagic stroke in mice. *Ann. Neurol.* 84 (6), 854–872. doi:10.1002/ana.25356

Kastenhuber, E. R., and Lowe, S. W. (2017). Putting p53 in context. *Cell* 170 (6), 1062–1078. doi:10.1016/j.cell.2017.08.028

Kato, S., Han, S. Y., Liu, W., Otsuka, K., Shibata, H., Kanamaru, R., et al. (2003). Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc. Natl. Acad. Sci. U. S. A.* 100 (14), 8424–8429. doi:10.1073/pnas.1431692100

Kim, J., Yu, L., Chen, W., Xu, Y., Wu, M., Todorova, D., et al. (2019). Wild-type p53 promotes cancer metabolic switch by inducing PUMA-dependent suppression of oxidative phosphorylation. *Cancer Cell* 35 (2), 191–203. doi:10.1016/j.ccell.2018.12.012

Knights, C. D., Catania, J., Di Giovanni, S., Muratoglu, S., Perez, R., Swartzbeck, A., et al. (2006). Distinct p53 acetylation cassettes differentially influence gene-expression patterns and cell fate. *J. Cell Biol.* 173 (4), 533–544. doi:10.1083/jcb.200512059

Kovacina, K. S., Park, G. Y., Bae, S. S., Guzzetta, A. W., Schaefer, E., Birnbaum, M. J., et al. (2003). Identification of a proline-rich Akt substrate as a 14-3-3 binding partner. *J. Biol. Chem.* 278 (12), 10189–10194. doi:10.1074/jbc.M210837200

Kruse, J. P., and Gu, W. (2008). SnapShot: p53 posttranslational modifications. *Cell* 133 (5), 930–30.e1. doi:10.1016/j.cell.2008.05.020

Kusiak, J. W., Izzo, J. A., and Zhao, B. (1996). Neurodegeneration in Alzheimer disease. Is apoptosis involved? *Mol. Chem. Neuropathol.* 28 (1-3), 153–162. doi:10.1007/BF02815217

Kwon, S. K., Saindane, M., and Baek, K. H. (2017). p53 stability is regulated by diverse deubiquitinating enzymes. *Biochim. Biophys. Acta Rev. Cancer* 1868 (2), 404–411. doi:10.1016/j.bbcan.2017.08.001

Lathia, J. D., Mattson, M. P., and Cheng, A. (2008). Notch: From neural development to neurological disorders. *J. Neurochem.* 107 (6), 1471–1481. doi:10.1111/j.1471-4159.2008.05715.x

Lee, C. W., Sorensen, T. S., ShikamaN.and La Thangue, N. B. (1998). Functional interplay between p53 and E2F through co-activator p300. *Oncogene* 16 (21), 2695–2710. doi:10.1038/sj.onc.1201818

Leker, R. R., Aharonowiz, M., Greig, N. H., and Ovadia, H. (2004). The role of p53-induced apoptosis in cerebral ischemia: Effects of the p53 inhibitor pifithrin alpha. *Exp. Neurol.* 187 (2), 478–486. doi:10.1016/j.expneurol.2004.01.030

Lin, J., Chen, J., ElenBaas, B., and Levine, A. J. (1994). Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. *Genes Dev.* 8 (10), 1235–1246. doi:10.1101/gad.8.10.1235

Linke, K., Mace, P. D., Smith, C. A., Vaux, D. L., Silke, J., and Day, C. L. (2008). Structure of the MDM2/MDMX RING domain heterodimer reveals dimerization is required for their ubiquitylation in trans. *Cell Death Differ.* 15 (5), 841–848. doi:10.1038/sj.cdd.4402309

Liu, G. Y., and Sabatini, D. M. (2020). mTOR at the nexus of nutrition, growth, ageing and disease. *Nat. Rev. Mol. Cell Biol.* 21 (4), 183–203. doi:10.1038/s41580-019-0199-y

Liu, X., Miller, C. W., Koeffler, P. H., and Berk, A. J. (1993). The p53 activation domain binds the TATA box-binding polypeptide in Holo-TFIID, and a neighboring p53 domain inhibits transcription. *Mol. Cell Biol.* 13 (6), 3291–3300. doi:10.1128/mcb.13.6.3291

Liu, Y., and Gu, W. (2021). The complexity of p53-mediated metabolic regulation in tumor suppression. *Semin. Cancer Biol.* 85, 4–32. doi:10.1016/j.semcancer.2021.03.010

Livingstone, L. R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T. D. (1992). Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell* 70 (6), 923–935. doi:10.1016/0092-8674(92)90243-6

Lou, J., Hao, Y., Lin, K., Lyu, Y., Chen, M., Wang, H., et al. (2020). Circular RNA CDR1as disrupts the p53/MDM2 complex to inhibit Gliomagenesis. *Mol. Cancer* 19 (1), 138. doi:10.1186/s12943-020-01253-y

Louis, D. N., Perry, A., Wesseling, P., Brat, D. J., Cree, I. A., Figarella-Branger, D., et al. (2021). The 2021 WHO classification of tumors of the central nervous system: A summary. *Neuro Oncol.* 23 (8), 1231–1251. doi:10.1093/neuonc/noab106

Ma, R., Taphoorn, M. J. B., and Plaha, P. (2021). Advances in the management of glioblastoma. *J. Neurol. Neurosurg. Psychiatry* 92 (10), 1103–1111. doi:10.1136/jnnp-2020-325334

Matsui, T., Ramasamy, K., Ingelsson, M., Fukumoto, H., Conrad, C., Frosch, M. P., et al. (2006). Coordinated expression of caspase 8, 3 and 7 mRNA in temporal cortex of alzheimer disease: Relationship to formic acid extractable abeta42 levels. *J. Neuropathol. Exp. Neurol.* 65 (5), 508–515. doi:10.1097/01.jnen.0000229238.05748.12

Mattson, M. P., and Magnus, T. (2006). Ageing and neuronal vulnerability. *Nat. Rev. Neurosci.* 7 (4), 278–294. doi:10.1038/nrn1886

Meister, A. (1995). Glutathione metabolism. *Methods Enzymol.* 251, 3–7. doi:10.1016/0076-6879(95)51106-7

Michael, D., and Oren, M. (2003). The p53-Mdm2 module and the ubiquitin system. *Semin. Cancer Biol.* 13 (1), 49–58. doi:10.1016/s1044-579x(02)00099-8

Moon, H. E., and Paek, S. H. (2015). Mitochondrial dysfunction in Parkinson's disease. *Exp. Neurobiol.* 24 (2), 103–116. doi:10.5607/en.2015.24.2.103

Moradimotlagh, A., Arefian, E., Rezazadeh Valojerdi, R., Ghaemi, S., Jamshidi Adegani, F., and Soleimani, M. (2020). MicroRNA-129 inhibits glioma cell growth by targeting CDK4, CDK6, and MDM2. *Mol. Ther. Nucleic Acids* 19, 759–764. doi:10.1016/j.omtn.2019.11.033

Morimura, T., Goitsuka, R., Zhang, Y., SaIto, I., RethM.and Kitamura, D. (2000). Cell cycle arrest and apoptosis induced by Notch1 in B cells. *J. Biol. Chem.* 275 (47), 36523–36531. doi:10.1074/jbc.M006415200

Naik, E., Michalak, E. M., Villunger, A., Adams, J. M., and Strasser, A. (2007). Ultraviolet radiation triggers apoptosis of fibroblasts and skin keratinocytes mainly via the BH3-only protein Noxa. *J. Cell Biol.* 176 (4), 415–424. doi:10.1083/jcb.200608070

Nakamizo, A., Amano, T., Zhang, W., Zhang, X. Q., Ramdas, L., Liu, T. J., et al. (2008). Phosphorylation of Thr18 and Ser20 of p53 in ad-p53-induced apoptosis. *Neuro Oncol.* 10 (3), 275–291. doi:10.1215/15228517-2008-015

Nakanishi, M., Kaneko, Y., MatsusHime, H., and Ikeda, K. (1999). Direct interaction of P21 cyclin-dependent kinase inhibitor with the retinoblastoma tumor suppressor protein. *Biochem. Biophys. Res. Commun.* 263 (1), 35–40. doi:10.1006/bbrc.1999.1296

Nakano, K., and Vousden, K. H. (2001). PUMA, a novel proapoptotic gene, is induced by p53. *Mol. Cell* 7 (3), 683–694. doi:10.1016/s1097-2765(01)00214-3

Ohyagi, Y., Asahara, H., Chui, D. H., Tsuruta, Y., Sakae, N., Miyoshi, K., et al. (2005). Intracellular Abeta42 activates p53 promoter: A pathway to neurodegeneration in alzheimer's disease. *Faseb J.* 19 (2), 255–257. doi:10.1096/fj.04-2637fje

Olivier, M., Hollstein, M., and Hainaut, P. (2010). TP53 mutations in human cancers: Origins, consequences, and clinical use. *Cold Spring Harb. Perspect. Biol.* 2 (1), a001008. doi:10.1101/cshperspect.a001008

Pariyar, R., Lamichhane, R., Jung, H. J., Kim, S. Y., and Seo, J. (2017). Sulfuretin attenuates MPP^+-Induced neurotoxicity through akt/gsk3β and ERK signaling pathways. *Int. J. Mol. Sci.* 18 (12), 2753. doi:10.3390/ijms18122753

Park, S. Y., Lee, J. H., Ha, M., Nam, J. W., and Kim, V. N. (2009). miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. *Nat. Struct. Mol. Biol.* 16 (1), 23–29. doi:10.1038/nsmb.1533

Phillips, H. S., Kharbanda, S., Chen, R., Forrest, W. F., Soriano, R. H., Wu, T. D., et al. (2006). Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 9 (3), 157–173. doi:10.1016/j.ccr.2006.02.019

Pickart, C. M. (2001). Mechanisms underlying ubiquitination. *Annu. Rev. Biochem.* 70, 503–533. doi:10.1146/annurev.biochem.70.1.503

Qi, X., Davis, B., Chiang, Y. H., Filichia, E., Barnett, A., Greig, N. H., et al. (2016). Dopaminergic neuron-specific deletion of p53 gene is neuroprotective in an experimental Parkinson's disease model. *J. Neurochem.* 138 (5), 746–757. doi:10.1111/jnc.13706

Qi, Y., Cheng, X., Jing, H., Yan, T., Xiao, F., Wu, B., et al. (2019). Effect of Alpinia oxyphylla-Schisandra chinensis herb pair on inflammation and apoptosis in Alzheimer's disease mice model. *J. Ethnopharmacol.* 237, 28–38. doi:10.1016/j.jep.2019.03.029

Raycroft, L., Wu, H. Y., and Lozano, G. (1990). Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. *Science* 249 (4972), 1049–1051. doi:10.1126/science.2144364

Rokavec, M., Li, H., Jiang, L., and Hermeking, H. (2014). The p53/miR-34 axis in development and disease. *J. Mol. Cell Biol.* 6 (3), 214–230. doi:10.1093/jmcb/mju003

Sargin, D., Botly, L. C. P., Higgs, G., Marsolais, A., Frankland, P. W., Egan, S. E., et al. (2013). Disrupting Jagged1-Notch signaling impairs spatial memory formation in adult mice. *Neurobiol. Learn Mem.* 103, 39–49. doi:10.1016/j.nlm.2013.03.001

Sato, H., TaMbaM.Ishii, T., and Bannai, S. (1999). Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. *J. Biol. Chem.* 274 (17), 11455–11458. doi:10.1074/jbc.274.17.11455

Seiler, A., Schneider, M., Forster, H., Roth, S., Wirth, E. K., Culmsee, C., et al. (2008). Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* 8 (3), 237–248. doi:10.1016/j.cmet.2008.07.005

Stewart, Z. A., Leach, S. D., and Pietenpol, J. A. (1999). P21(Waf1/Cip1) inhibition of cyclin E/Cdk2 activity prevents endoreduplication after mitotic spindle disruption. *Mol. Cell Biol.* 19 (1), 205–215. doi:10.1128/mcb.19.1.205

Talebi, M., Talebi, M., Kakouri, E., Farkhondeh, T., Pourbagher-Shahri, A. M., Tarantilis, P. A., et al. (2021). Tantalizing role of p53 molecular pathways and its coherent medications in neurodegenerative diseases. *Int. J. Biol. Macromol.* 172, 93–103. doi:10.1016/j.ijbiomac.2021.01.042

Tortora, G., Caputo, R., Damiano, V., Bianco, R., Chen, J., Agrawal, S., et al. (2000). A novel MDM2 anti-sense oligonucleotide has anti-tumor activity and potentiates cytotoxic drugs acting by different mechanisms in human colon cancer. *Int. J. Cancer* 88 (5), 804–809. doi:10.1002/1097-0215(20001201)88:5<804::aid-ijc19>3.0.co;2-z

Unger, T., Sionov, R. V., MoallEm, E., Yee, C. L., Howley, P. M., OrenM., et al. (1999). Mutations in serines 15 and 20 of human p53 impair its apoptotic activity. *Oncogene* 18 (21), 3205–3212. doi:10.1038/sj.onc.1202656

van Lookeren Campagne, M., and Gill, R. (1998). Increased expression of cyclin G1 and P21WAF1/CIP1 in neurons following transient forebrain ischemia: Comparison with early DNA damage. *J. Neurosci. Res.* 53 (3), 279–296. doi:10.1002/(SICI)1097-4547(19980801)53:3<279::AID-JNR2>3.0.CO;2-7

Vassilev, L. T., Vu, B. T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., et al. (2004). *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 303 (5659), 844–848. doi:10.1126/science.1092472

Venderova, K., and Park, D. S. (2012). Programmed cell death in Parkinson's disease. *Cold Spring Harb. Perspect. Med.* 2 (8), a009365. doi:10.1101/cshperspect.a009365

Vogelstein, B., Lane, D., and Levine, A. J. (2000). Surfing the p53 network. *Nature* 408 (6810), 307–310. doi:10.1038/35042675

Walker, K. K., and Levine, A. J. (1996). Identification of a novel p53 functional domain that is necessary for efficient growth suppression. *Proc. Natl. Acad. Sci. U. S. A.* 93 (26), 15335–15340. doi:10.1073/pnas.93.26.15335

Watanabe, H., Ohta, S., Kumon, Y., Sakaki, S., and SakanakaM. (1999). Increase in p53 protein expression following cortical infarction in the spontaneously hypertensive rat. *Brain Res.* 837 (1-2), 38–45. doi:10.1016/s0006-8993(99)01652-2

Watson, I. R., and Irwin, M. S. (2006). Ubiquitin and ubiquitin-like modifications of the p53 family. *Neoplasia* 8 (8), 655–666. doi:10.1593/neo.06439

Wiza, C., Chadt, A., Blumensatt, M., Kanzleiter, T., Herzfeld De Wiza, D., Horrighs, A., et al. (2014). Over-expression of PRAS40 enhances insulin sensitivity in skeletal muscle. *Arch. Physiol. Biochem.* 120 (2), 64–72. doi:10.3109/13813455.2014.894076

Xie, R., Wang, P., Cheng, M., Sapolsky, R., Ji, X., and Zhao, H. (2014). Mammalian target of rapamycin cell signaling pathway contributes to the protective effects of ischemic postconditioning against stroke. *Stroke* 45 (9), 2769–2776. doi:10.1161/STROKEAHA.114.005406

Xie, W., Zhou, P., Sun, Y., Meng, X., Dai, Z., Sun, G., et al. (2018). Protective effects and target network analysis of ginsenoside Rg1 in cerebral ischemia and reperfusion injury: A comprehensive overview of experimental studies. *Cells* 7 (12), 270. doi:10.3390/cells7120270

Xiong, X., Xie, R., Zhang, H., Gu, L., Xie, W., Cheng, M., et al. (2014). PRAS40 plays a pivotal role in protecting against stroke by linking the Akt and mTOR pathways. *Neurobiol. Dis.* 66, 43–52. doi:10.1016/j.nbd.2014.02.006

Yang, W. S., SriRamaratnam, R., Welsch, M. E., Shimada, K., Skouta, R., Viswanathan, V. S., et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156 (1-2), 317–331. doi:10.1016/j.cell.2013.12.010

Yang, X., Klein, R., Tian, X., Cheng, H. T., Kopan, R., and Shen, J. (2004). Notch activation induces apoptosis in neural progenitor cells through a p53-dependent pathway. *Dev. Biol.* 269 (1), 81–94. doi:10.1016/j.ydbio.2004.01.014

Yonekura, I., Takai, K., Asai, A., Kawahara, N., and Kirino, T. (2006). p53 potentiates hippocampal neuronal death caused by global ischemia. *J. Cereb. Blood Flow. Metab.* 26 (10), 1332–1340. doi:10.1038/sj.jcbfm.9600293

Yu, J., Zhang, L., Hwang, P. M., Kinzler, K. W., and Vogelstein, B. (2001). PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol. Cell* 7 (3), 673–682. doi:10.1016/s1097-2765(01)00213-1

Yu, J., and Zhang, L. (2008). PUMA, a potent killer with or without p53. *Oncogene* 27 (1), S71–S83. doi:10.1038/onc.2009.45

Zhang, C. Z., Zhang, J. X., Zhang, A. L., Shi, Z. D., Han, L., Jia, Z. F., et al. (2010). MiR-221 and miR-222 target PUMA to induce cell survival in glioblastoma. *Mol. Cancer* 9, 229. doi:10.1186/1476-4598-9-229

Zhang, J., Shen, L., and Sun, L. Q. (2015). The regulation of radiosensitivity by p53 and its acetylation. *Cancer Lett.* 363 (2), 108–118. doi:10.1016/j.canlet.2015.04.015

Zhang, Y., Lei, L., Zhou, H., Lu, X., Cai, F., and Li, T. (2022). Roles of micro ribonucleic acids in astrocytes after cerebral stroke. *Front. Cell Neurosci.* 16, 890762. doi:10.3389/fncel.2022.890762

Zhang, Y., Lu, X., Tai, B., Li, W., and Li, T. (2021). Ferroptosis and its multifaceted roles in cerebral stroke. *Front. Cell Neurosci.* 15, 615372. doi:10.3389/fncel.2021.615372

Zhao, J., Dong, Y., Chen, X., Xiao, X., Tan, B., Chen, G., et al. (2021). p53 inhibition protects against neuronal ischemia/reperfusion injury by the p53/PRAS40/mTOR pathway. *Oxid. Med. Cell Longev.* 2021, 4729465. doi:10.1155/2021/4729465

Zhao, Y., Aguilar, A., Bernard, D., and Wang, S. (2015). Small-molecule inhibitors of the MDM2-p53 protein-protein interaction (MDM2 Inhibitors) in clinical trials for cancer treatment. *J. Med. Chem.* 58 (3), 1038–1052. doi:10.1021/jm501092z

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p53 mutation and deletion contribute to tumor immune evasion

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TP53 (or p53) is widely accepted to be a tumor suppressor. Upon various cellular stresses, p53 mediates cell cycle arrest and apoptosis to maintain genomic stability. p53 is also discovered to suppress tumor growth through regulating metabolism and ferroptosis. However, p53 is always lost or mutated in human and the loss or mutation of p53 is related to a high risk of tumors. Although the link between p53 and cancer has been well established, how the different p53 status of tumor cells help themselves evade immune response remains largely elusive. Understanding the molecular mechanisms of different status of p53 and tumor immune evasion can help optimize the currently used therapies. In this context, we discussed the how the antigen presentation and tumor antigen expression mode altered and described how the tumor cells shape a suppressive tumor immune microenvironment to facilitate its proliferation and metastasis.

KEYWORDS

p53 mutation, p53 deletion, tumor immune evasion, tumor immune microenvironment, MHC

Introduction

Genome instability is one of the hallmarks of cancer (Negrini et al., 2010; Hanahan and Weinberg, 2011). TP53 (or p53) is a vital tumor suppressor as it is the key regulator of DNA replication stress and DNA repair (Gaillard et al., 2015; Adriaens et al., 2016; Lindstrom et al., 2022) to maintain genomic stability. p53 responds to diverse cellular stresses, such as DNA damage, oxidative stress and oncogenic signaling (Hafner et al., 2019; Boutelle and Attardi, 2021). In unstressed, non-transformed cells, the expression and activity of p53 are blocked by its negative regulator MDM2 protein to be maintained at a low level (Haupt et al., 1997; Kubbutat et al., 1997; Shieh et al., 1997). On the contrary, the p53-MDM2 interaction will be lost and the expression of p53 is upregulated in stressed cells (Aubrey et al., 2018). Upregulated p53 mediates cell cycle arrest and apoptosis (Engeland, 2018) to eliminate damaged cells. p53 has a complicated link with the death or survival of tumor cells through regulating metabolism (Liu and Gu, 2021). Ferroptosis, an iron-dependent mode of death (Dixon et al., 2012) associated with metabolism, has also been recently found to be a p53-regulated activity to inhibit tumor growth (Jiang et al., 2015; Liu and Gu, 2022) (Figure 1).

The function of the immune system in control of cancer has been realized (Keast, 1970). Both the elements of innate immune and adaptive immune participate in anti-tumor activities, such as $CD4^+$ T cells, $CD8^+$ T cells, and natural killer (NK) cells. The immune response to cancer is thought to be activated in a tumor genome-dependent manner (Chen and Mellman, 2017). Tumor antigens originated from specific gene mutations (Epping and

Bernards, 2006) are presented by dendritic (DC) cells or directly presented by tumor cells (Jhunjhunwala et al., 2021) for priming of $CD8^+$ T cells to eliminate tumor cells. Simultaneously, tumor cells escape from immune attack through altering internal genes and shaping external environment, and p53 is one of the key points.

TP53 mutation is strongly associated with a risk of cancer (Harris, 1995; Willenbrink et al., 2020). Previous researches in transcriptome and proteome have demonstrated that TP53 mutation exists broadly in patients suffering tumors, such as urothelial carcinoma of the bladder (Xu et al., 2022), lung cancer (Chen and Roumeliotis, 2020; Gillette et al., 2020), and mutant p53 (hereafter referred to as "mutp53") always results in poor prognosis (Cao et al., 2021). The mutp53 displays various responses in cellular activity (Muller and Vousden, 2013), mainly dominant-negative effects compared to wild-type p53 (wt-p53) (Muller and Vousden, 2014). The loss of p53 gene also results in developing more advanced carcinomas than $p53^{+/+}$ and $p53^{+/-}$ in mice skin cancer models (Guinea-Viniegra et al., 2012). Restoring the function or expression of p53 has been proved to inhibit tumor progression and even reduce tumor size in both *in vivo* and *in vitro* experiments (Ventura et al., 2007; Guinea-Viniegra et al., 2012).

Based on current research, the hallmarks of tumors with different status of p53 is clear, but how the tumor cells with different p53 status survived from immune surveillance remains largely elusive. Here, we focus on the complicated molecular network of tumor evasion derived from different status of p53 and explore new options of immunotherapy.

The p53 mutation regulates the MHC molecules and reduces immunogenicity of tumor cells

Major histocompatibility complex (MHC) molecules expressed on cell surface present peptides to T cells to motivate immune responses. MHC molecules can be divided into two major classes. MHC Class I mainly presents peptides came from intracellular proteins (Jhunjhunwala et al., 2021), which prevent cells from malignant proliferation and stop cancer formation, while MHC Class II presents extracellular proteins to protect cells from infection. MHC I is formed by four domains. The α1, α2, and α3 domain form a heavy chain, and the β2m domain forms a light chain. After the heavy chain combine with β2m, the complex binds to peptides provided by the transporter associated with antigen processing (TAP) and is transported to the cell surface *via* the Golgi network (Flutter and Gao, 2004; Cresswell et al., 2005). Under most

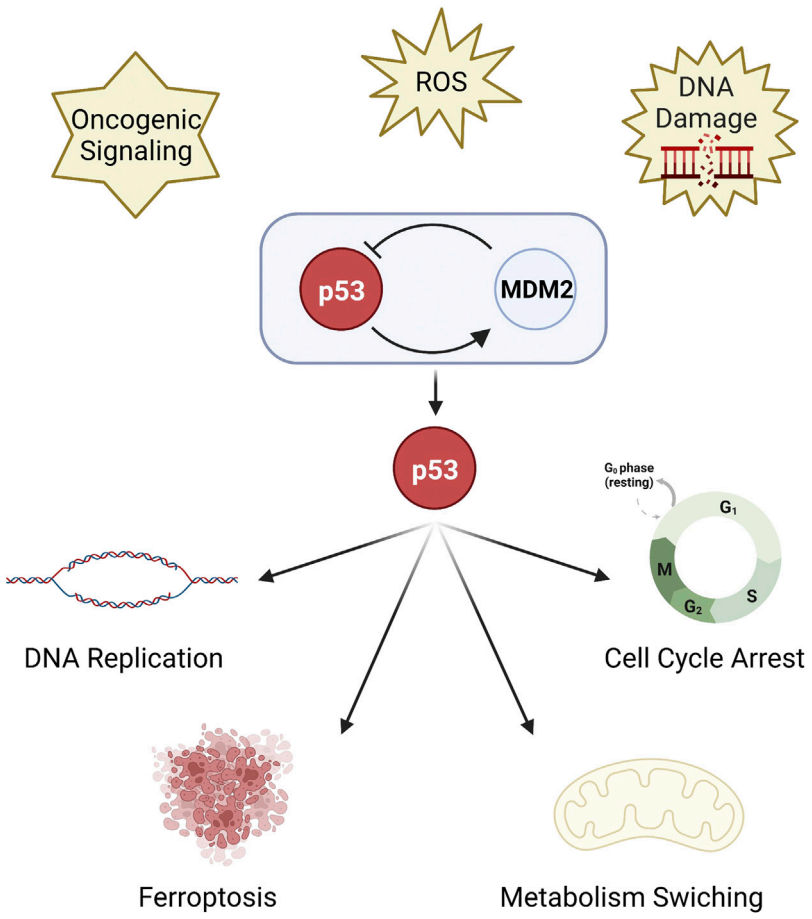

FIGURE 1
The function of p53. p53 is a key regulator of DNA replication stress and DNA repair. p53 is inhibited by MDM2 protein, but upregulated under stress. Upregulated p53 mediates cell cycle arrest and DNA replication, metabolism switching, and ferroptosis.

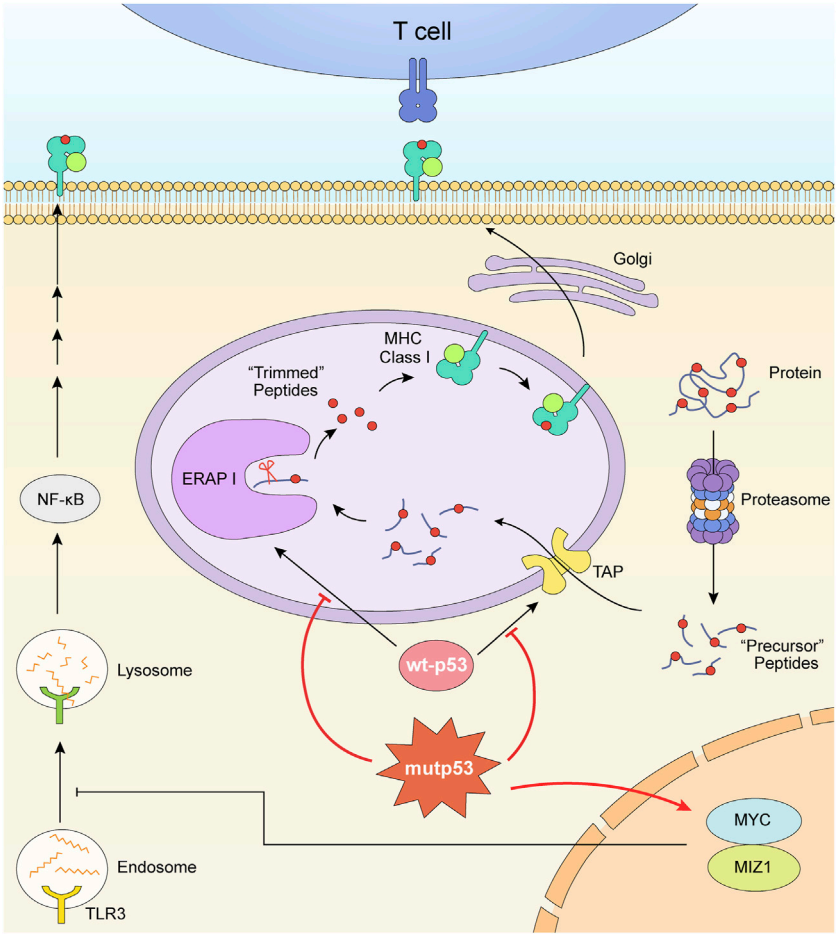

FIGURE 2
Mutant p53 downregulates the expression of MHC I. The formation and expression of MHC I is related with TAP and ERAP1. TAP transports peptides degraded by proteasome into ER. ERAP1 trims these precursor peptides to be optimal length for assembling with MHC. The dysfunctional p53 lost its TAP1 and ERAP1 activation function. What's more, the p53 mutation and deletion play roles in upregulating MYC oncoprotein, consequently inhibited the activation of downstream MHC I.

circumstances, tumor cells lack of the expression of MHC molecules to decrease their immunogenicity.

Tumor cells that lack p53 exhibit markedly lower MHC I molecules (Bubeník, 2004) (Figure 2). The dysfunctional p53 lost its TAP1 activation function. In normal cells, TAP1 is induced by endogenous wild-type p53 (wt-p53) to enhance the transport and the expression of surface MHC-peptide complexes, but not in mutant p53 (R249S) cells and p53-null like HCT116E6 cells (Zhu et al., 1999). Similarly, endoplasmic reticulum aminopeptidase 1 (ERAP1) is another p53-target gene. ERAP1 acts as a molecular scissor to trim N-terminal extended peptides to be optimal length for assembling with MHC I (Falk and Rötzschke, 2002; Reeves et al., 2020). In human colon carcinoma cell lines, it has been proved that the cognate response element of ERAP1 gene is not accessible to bind silenced p53. The expression of MHC I consequently decreased. (Wang et al., 2013). Wt-p53 limits tumor growth *via* repressing the myelocytomatosis (Myc) oncogene transcription (Olivero et al., 2020). But the p53 deletion could increase the expression of Myc (Olivero et al., 2020) and the p53-R249S mutation could enhance the activity of Myc (Liao et al., 2017). Upregulated MYC prevented nuclear-derived double-stranded RNA from being recognized by toll-like receptor 3 (TLR3), consequently inhibited the activation of downstream MHC I (Krenz et al., 2021).

Thus, one of the treatments is to upregulate MHC I expression relying on the re-activation of p53. Pharmacologically activated p53 induced by MDM2 inhibitors enhanced the expression of endogenous retroviruses (ERV). The derepression of ERV triggered ERV-dsRNA-interferon (IFN) pathway followed by activation of antigen processing and presenting genes, including B2M, HLA-A, HLA-B, and HLA-C which encode MHC class I molecules (Zhou et al., 2021). Besides, it is also demonstrated that the dual-targeting PI3K and HDAC inhibitor BEBT-908 can promote ferroptosis of cancer cells by hyperacetylating p53 and promoting the expression of ferroptotic signaling (Liu et al., 2019; Fan et al., 2021). Acetylation-modified p53 in tumor cells induced the upregulation of MHC I *via* signal transducer and activator of transcription (STAT) one signaling pathway (Fan et al., 2021).

Another mechanism that p53 impacts immune escape is through affecting the recognition of MHC molecules. The different mutant p53 status interfere with TCR-MHC

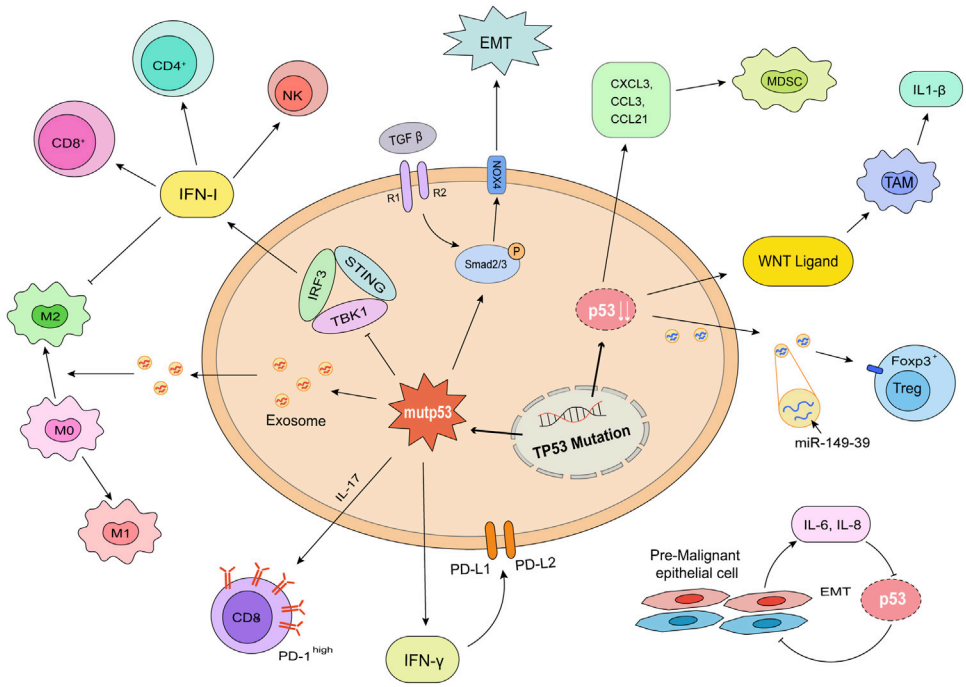

FIGURE 3
p53 mutation and deletion shape an immunosuppressive environment. The consequences of mutation (left) or deletion (right) of p53 in tumor cells are shown. Mutp53 decreased the release of IFN I, consequently decreased the infiltration of CD4$^+$ T cells, CD8$^+$ T cells, and NK cells. Mutp53 promoted the formation of exhausted CD8$^+$ T cells through IL-17 signaling. The increased IFNγ played a role in the expression of immunosuppressive ligands, PD-L1 and PD-L2. Mutant p53 has a synergistic effect with TGF-β to promote EMT. p53 deletion upregulated the releasing of WNT ligands, CXCL1, CCL3, CCL21, and miR-149–39 to enhance the differentiation of TAMs and Tregs. Pre-malignant epithelial cells would release IL-6 and IL-8 to promote EMT. This process promoted tumor formation and metastasis.

identification as endogenous proteins presented by MHC I *via* proteasome. By detecting the secretion of IFNγ, tumor necrosis factor (TNF)-α, and the proportion of CD69$^+$ T cells, it was found that p53-bearing destabilizing mutations, such as R175H and Y220C mutations, are recognized more efficiently and activated more p53 target T cells than G245S mutation (Shamalov et al., 2017).

As for MHC II, it is found MHC II mediated the T-cell response to acute myeloid leukemia (AML) after allogeneic hematopoietic cell transplantation. The p53 deletion caused the downregulation of MHC II and tumor necrosis factor related apoptosis-inducing ligand receptor one and receptor 2 (TRAIL-R1/2) which induced immune evasion of AML (Chitlur, 2022; Ho et al., 2022).

How does p53 mutation or deletion shape an immunosuppressive environment?

Over the past few centuries, researchers came to realize tumors are more than a group of malignant proliferating cells, but a complex "organ" including tumor cells, stromal cells, immune cells, extracellular matrix, vessels, cytokines, chemokines, as well as other metabolic products (Fu et al., 2021). It has been found that tumor cells transformed themselves (Bottcher et al., 2018) at gene and transcriptome levels (Bi et al., 2021; Sun et al., 2021) to shape a surrounding which is suitable for proliferation and differentiation. In this part, we discussed how the changes of p53 influence the cytokines secreting, suppressive ligands expression, and immunocytes with inhibitive function differentiation (Figure 3).

The p53 mutation or deletion induces immunosuppressive cytokines and downregulates proinflammatory factors

Cytokines are the main proteins produced and secreted by many different cell types. They mediate immune system responses (Borish and Steinke, 2003) and communication between cells and immune system components (Propper and Balkwill, 2022). For one thing, cytokines can act directly on tumor cells to promote or inhibit their growth; for another, they can also influence the status of tumors by recruiting immune cells or stromal cells. Responses caused by different cytokines also have synergy or confrontation effects (Salazar-Onfray et al., 2007). All above combined the complexity of cytokines therapy (Conlon et al., 2019). Understanding the regulatory mechanism of cytokines may help enhance clinical benefits and reduce adverse reactions. Using RNA interference to reactivate p53 briefly in the p53-dificient mouse liver carcinoma model, (Xue et al., 2007) found that tumor proliferation is restricted and dependent on the cellular senescence program and consequently increased inflammation cytokines. We hypothesized that the

influence of p53 in different status on tumor immunity may be achieved through the influence of cytokines.

Type I IFN (IFN I) is an important cytokine whose response plays significant roles in antiviral innate immune and anti-tumor adaptive immune (Woo et al., 2015). One of the mechanisms to active immunity is to upregulate the expression of IFN-stimulated genes (ISGs), thereby giving rise to ISG DC cells resembling type 1 DC cells. ISG DC cells present intact tumor-derived peptide-MHC I to reactivate antitumor immunity (Corrales et al., 2016; Duong et al., 2022). The attenuation of IFN I response is conducive to tumor evasion. Current studies confirmed that IFN I response is activated by stimulator of IFN genes (STING) pathway. Cells with mutp53 suppress downstream signaling of cGAS/STING. Mutp53 prevents STING-IRF3-TBK1 trimeric complex formation and IFN regulatory factor 3 (IRF3) activation *via* interacting with TANK binding protein kinase 1 (TBK1) (Balka et al., 2020; Ghosh et al., 2021), consequently downregulates the release of IFN I.

Interleukin-6 (IL-6) is an important trigger of tumor-promoting inflammation. Tumor cells exposed to IL-6 activate the oncogenic STAT3 transcription factor to promote epithelial-to-mesenchymal transition (EMT), which is the first step for tumor cell migration. MiR-34a, activated by p53, is a major inhibitor through targeting IL-6R (Rokavec et al., 2014). In studies investigating aging and carcinogenesis, it was found that pre-malignant epithelial cells induce EMT through a paracrine mechanism of IL-6 and IL-8. IL-6, and IL-8 caused the loss of p53 in normal cells and the p53 deficiency exacerbated the pro-malignant secretory activity, forming a vicious cycle (Downward et al., 2008). Whereas co-expression of wt-p53 and NF-κB in tumor associated macrophages (TAMs) can enhance the survival of tumor cells through secreting IL-6, CXCL-1, and promoting tumor associated neutrophils recruitment (Lowe et al., 2014).

Transforming growth factor-β (TGF-β) is an immunosuppressive cytokine that has both positive and negative roles in tumor formation (Yang et al., 2010). The canonical response of TGF-β is the phosphorylation of SMAD2 and SMAD3, which then combine with SMAD4 to mediate growth inhibition (Yang et al., 2010). TGF-β acts as a tumor suppressor and the loss of TGF-β signaling effectors is the molecular basis to develop tumors. A study in prostate cancer discovered the TGF-βRII and Smad4 in tumor cells taper off during the progression process (Zeng et al., 2004). TGF-β also acts as a tumor promoter owing to its immune suppressive effects (Batlle and Massague, 2019). TGF-β inhibits the differentiation and proliferation of effector T cells (Teffs) but enhances the fraction of regulatory T cells (Tregs) and other suppressive cells through the phosphorylation of Smad family proteins. Blocking TGF-β in breast cancer cell lines was effectively to counteract its effects (Yi et al., 2021).

The dual functions of TGF-β in tumor cell survival are interconnected with different status of p53. TGF-β mediates the inhibition of p53 and DNA damage response to conduce to tumor progression. These effects are achieved through the downstream signals of miR-100 and miR-125b upregulated by SMAD2/3 transcription factors (Ottaviani et al., 2018). Furthermore, TGF-β1 antagonizes p53-induced apoptosis in precancerous cells *via* switching the viral E2-associated factor 4 (E2F-4)/p107 complex to Smad/E2F-4 corepressor, which represses transcription and translation of p53 (Lopez-Diaz et al., 2013). Equally, both wt-p53 and mutp53 regulate the TGF-β-mediated human lung and breast EMT by affecting TGF-β/SMAD3-mediated signaling. By restricting the expression of Nox4, a NADPH oxidase, wt-p53 downregulates downstream focal adhesion kinase phosphorylation to reduce migration. On the contrary, mutp53 has a synergistic effect with TGF-β to upregulate Nox4 and promotes tumor cells evasion (Boudreau et al., 2014).

The p53 mutation or deletion upregulates immunosuppressive ligands

Immunosuppressive ligands, also known as immune checkpoints, such as programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), have attracted much attention since their discoverers won the 2018 Nobel Prize in Physiology or Medicine. Immune Checkpoints are inhibitory pathways to maintain the persistence of immune response and the stability of the internal environment based on self-tolerance (Abril-Rodriguez and Ribas, 2017). Currently, there are several immune checkpoint inhibitors (ICIs) proved by FDA for clinical treatment. The representative examples are ipilimumab for metastatic melanoma (Langer et al., 2007; Kirkwood et al., 2008; Weber et al., 2008), nivolumab for non-small cell lung cancer (Topalian et al., 2012; Hellmann et al., 2019; Forde et al., 2022) and avelumab for urothelial carcinoma (Powles et al., 2020; Powles et al., 2021). However, immune therapy still has limits. A large fraction of patients has no response to immune therapy and many patients responding develop drug resistance after several treatment cycles (Chowdhury et al., 2018). Understanding the intrinsic mechanisms of immune checkpoints expression can therefore deepen our understanding of tumor immune escape and help develop new treatment options.

It is discovered that PD-L1, PD-L2, and CTLA-4 expression is significantly increased in patients with TP53 mutations in both clinical samples and mouse models of hematologic neoplasms (Pascual et al., 2019; Sallman et al., 2020). Similar findings have been discovered in solid tumors (Thiem et al., 2019; Sun et al., 2020). Furthermore, other co-inhibitory receptors, such as PD-1, T-cell immunoglobulin and mucin-domain containing-3 (TIM3), and lymphocyte-activation gene 3 (LAG3) are co-expressed on tumor-infiltrating lymphocyte cells (Williams et al., 2019). Based on these specific subtypes of T cells and tumor cells, TP53 mutations are one of the indicators in predicting efficacy in patients treated with ICIs (Chen et al., 2019; Sun et al., 2020).

PD-L1 expression is regulated by multiple pathways. The gain-of-function mutant p53 upregulated IL-17 signaling and induced the transformation of infiltrating T cells into exhausted $CD8^+$ T cells to counteract the effects of PD-1 inhibitors (Wang J. et al., 2021). Besides, PD-L1 expression is upregulated by IFN-γ induced immune response, and both wt- and mut-p53 work in this process. It isn't the activity, but the expression level of p53 or mutp53 impacts PD-L1 expression activated by IFN-γ (Thiem et al., 2019). The non-coding RNA miR-34, transcriptionally induced by p53, is also proved to negatively regulate PD-L1 (Cortez et al., 2016). In addition, p53 transcriptionally induces the expression of PTEN gene, an inhibitor of PI3K/Akt/mTOR pathway, which

downregulates PD-L1 and maintains immune response (Hays and Bonavida, 2019). For the p53 deletion losing inhibition of PD-L1, it is conducive to tumor immune evasion.

The p53 mutation or deletion promotes immunosuppressive cells differentiation

Tumor immune microenvironment (TIME) refers to immunological components with tumors (Fu et al., 2021). Various immune cells are the major participators of immune response. Notably, patients with TP53 mutations display non-T cell infiltrated phenotype. The numbers of cytotoxic T cells, helper T cells, as well as NK cells significantly reduced. Meanwhile, highly immunosuppressive Tregs (Sallman et al., 2020) and M2 macrophages (Wang et al., 2021) are expanded in cases with TP53 mutations.

Some indirect evidence suggests different status of p53 impact the intensity of immune response. The adaptive immune response is enhanced in the mouse model of colon cancer treated with HDM201, a selective MDM2 inhibitor. After the HDM201 treatment, the percentage of DC cells and $CD8^+$ T cells increased in a p53-dependent manner (Wang et al., 2021). Moreover, p53 activation combined with immune checkpoint blockade therapy has been found to make breakthroughs in a variety of tumors. Studies in hepatocellular carcinoma (HCC) demonstrated the number of infiltrating $CD8^+$ T cells and the fraction of activated $CD8^+$ T cells is significantly increased after the combination therapy (Xiao et al., 2022).

Tregs are the major suppressive cells in controlling immune tolerance and homeostasis of immune system. Tregs are considered as tumor-promoting cells (Ye et al., 2012) because of suppressing anti-tumor Teffs response by releasing inhibitory cytokines such as IL-10, TGF-β, and IL-35 (Li et al., 2020). The relationship between Tregs and p53 is complex. Patients treated with p53 vaccination displayed decreased frequencies of Tregs and the 2-year disease-free survival reached 88% (Schuler et al., 2014). High doses of p53-derived peptide inhibited the Tregs differentiation and immunosuppressive function *in vitro* (Mandapathil et al., 2013). However, a research found the lack of p53 in rheumatoid arthritis compromised Tregs differentiation because of the decreasing activity of STAT-5 (Park et al., 2013). Depletion of highly activated and strongly suppressive tumor-infiltrating Tregs contributes to clinical outcomes of immunotherapy (Van Damme et al., 2021). These phenomena suggest that wt-p53 in the normal state promotes Tregs differentiation to control immune response, but under the tumor environment, p53 tends to suppress Tregs action to limit tumors.

The suppressive effects in Tregs cells is mainly dependent on the expression and function of the transcription factor forkhead box P3 (Foxp3) (Ohkura and Sakaguchi, 2020). Further, the lineage stability of Tregs is closely related to the PI3K/Akt pathway and its suppressor PTEN (Huynh et al., 2015). As is mentioned above, the lack of p53 consequently promotes the accumulation of inactivated PTEN, thus activating PI3K/Akt signaling and the Tregs differentiation (Kang et al., 2017). Foxp3 also can be regulated by miR-149-39. A study in esophageal cancer found long non-coding RNA (lncRNA) maternally expressed gene 3 (MEG3) upregulates MDM2, the inhibitor of p53. The decreased p53 is unable to generate sufficient miR-149-3p to limit transcription of Foxp3, but upregulate Tregs (Xu et al., 2021).

Myeloid-derived suppressor cell (MDSC) is another important member of the suppressive immune microenvironment, which produces reactive oxygen species and other cytokines to inhibit T cell mediated immune response (Li et al., 2021). p53 mediates the quantity and quality of MDSC in TIME. The p53 deletion enhanced the recruitment of suppressive myeloid $CD11b^+$ cells through upregulating the expression of CXCR3/CCR2-associated chemokines and macrophage colony-stimulating factor (M-CSF) (Blagih et al., 2020). The destabilizing p53 prevents MDSCs from ferroptosis through upregulating Heme Oxygenase-1 (Hmox1) expression to suppress lipid reactive oxygen species production (Zhu et al., 2021). The dysfunctional p53 promotes the expansion of lymphoid-like stromal network, which increased the expression of CXCL1, CCL3, and CCL21 to recruit more immunosuppressive populations, especially MDSCs (Guo et al., 2013).

TAMs are major tumor-infiltrating cells mediated a variety of cellular activities such as tumor cytotoxicity (Chen et al., 2018), angiogenesis, and lymphangiogenesis (Volk-Draper et al., 2019). TAMs can be simply divided into tumor killing M1 type and tumor promoting M2 type based on their response to tumors. The transform mechanism between M1 and M2 that remains a mystery is a research hotspot, and p53 plays a role in the process. As mentioned above, the co-expression of p53 and NF-κB in TAMs promotes the secretion of IL-6 and CXCL-1 to promote tumor cell survival (Lowe et al., 2014). Tumor cells lack p53 also release WNT ligands to stimulate TAMs to produce IL-1β. The IL-1β triggers an inflammatory cascade throughout the body and drives tumor metastasis (Wellenstein et al., 2019). In macrophages, p53 acetylation induced M1 polarization to maintain iron homeostasis (Zhou et al., 2018). *In vitro* co-cultures of M0 macrophages with H358 (a p53-null cell line) exosomes demonstrated that exosome-induced M2 polarization may be p53 independent (Pritchard et al., 2020). Besides, upregulated wt-p53 altered miRNA levels in the exosomes and promoted macrophage repolarization towards a more pro-inflammatory/antitumor M1 phenotype (Trivedi et al., 2016). Equally, TAMs will affect p53 during the response. The expression of VEGF-C and its receptor VEGFR3 promoted by TAMs results in the loss of p53 and PTEN in tumor cells, which contributes to tumor resistance (Li et al., 2017).

Target p53: New therapeutic strategies for immunotherapy

In recent years, immunotherapy including ICIs, cancer vaccination, and adoptive cell therapy has revolutionized the treatment of cancer. As introduced above, the p53 mutation or deletion play a central role in tumor immune evasion, so reactivating wt-p53 or restoring tumor suppressive function of mutp53 are promising anti-tumor immunotherapy strategies.

Scientists has appreciated the antigenic character of p53 since 1990s and developed p53-based vaccines (DeLeo and Appella, 2020). A novel phase Ib clinical trial of adjuvant p53 peptide-loaded DC

cells demonstrated that the DC–p53 vaccine triggered a p53-specific immune response in 11 patients with head and neck squamous cell carcinoma, out of the 16 patients treated (Schuler et al., 2014). In another phase I trial, patients with platinum-resistant ovarian cancer received a combination regimen of a Modified Vaccinia Ankara vaccine delivering wild-type human p53 (p53MVA) and gemcitabine chemotherapy. It is found that p53MVA could elevate p53-reactive CD4 and CD8 T-cell responses, and patients with greatest expansion of T cells had longer progression-free survival (PFS) (Hardwick et al., 2018). Moreover, restoring p53 expression by p53 mRNA nanomedicine (Xiao et al., 2022) or directly introducing wt-p53 gene could reprogram the TME and sensitize tumors to anti-PD-1 therapy in mice experiments (Kim et al., 2019).

Targeting p53-MDM2 pathway is another strategy. Combination of reactivation of wt-p53 and ICIs is also proved to have a better anti-tumor effect. Wang and co-workers demonstrated that HDM201, a potent and selective second-generation MDM2 inhibitor, could trigger adaptive immunity and develop durable, antigen-specific memory T cells in a p53-dependent manner. Combination of HDM201 and PD-1/PD-L1 blockade is more efficient for complete tumor regressions (Wang et al., 2021). Similarly, Fang et al. (2019) also discovered that p53 activation by APG-115 would reduce the number and proportion of immunosuppressive M2 macrophage and has a synergistic effect with PD-1 blockade in anti-tumor.

It isn't difficult to find from the previous studies that p53 has a strong connection with TIME (Hassin and Oren, 2022). The p53 mutation and deletion in tumor cells trends to form a tumor promoting microenvironment. Growing evidence indicated that p53 could be an effective target to deal with immune evasion.

Concluding remarks

p53 is a star molecular which mediates multiple cellular activities of tumors and attracts much attention since its discovery. Scientists gradually realized the better treatment is to recover normal immune response, not only to enhance immune response. Owing to the essential role of p53 mutation and deletion in tumor immune evasion, reactivation of expression and function of p53 to reshape TIME and restore anti-tumor immunity may be efficient treatment for anti-tumor. Although scientists have spent almost 30 years to develop p53-based therapies, p53 is still a mystery protein attracting our attention. Great expectations of targeting p53 and unstable efficacy urge us to have a deeper understanding of it.

Author contributions

RY and HQG designed the study. SYL and TYL wrote and edited the manuscript. JXJ and SYL drew the figures. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abril-Rodriguez, G., and Ribas, A. (2017). SnapShot: Immune checkpoint inhibitors. *Cancer Cell* 31 (6), 848–848.e1. doi:10.1016/j.ccell.2017.05.010

Adriaens, C., Standaert, L., Barra, J., Latil, M., Verfaillie, A., Kalev, P., et al. (2016). p53 induces formation of NEAT1 lncRNA-containing paraspeckles that modulate replication stress response and chemosensitivity. *Nat. Med.* 22 (8), 861–868. doi:10.1038/nm.4135

Aubrey, B. J., Kelly, G. L., Janic, A., Herold, M. J., and Strasser, A. (2018). How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? *Cell Death Differ.* 25 (1), 104–113. doi:10.1038/cdd.2017.169

Balka, K. R., Louis, C., Saunders, T. L., Smith, A. M., Calleja, D. J., D'Silva, D. B., et al. (2020). TBK1 and IKKε act redundantly to mediate STING-induced NF-κB responses in myeloid cells. *Cell Rep.* 31 (1), 107492. doi:10.1016/j.celrep.2020.03.056

Batlle, E., and Massague, J. (2019). Transforming growth factor-beta signaling in immunity and cancer. *Immunity* 50 (4), 924–940. doi:10.1016/j.immuni.2019.03.024

Bi, K., He, M. X., Bakouny, Z., Kanodia, A., Napolitano, S., Wu, J., et al. (2021). Tumor and immune reprogramming during immunotherapy in advanced renal cell carcinoma. *Cancer Cell* 39 (5), 649–661.e5. doi:10.1016/j.ccell.2021.02.015

Blagih, J., Zani, F., Chakravarty, P., Hennequart, M., Pilley, S., Hobor, S., et al. (2020). Cancer-specific loss of p53 leads to a modulation of myeloid and T cell responses. *Cell Rep.* 30 (2), 481–496.e6. doi:10.1016/j.celrep.2019.12.028

Borish, L. C., and Steinke, J. W. (2003). 2. Cytokines and chemokines. *J. Allergy Clin. Immunol.* 111, S460–S475. doi:10.1067/mai.2003.108

Bottcher, J. P., Bonavita, E., Chakravarty, P., Blees, H., Cabeza-Cabrerizo, M., Sammicheli, S., et al. (2018). NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell* 172 (5), 1022–1037.e14. doi:10.1016/j.cell.2018.01.004

Boudreau, H. E., Casterline, B. W., Burke, D. J., and Leto, T. L. (2014). Wild-type and mutant p53 differentially regulate NADPH oxidase 4 in TGF-beta-mediated migration of human lung and breast epithelial cells. *Br. J. Cancer* 110 (10), 2569–2582. doi:10.1038/bjc.2014.165

Boutelle, A. M., and Attardi, L. D. (2021). p53 and tumor suppression: It takes a network. *Trends Cell Biol.* 31 (4), 298–310. doi:10.1016/j.tcb.2020.12.011

Bubeník, J. (2004). MHC class I down-regulation: Tumour escape from immune surveillance? (review). *Int. J. Oncol.* 25 (2), 487–491. doi:10.3892/ijo.25.2.487

Cao, L., Huang, C., Cui Zhou, D., Hu, Y., Lih, T. M., Savage, S. R., et al. (2021). Proteogenomic characterization of pancreatic ductal adenocarcinoma. *Cell* 184 (19), 5031–5052.e26. doi:10.1016/j.cell.2021.08.023

Chen, D. S., and Mellman, I. (2017). Elements of cancer immunity and the cancer-immune set point. *Nature* 541 (7637), 321–330. doi:10.1038/nature21349

Chen, D., Xie, J., Fiskesund, R., Dong, W., Liang, X., Lv, J., et al. (2018). Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype. *Nat. Commun.* 9 (1), 873. doi:10.1038/s41467-018-03225-9

Chen, Y., Chen, G., Li, J., Huang, Y. Y., Li, Y., Lin, J., et al. (2019). Association of tumor protein p53 and ataxia-telangiectasia mutated comutation with response to immune checkpoint inhibitors and mortality in patients with non-small cell lung cancer. *JAMA Netw. Open* 2 (9), e1911895. doi:10.1001/jamanetworkopen.2019.11895

Chen, Y. J., and Roumeliotis, T. I. (2020). Proteogenomics of non-smoking lung cancer in east asia delineates molecular signatures of pathogenesis and progression. *Cell* 182 (1), 226–244.e17. doi:10.1016/j.cell.2020.06.012

Chitlur, M. (2022). Desmopressin revisited in mild hemophilia A. *Blood* 140 (10), 1063–1064. doi:10.1182/blood.2022017652

Chowdhury, P. S., Chamoto, K., and Honjo, T. (2018). Combination therapy strategies for improving PD-1 blockade efficacy: A new era in cancer immunotherapy. *J. Intern Med.* 283 (2), 110–120. doi:10.1111/joim.12708

Conlon, K. C., Miljkovic, M. D., and Waldmann, T. A. (2019). Cytokines in the treatment of cancer. *J. Interferon Cytokine Res.* 39 (1), 6–21. doi:10.1089/jir.2018.0019

Corrales, L., Matson, V., Flood, B., Spranger, S., and Gajewski, T. F. (2016). Innate immune signaling and regulation in cancer immunotherapy. *Cell Res.* 27 (1), 96–108. doi:10.1038/cr.2016.149

Cortez, M. A., Ivan, C., Valdecanas, D., Wang, X., Peltier, H. J., Ye, Y., et al. (2016). PDL1 Regulation by p53 via miR-34. *J. Natl. Cancer Inst.* 108 (1), djv303. doi:10.1093/jnci/djv303

Cresswell, P., Ackerman, A. L., Giodini, A., Peaper, D. R., and Wearsch, P. A. (2005). Mechanisms of MHC class I-restricted antigen processing and cross-presentation. *Immunol. Rev.* 207, 145–157. doi:10.1111/j.0105-2896.2005.00316.x

DeLeo, A. B., and Appella, E. (2020). The p53 saga: Early steps in the Development of tumor immunotherapy. *J. Immunol.* 204 (9), 2321–2328. doi:10.4049/jimmunol.1901343

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell* 149 (5), 1060–1072. doi:10.1016/j.cell.2012.03.042

Downward, J., Coppé, J-P., Patil, C. K., Sun, Y., Munoz, D. P., Goldstein, J., et al. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* 6 (12), 2853–2868. doi:10.1371/journal.pbio.0060301

Duong, E., Fessenden, T. B., Lutz, E., Dinter, T., Yim, L., Blatt, S., et al. (2022). Type I interferon activates MHC class I-dressed CD11b(+) conventional dendritic cells to promote protective anti-tumor CD8(+) T cell immunity. *Immunity* 55 (2), 308–323.e9. doi:10.1016/j.immuni.2021.10.020

Engeland, K. (2018). Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. *Cell Death Differ.* 25 (1), 114–132. doi:10.1038/cdd.2017.172

Epping, M. T., and Bernards, R. (2006). A causal role for the human tumor antigen preferentially expressed antigen of melanoma in cancer. *Cancer Res.* 66 (22), 10639–10642. doi:10.1158/0008-5472.CAN-06-2522

Falk, K., and Rötzschke, O. (2002). The final cut: how ERAP1 trims MHC ligands to size. *Nat. Immunol.* 3, 1121–1122. doi:10.1038/ni1202-1121

Fan, F., Liu, P., Bao, R., Chen, J., Zhou, M., Mo, Z., et al. (2021). A Dual PI3K/HDAC inhibitor induces immunogenic ferroptosis to potentiate cancer immune checkpoint therapy. *Cancer Res.* 81 (24), 6233–6245. doi:10.1158/0008-5472.CAN-21-1547

Fang, D. D., Tang, Q., Kong, Y., Wang, Q., Gu, J., Fang, X., et al. (2019). MDM2 inhibitor APG-115 synergizes with PD-1 blockade through enhancing antitumor immunity in the tumor microenvironment. *J. Immunother. Cancer* 7 (1), 327. doi:10.1186/s40425-019-0750-6

Flutter, B., and Gao, B. (2004). MHC class I antigen presentation-recently trimmed and well presented. *Cell Mol. Immunol.* 1 (1), 22–30.

Forde, P. M., Spicer, J., Lu, S., Provencio, M., Mitsudomi, T., Awad, M. M., et al. (2022). Neoadjuvant nivolumab plus chemotherapy in resectable lung cancer. *N. Engl. J. Med.* 386 (21), 1973–1985. doi:10.1056/NEJMoa2202170

Fu, T., Dai, L. J., Wu, S. Y., Xiao, Y., Ma, D., Jiang, Y. Z., et al. (2021). Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic response. *J. Hematol. Oncol.* 14 (1), 98. doi:10.1186/s13045-021-01103-4

Gaillard, H., Garcia-Muse, T., and Aguilera, A. (2015). Replication stress and cancer. *Nat. Rev. Cancer* 15 (5), 276–289. doi:10.1038/nrc3916

Ghosh, M., Saha, S., Bettke, J., Nagar, R., Parrales, A., Iwakuma, T., et al. (2021). Mutant p53 suppresses innate immune signaling to promote tumorigenesis. *Cancer Cell* 39 (4), 494–508.e5. doi:10.1016/j.ccell.2021.01.003

Gillette, M. A., Satpathy, S., Cao, S., Dhanasekaran, S. M., Vasaikar, S. V., Krug, K., et al. (2020). Proteogenomic characterization reveals therapeutic vulnerabilities in lung adenocarcinoma. *Cell* 182 (1), 200–225.e35. doi:10.1016/j.cell.2020.06.013

Guinea-Viniegra, J., Zenz, R., Scheuch, H., Jimenez, M., Bakiri, L., Petzelbauer, P., et al. (2012). Differentiation-induced skin cancer suppression by FOS, p53, and TACE/ADAM17. *J. Clin. Invest.* 122 (8), 2898–2910. doi:10.1172/JCI63103

Guo, G., Marrero, L., Rodriguez, P., Del Valle, L., Ochoa, A., and Cui, Y. (2013). Trp53 inactivation in the tumor microenvironment promotes tumor progression by expanding the immunosuppressive lymphoid-like stromal network. *Cancer Res.* 73 (6), 1668–1675. doi:10.1158/0008-5472.CAN-12-3810

Hafner, A., Bulyk, M. L., Jambhekar, A., and Lahav, G. (2019). The multiple mechanisms that regulate p53 activity and cell fate. *Nat. Rev. Mol. Cell Biol.* 20 (4), 199–210. doi:10.1038/s41580-019-0110-x

Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell* 144 (5), 646–674. doi:10.1016/j.cell.2011.02.013

Hardwick, N. R., Frankel, P., Ruel, C., Kilpatrick, J., Tsai, W., Kos, F., et al. (2018). p53-Reactive T cells are associated with clinical benefit in patients with platinum-resistant epithelial ovarian cancer after treatment with a p53 vaccine and gemcitabine chemotherapy. *Clin. Cancer Res.* 24 (6), 1315–1325. doi:10.1158/1078-0432.CCR-17-2709

Harris, C. C. (1995). 1995 deichmann lecture-p53 tumor suppressor gene: At the crossroads of molecular carcinogenesis, molecular epidemiology and cancer risk assessment. *Toxicol. Lett.* 82-83, 1–7. doi:10.1016/0378-4274(95)03643-1

Hassin, O., and Oren, M. (2022). Drugging p53 in cancer: one protein, many targets. *Nat. Rev. Drug Discov.* 22, 127–144. doi:10.1038/s41573-022-00571-8

Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997). Mdm2 promotes the rapid degradation of p53. *Nature* 387 (6630), 296–299. doi:10.1038/387296a0

Hays, E., and Bonavida, B. (2019). YY1 regulates cancer cell immune resistance by modulating PD-L1 expression. *Drug Resist. Updat.* 43, 10–28. doi:10.1016/j.drup.2019.04.001

Hellmann, M. D., Paz-Ares, L., Bernabe Caro, R., Zurawski, B., Kim, S. W., Carcereny Costa, E., et al. (2019). Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. *N. Engl. J. Med.* 381 (21), 2020–2031. doi:10.1056/NEJMoa1910231

Ho, J. N. H. G., Schmidt, D., Lowinus, T., Ryoo, J., Dopfer, E. P., Gonzalo Nunez, N., et al. (2022). Targeting MDM2 enhances antileukemia immunity after allogeneic transplantation via MHC-II and TRAIL-R1/2 upregulation. *Blood* 140 (10), 1167–1181. doi:10.1182/blood.2022016082

Huynh, A., DuPage, M., Priyadharshini, B., Sage, P. T., Quiros, J., Borges, C. M., et al. (2015). Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. *Nat. Immunol.* 16 (2), 188–196. doi:10.1038/ni.3077

Jhunjhunwala, S., Hammer, C., and Delamarre, L. (2021). Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. *Nat. Rev. Cancer* 21 (5), 298–312. doi:10.1038/s41568-021-00339-z

Jiang, L., Kon, N., Li, T., Wang, S. J., Su, T., Hibshoosh, H., et al. (2015). Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520 (7545), 57–62. doi:10.1038/nature14344

Kang, Y. J., Balter, B., Csizmadia, E., Haas, B., Sharma, H., Bronson, R., et al. (2017). Contribution of classical end-joining to PTEN inactivation in p53-mediated glioblastoma formation and drug-resistant survival. *Nat. Commun.* 8, 14013. doi:10.1038/ncomms14013

Keast, D. (1970). Immunosurveillance and cancer. *Lancet* 2 (7675), 710–712. doi:10.1016/s0140-6736(70)91973-2

Kim, S. S., Harford, J. B., Moghe, M., Slaughter, T., Doherty, C., and Chang, E. H. (2019). A tumor-targeting nanomedicine carrying the p53 gene crosses the blood-brain barrier and enhances anti-PD-1 immunotherapy in mouse models of glioblastoma. *Int. J. Cancer* 145 (9), 2535–2546. doi:10.1002/ijc.32531

Kirkwood, J. M., Tarhini, A. A., Panelli, M. C., Moschos, S. J., Zarour, H. M., Butterfield, L. H., et al. (2008). Next generation of immunotherapy for melanoma. *J. Clin. Oncol.* 26 (20), 3445–3455. doi:10.1200/JCO.2007.14.6423

Krenz, B., Gebhardt-Wolf, A., Ade, C. P., Gaballa, A., Roehrig, F., Vendelova, E., et al. (2021). MYC- and MIZ1-Dependent vesicular transport of Double-strand RNA controls immune evasion in pancreatic Ductal adenocarcinoma. *Cancer Res.* 81 (16), 4242–4256. doi:10.1158/0008-5472.CAN-21-1677

Kubbutat, M. H., Jones, S. N., and Vousden, K. H. (1997). Regulation of p53 stability by Mdm2. *Nature* 387 (6630), 299–303. doi:10.1038/387299a0

Langer, L. F., Clay, T. M., and Morse, M. A. (2007). Update on anti-CTLA-4 antibodies in clinical trials. *Expert Opin. Biol. Ther.* 7 (8), 1245–1256. doi:10.1517/14712598.7.8.1245

Li, Y., Weng, Y., Zhong, L., Chong, H., Chen, S., Sun, Y., et al. (2017). VEGFR3 inhibition chemosensitizes lung adenocarcinoma A549 cells in the tumor-associated macrophage microenvironment through upregulation of p53 and PTEN. *Oncol. Rep.* 38 (5), 2761–2773. doi:10.3892/or.2017.5969

Li, C., Jiang, P., Wei, S., Xu, X., and Wang, J. (2020). Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. *Mol. Cancer* 19 (1), 116. doi:10.1186/s12943-020-01234-1

Li, T., Liu, T., Zhu, W., Xie, S., Zhao, Z., Feng, B., et al. (2021). Targeting MDSC for immune-checkpoint blockade in cancer immunotherapy: Current progress and new prospects. *Clin. Med. Insights Oncol.* 15, 11795549211035540. doi:10.1177/11795549211035540

Liao, P., Zeng, S. X., Zhou, X., Chen, T., Zhou, F., Cao, B., et al. (2017). Mutant p53 gains its function via c-myc activation upon CDK4 phosphorylation at serine 249 and consequent PIN1 binding. *Mol. Cell* 68 (6), 1134–1146.e6. doi:10.1016/j.molcel.2017.11.006

Lindstrom, M. S., Bartek, J., and Maya-Mendoza, A. (2022). p53 at the crossroad of DNA replication and ribosome biogenesis stress pathways. *Cell Death Differ.* 29 (5), 972–982. doi:10.1038/s41418-022-00999-w

Liu, Y., and Gu, W. (2021). The complexity of p53-mediated metabolic regulation in tumor suppression. *Seminars Cancer Biol.* 85, 4–32. doi:10.1016/j.semcancer.2021.03.010

Liu, Y., and Gu, W. (2022). p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell Death Differ.* 29 (5), 895–910. doi:10.1038/s41418-022-00943-y

Liu, Y., Tavana, O., and Gu, W. (2019). p53 modifications: exquisite decorations of the powerful guardian. *J. Mol. Cell Biol.* 11 (7), 564–577. doi:10.1093/jmcb/mjz060

Lopez-Diaz, F. J., Gascard, P., Balakrishnan, S. K., Zhao, J., Del Rincon, S. V., Spruck, C., et al. (2013). Coordinate transcriptional and translational repression of p53 by TGF-β1 impairs the stress response. *Mol. Cell* 50 (4), 552–564. doi:10.1016/j.molcel.2013.04.029

Lowe, J. M., Menendez, D., Bushel, P. R., Shatz, M., Kirk, E. L., Troester, M. A., et al. (2014). p53 and NF-κB coregulate proinflammatory gene responses in human macrophages. *Cancer Res.* 74 (8), 2182–2192. doi:10.1158/0008-5472.CAN-13-1070

Mandapathil, M., Visus, C., Finn, O. J., Lang, S., and Whiteside, T. L. (2013). Generation and immunosuppressive functions of p53-induced human adaptive regulatory T cells. *Oncoimmunology* 2 (7), e25514. doi:10.4161/onci.25514

Muller, P. A., and Vousden, K. H. (2013). p53 mutations in cancer. *Nat. Cell Biol.* 15 (1), 2–8. doi:10.1038/ncb2641

Muller, P. A., and Vousden, K. H. (2014). Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell* 25 (3), 304–317. doi:10.1016/j.ccr.2014.01.021

Negrini, S., Gorgoulis, V. G., and Halazonetis, T. D. (2010). Genomic instability-an evolving hallmark of cancer. *Nat. Rev. Mol. Cell Biol.* 11 (3), 220–228. doi:10.1038/nrm2858

Ohkura, N., and Sakaguchi, S. (2020). Transcriptional and epigenetic basis of treg cell development and function: its genetic anomalies or variations in autoimmune diseases. *Cell Res.* 30 (6), 465–474. doi:10.1038/s41422-020-0324-7

Olivero, C. E., Martinez-Terroba, E., Zimmer, J., Liao, C., Tesfaye, E., Hooshdaran, N., et al. (2020). p53 activates the long noncoding RNA Pvt1b to inhibit Myc and suppress tumorigenesis. *Mol. Cell* 77 (4), 761–774.e8. doi:10.1016/j.molcel.2019.12.014

Ottaviani, S., Stebbing, J., Frampton, A. E., Zagorac, S., Krell, J., de Giorgio, A., et al. (2018). TGF-beta induces miR-100 and miR-125b but blocks let-7a through LIN28B controlling PDAC progression. *Nat. Commun.* 9 (1), 1845. doi:10.1038/s41467-018-03962-x

Park, J. S., Lim, M. A., Cho, M. L., Ryu, J. G., Moon, Y. M., Jhun, J. Y., et al. (2013). p53 controls autoimmune arthritis via STAT-mediated regulation of the Th17 cell/Treg cell balance in mice. *Arthritis Rheum.* 65 (4), 949–959. doi:10.1002/art.37841

Pascual, M., Mena-Varas, M., Robles, E. F., Garcia-Barchino, M. J., Panizo, C., Hervas-Stubbs, S., et al. (2019). PD-1/PD-L1 immune checkpoint and p53 loss facilitate tumor progression in activated B-cell diffuse large B-cell lymphomas. *Blood* 133 (22), 2401–2412. doi:10.1182/blood.2018889931

Powles, T., Park, S. H., Voog, E., Caserta, C., Valderrama, B. P., Gurney, H., et al. (2020). Avelumab maintenance therapy for advanced or metastatic urothelial carcinoma. *N. Engl. J. Med.* 383 (13), 1218–1230. doi:10.1056/NEJMoa2002788

Powles, T., Sridhar, S. S., Loriot, Y., Bellmunt, J., Mu, X. J., Ching, K. A., et al. (2021). Avelumab maintenance in advanced urothelial carcinoma: biomarker analysis of the phase 3 JAVELIN bladder 100 trial. *Nat. Med.* 27 (12), 2200–2211. doi:10.1038/s41591-021-01579-0

Pritchard, A., Tousif, S., Wang, Y., Hough, K., Khan, S., Strenkowski, J., et al. (2020). Lung tumor cell-Derived exosomes promote M2 macrophage polarization. *Cells* 9 (5), 1303. doi:10.3390/cells9051303

Propper, D. J., and Balkwill, F. R. (2022). Harnessing cytokines and chemokines for cancer therapy. *Nat. Rev. Clin. Oncol.* 19 (4), 237–253. doi:10.1038/s41571-021-00588-9

Reeves, E., Islam, Y., and James, E. (2020). ERAP1: A potential therapeutic target for a myriad of diseases. *Expert Opin. Ther. Targets* 24 (6), 535–544. doi:10.1080/14728222.2020.1751821

Rokavec, M., Oner, M. G., Li, H., Jackstadt, R., Jiang, L., Lodygin, D., et al. (2014). IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J. Clin. Invest.* 124 (4), 1853–1867. doi:10.1172/JCI73531

Salazar-Onfray, F., Lopez, M. N., and Mendoza-Naranjo, A. (2007). Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape. *Cytokine Growth Factor Rev.* 18 (1-2), 171–182. doi:10.1016/j.cytogfr.2007.01.015

Sallman, D. A., McLemore, A. F., Aldrich, A. L., Komrokji, R. S., McGraw, K. L., Dhawan, A., et al. (2020). TP53 mutations in myelodysplastic syndromes and secondary AML confer an immunosuppressive phenotype. *Blood* 136 (24), 2812–2823. doi:10.1182/blood.2020006158

Schuler, P. J., Harasymczuk, M., Visus, C., Deleo, A., Trivedi, S., Lei, Y., et al. (2014). Phase I dendritic cell p53 peptide vaccine for head and neck cancer. *Clin. Cancer Res.* 20 (9), 2433–2444. doi:10.1158/1078-0432.CCR-13-2617

Shamalov, K., Levy, S. N., Horovitz-Fried, M., and Cohen, C. J. (2017). The mutational status of p53 can influence its recognition by human T-cells. *Oncoimmunology* 6 (4), e1285990. doi:10.1080/2162402X.2017.1285990

Shieh, S. Y., Ikeda, M., Taya, Y., and Prives, C. (1997). DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell* 91 (3), 325–334. doi:10.1016/s0092-8674(00)80416-x

Sun, H., Liu, S. Y., Zhou, J. Y., Xu, J. T., Zhang, H. K., Yan, H. H., et al. (2020). Specific TP53 subtype as biomarker for immune checkpoint inhibitors in lung adenocarcinoma. *EBioMedicine* 60, 102990. doi:10.1016/j.ebiom.2020.102990

Sun, Y. F., Wu, L., Liu, S. P., Jiang, M. M., Hu, B., Zhou, K. Q., et al. (2021). Dissecting spatial heterogeneity and the immune-evasion mechanism of CTCs by single-cell RNA-seq in hepatocellular carcinoma. *Nat. Commun.* 12 (1), 4091. doi:10.1038/s41467-021-24386-0

Thiem, A., Hesbacher, S., Kneitz, H., di Primio, T., Heppt, M. V., Hermanns, H. M., et al. (2019). IFN-gamma-induced PD-L1 expression in melanoma depends on p53 expression. *J. Exp. Clin. Cancer Res.* 38 (1), 397. doi:10.1186/s13046-019-1403-9

Topalian, S. L., Hodi, F. S., Brahmer, J. R., Gettinger, S. N., Smith, D. C., McDermott, D. F., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366 (26), 2443–2454. doi:10.1056/NEJMoa1200690

Trivedi, M., Talekar, M., Shah, P., Ouyang, Q., and Amiji, M. (2016). Modification of tumor cell exosome content by transfection with wt-p53 and microRNA-125b expressing plasmid DNA and its effect on macrophage polarization. *Oncogenesis* 5 (8), e250. doi:10.1038/oncsis.2016.52

Van Damme, H., Dombrecht, B., Kiss, M., Roose, H., Allen, E., Van Overmeire, E., et al. (2021). Therapeutic depletion of CCR8(+) tumor-infiltrating regulatory T cells elicits antitumor immunity and synergizes with anti-PD-1 therapy. *J. Immunother. Cancer* 9 (2), e001749. doi:10.1136/jitc-2020-001749

Ventura, A., Kirsch, D. G., McLaughlin, M. E., Tuveson, D. A., Grimm, J., Lintault, L., et al. (2007). Restoration of p53 function leads to tumour regression *in vivo*. *Nature* 445 (7128), 661–665. doi:10.1038/nature05541

Volk-Draper, L., Patel, R., Bhattarai, N., Yang, J., Wilber, A., DeNardo, D., et al. (2019). Myeloid-Derived lymphatic endothelial cell progenitors significantly contribute to lymphatic metastasis in clinical breast cancer. *Am. J. Pathol.* 189 (11), 2269–2292. doi:10.1016/j.ajpath.2019.07.006

Wang, B., Niu, D., Lai, L., and Ren, E. C. (2013). p53 increases MHC class I expression by upregulating the endoplasmic reticulum aminopeptidase ERAP1. *Nat. Commun.* 4, 2359. doi:10.1038/ncomms3359

Wang, H. Q., Mulford, I. J., Sharp, F., Liang, J., Kurtulus, S., Trabucco, G., et al. (2021). Inhibition of MDM2 promotes antitumor responses in p53 wild-type cancer cells through their interaction with the immune and stromal microenvironment. *Cancer Res.* 81 (11), 3079–3091. doi:10.1158/0008-5472.CAN-20-0189

Wang, J., Hu, Y., Escamilla-Rivera, V., Gonzalez, C. L., Tang, L., Wang, B., et al. (2021). Epithelial mutant p53 promotes resistance to anti-PD-1-mediated oral cancer immunoprevention in carcinogen-induced mouse models. *Cancers (Basel)* 13 (6), 1471. doi:10.3390/cancers13061471

Weber, J. S., O'Day, S., Urba, W., Powderly, J., Nichol, G., Yellin, M., et al. (2008). Phase I/II study of ipilimumab for patients with metastatic melanoma. *J. Clin. Oncol.* 26 (36), 5950–5956. doi:10.1200/JCO.2008.16.1927

Wellenstein, M. D., Coffelt, S. B., Duits, D. E. M., van Miltenburg, M. H., Slagter, M., de Rink, I., et al. (2019). Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature* 572 (7770), 538–542. doi:10.1038/s41586-019-1450-6

Willenbrink, T. J., Ruiz, E. S., Cornejo, C. M., Schmults, C. D., Arron, S. T., and Jambusaria-Pahlajani, A. (2020). Field cancerization: Definition, epidemiology, risk factors, and outcomes. *J. Am. Acad. Dermatol.* 83 (3), 709–717. doi:10.1016/j.jaad.2020.03.126

Williams, P., Basu, S., Garcia-Manero, G., Hourigan, C. S., Oetjen, K. A., Cortes, J. E., et al. (2019). The distribution of T-cell subsets and the expression of immune checkpoint receptors and ligands in patients with newly diagnosed and relapsed acute myeloid leukemia. *Cancer* 125 (9), 1470–1481. doi:10.1002/cncr.31896

Woo, S. R., Corrales, L., and Gajewski, T. F. (2015). Innate immune recognition of cancer. *Annu. Rev. Immunol.* 33, 445–474. doi:10.1146/annurev-immunol-032414-112043

Xiao, Y., Chen, J., Zhou, H., Zeng, X., Ruan, Z., Pu, Z., et al. (2022). Combining p53 mRNA nanotherapy with immune checkpoint blockade reprograms the immune microenvironment for effective cancer therapy. *Nat. Commun.* 13 (1), 758. doi:10.1038/s41467-022-28279-8

Xu, Q. R., Tang, J., Liao, H. Y., Yu, B. T., He, X. Y., Zheng, Y. Z., et al. (2021). Long non-coding RNA MEG3 mediates the miR-149-3p/FOXP3 axis by reducing

p53 ubiquitination to exert a suppressive effect on regulatory T cell differentiation and immune escape in esophageal cancer. *J. Transl. Med.* 19 (1), 264. doi:10.1186/s12967-021-02907-1

Xu, N., Yao, Z., Shang, G., Ye, D., Wang, H., Zhang, H., et al. (2022). Integrated proteogenomic characterization of urothelial carcinoma of the bladder. *J. Hematol. Oncol.* 15 (1), 76. doi:10.1186/s13045-022-01291-7

Xue, W., Zender, L., Miething, C., Dickins, R. A., Hernando, E., Krizhanovsky, V., et al. (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 445 (7128), 656–660. doi:10.1038/nature05529

Yang, L., Pang, Y., and Moses, H. L. (2010). TGF-beta and immune cells: An important regulatory axis in the tumor microenvironment and progression. *Trends Immunol.* 31 (6), 220–227. doi:10.1016/j.it.2010.04.002

Ye, J., Huang, X., Hsueh, E. C., Zhang, Q., Ma, C., Zhang, Y., et al. (2012). Human regulatory T cells induce T-lymphocyte senescence. *Blood* 120 (10), 2021–2031. doi:10.1182/blood-2012-03-416040

Yi, M., Zhang, J., Li, A., Niu, M., Yan, Y., Jiao, Y., et al. (2021). The construction, expression, and enhanced anti-tumor activity of YM101: A bispecific antibody simultaneously targeting TGF-β and PD-L1. *J. Hematol. Oncol.* 14 (1), 27. doi:10.1186/s13045-021-01045-x

Zeng, L., Rowland, R. G., Lele, S. M., and Kyprianou, N. (2004). Apoptosis incidence and protein expression of p53, TGF-beta receptor II, p27Kip1, and Smad4 in benign, premalignant, and malignant human prostate. *Hum. Pathol.* 35 (3), 290–297. doi:10.1016/j.humpath.2003.11.001

Zhou, Y., Que, K. T., Zhang, Z., Yi, Z. J., Zhao, P. X., You, Y., et al. (2018). Iron overloaded polarizes macrophage to proinflammation phenotype through ROS/acetyl-p53 pathway. *Cancer Med.* 7 (8), 4012–4022. doi:10.1002/cam4.1670

Zhou, X., Singh, M., Sanz Santos, G., Guerlavais, V., Carvajal, L. A., Aivado, M., et al. (2021). Pharmacologic activation of p53 triggers viral mimicry response thereby abolishing tumor immune evasion and promoting antitumor immunity. *Cancer Discov.* 11 (12), 3090–3105. doi:10.1158/2159-8290.CD-20-1741

Zhu, K., Wang, J., Zhu, J., Jiang, J., Shou, J., and Chen, X. (1999). p53 induces TAP1 and enhances the transport of MHC class I peptides. *Oncogene* 18 (54), 7740–7747. doi:10.1038/sj.onc.1203235

Zhu, H., Klement, J. D., Lu, C., Redd, P. S., Yang, D., Smith, A. D., et al. (2021). Asah2 represses the p53-hmox1 Axis to protect myeloid-Derived suppressor cells from ferroptosis. *J. Immunol.* 206 (6), 1395–1404. doi:10.4049/jimmunol.2000500

Glossary

NK cell Natural killer cell

DC cell Dendritic cell

Mutp53 Mutant p53

Wt-p53 Wild-type p53

MHC Major histocompatibility complex

TAP Transporter associated with antigen processing

ERAP1 Endoplasmic reticulum aminopeptidase 1

Myc Myelocytomatosis

TLR3 Toll-like receptor 3

ERV Endogenous retroviruses

IFN Interferon

STAT Signal transducer and activator of transcription

TCR T cell receptor

TNF Tumor necrosis factor

AML Acute myeloid leukemia

TRAIL-R1/2 Tumor necrosis factor related apoptosis-inducing ligand receptor 1 and receptor 2

ISGs IFN-stimulated genes

STING Stimulator of IFN genes

IRF3 IFN regulatory factor 3

TBK1 TANK binding protein kinase 1

IL-6 Interleukin-6

EMT Epithelial-to-mesenchymal transition

TAMs Tumor accosicated macrophages

TGF-β Transforming growth factor-β

Teffs Effector T cells

Tregs Regulatory T cells

E2F-4 Viral E2-associated factor 4

PD-1 Programmed cell death protein 1

CTLA-4 Cytotoxic T lymphocyte-associated antigen-4

ICIs Immune checkpoint inhibitors

TIM3 T-cell immunoglobulin and mucin-domain containing-3

LAG3 Lymphocyte-activation gene 3

TIME Tumor immune microenvironment

Foxp3 Factor forkhead box P3

LncRNA Long non-coding RNA

MEG3 Maternally expressed gene 3

MDSC Myeloid-derived suppressor cell

M-CSF Macrophage colony-stimulating factor

Hmox1 Heme oxygenase-1

p53MVA Modified vaccinia ankara vaccine delivering wild-type human p53

PFS Progression-free survival.

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Wild-type and mutant p53 in cancer-related ferroptosis. A matter of stress management?

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Cancer cells within tumor masses are chronically exposed to stress caused by nutrient deprivation, oxygen limitation, and high metabolic demand. They also accumulate hundreds of mutations, potentially generating aberrant proteins that can induce proteotoxic stress. Finally, cancer cells are exposed to various damages during chemotherapy. In a growing tumor, transformed cells eventually adapt to these conditions, eluding the death-inducing outcomes of signaling cascades triggered by chronic stress. One such extreme outcome is ferroptosis, a form of iron-dependent non-apoptotic cell death mediated by lipid peroxidation. Not surprisingly, the tumor suppressor p53 is involved in this process, with evidence suggesting that it acts as a pro-ferroptotic factor and that its ferroptosis-inducing activity may be relevant for tumor suppression. Missense alterations of the TP53 gene are extremely frequent in human cancers and give rise to mutant p53 proteins (mutp53) that lose tumor suppressive function and can acquire powerful oncogenic activities. This suggests that p53 mutation provides a selective advantage during tumor progression, raising interesting questions on the impact of p53 mutant proteins in modulating the ferroptotic process. Here, we explore the role of p53 and its cancer-related mutants in ferroptosis, using a perspective centered on the resistance/sensitivity of cancer cells to exogenous and endogenous stress conditions that can trigger ferroptotic cell death. We speculate that an accurate molecular understanding of this particular axis may improve cancer treatment options.

KEYWORDS

hypoxia, autophagy, UPR, unfolded protein response, p53 tumor suppressor, ER stress, ferroptosis, stress response pathways

Introduction

TP53 is possibly the most frequently altered gene in human cancers (Kandoth et al., 2013). The encoded p53 protein is a powerful tumor suppressor, and its loss-of-function is associated with cancer development and progression (Levine, 2020). Intriguingly, the majority of TP53 mutations are missense, encoding full-length proteins (mutp53) that are stably expressed in tumor cells. The pervasive retention of mutp53 in cancer suggests a selective advantage; indeed, missense p53 mutants have been reported to foster cancer cell proliferation, invasion, metastasis, and chemoresistance (Pilley et al., 2021; Dolma and Muller, 2022). Various oncogenic phenotypes and mechanisms of action, transcriptional and non-transcriptional, have been described for mutant p53 (Bellazzo et al., 2018; Kim and

Lozano, 2018); nonetheless, our understanding of the real impact of mutp53 in cancer formation and progression remains incomplete.

An interesting hypothesis is that mutp53, similarly to its wild-type counterpart, may sense transformation-related cellular stresses and coordinate adaptive responses that help tumor progression (D'Orazi and Cirone, 2019; Mantovani et al., 2019). Such indirect action, dependent on multiple unpredictable circumstances, could explain why missense TP53 mutations are pervasively selected in tumors, but depletion of mutp53 in cancer cell lines and preclinical models gives variable and often contradictory results (Kennedy and Lowe, 2022; Wang et al., 2022).

Cancer cells within tumors experience multiple adverse conditions: nutrient and oxygen shortage, high metabolic demand, increased mutation rate, and chemotherapy-induced DNA damage. They eventually adapt to chronic stress, often hijacking stress-response pathways to favor homeostasis and survival. For instance, aberrant activation of the unfolded protein response can facilitate cancer progression by inducing epithelial mesenchymal transition, stimulating angiogenesis, and supporting tumor cell dormancy (Senft and Ronai, 2016; Limia et al., 2019). Some mechanisms by which mutp53 can help cancer cells adapt to cancer-related stress are beginning to emerge from tissue culture and animal models; characterizing such mechanisms may open new opportunities for targeted therapy.

Cancer-related stress conditions can directly or indirectly cause ferroptosis, a cell death process resulting from intracellular accumulation of lipid peroxides. Ferroptosis is under intense study due to its potential anti-cancer activity, especially in apoptosis-resistant tumors (Friedmann Angeli et al., 2019; Lei et al., 2022; Rodriguez et al., 2022). In fact, due to their altered metabolism, cancer cells are susceptible to ferroptosis and highly dependent on protective systems for survival; genes and pathways involved in such processes, therefore, could be targeted to improve chemotherapy. Not surprisingly, wild-type p53 has been reported to modulate ferroptosis in tumor models, possibly affecting response to treatment. The emerging relevance of the p53-ferroptosis axis inevitably raises important questions about the impact of cancer-associated mutant p53 in this phenomenon.

Ferroptosis

The term 'ferroptosis' describes a form of non-apoptotic cell death characterized by iron-dependent production of Lipid-ROS responsible for cell killing (Dixon et al., 2012). Since its first description, the number of papers studying ferroptosis has increased exponentially (Stockwell, 2022) confirming its involvement in both physiological and pathological events ranging from development, immune functions and tumor suppression, to neurodegeneration, autoimmunity and tumorigenesis (Jiang et al., 2021).

Lipid-ROS are the main executioners of ferroptosis, produced by intracellular iron accumulation, promoting peroxidation of PL-PUFA through Fenton reactions (Shah et al., 2018). The cellular labile iron pool required to stimulate ferroptosis can be the result of either increased iron import from the extracellular compartment, or released by autophagy-mediated degradation of ferritin (ferritinophagy) (Hou et al., 2016). Also iron-containing enzymes, such as ALOXs and POR, can promote lipid peroxidation, driving ferroptosis (Yang and Stockwell, 2016; Gagliardi et al., 2020; Zou et al., 2020).

On the other hand, biological processes protecting cells from Lipid-ROS must be concomitantly inhibited. GPX4 is the main intracellular factor responsible for Lipid-ROS reduction, using GSH as cofactor (Seiler et al., 2008). Thus, inhibition of GPX4 activity (e.g., through RSL3 administration), or impairment of GSH production through inhibition of the transmembrane glutamate/cystine antiporter "System Xc^-", will result in Lipid-ROS accumulation and ferroptosis (Dixon et al., 2012).

A key component of "System Xc^-" is the solute transporter SLC7A11, frequently overexpressed in human malignancies, representing a potential target for ferroptosis-based therapies. In addition, Lipid-ROS can be detoxified by GPX4-independent factors such as FSP1 (Bersuker et al., 2019; Doll et al., 2019), DHODH (Mao et al., 2021), GCHI/BH4 (Kraft et al., 2020), and AKRs (Gagliardi et al., 2019; Gagliardi et al., 2020). The precise molecular mechanism(s) by which membrane-bound Lipid-ROS execute the death process remains unclear; one hypothesis is that they destabilize the plasma membrane structure, dysregulating its permeability (Figure 1).

P53 behaves primarily as a pro-ferroptotic factor, since it negatively regulates SLC7A11, increasing sensitivity to ferroptosis (Jiang et al., 2015b). P53 also controls the expression of enzymes involved in polyamine, glutamine, and iron metabolism, facilitating cell death by ferroptosis inducers. Importantly, using mouse models, the pro-ferroptotic activity of p53 was elegantly demonstrated to be sufficient for tumor suppression *in vivo* (Wang et al., 2016). Under certain conditions, however, p53 can also inhibit ferroptosis facilitating ROS detoxification and lipid homeostasis, limiting their pro-oncogenic action (Liu et al., 2020; Liu and Gu, 2022b).

Less is known on the impact of mutant p53 in the ferroptotic process. The consensus is that it increases sensitivity to ferroptosis, since mutp53 efficiently represses SLC7A11 (Gnanapradeepan et al., 2018; Magri et al., 2021). However, there is contradictory evidence. For instance, the drug APR-246 can induce ferroptosis more efficiently in blood cancer cells with mutp53 (Birsen et al., 2021; Fujihara et al., 2022; Hong et al., 2022). Although the pro-ferroptotic action of APR-246 is independent of p53 (Liu et al., 2017; Magri et al., 2021; Fujihara et al., 2022), the drug is a powerful inhibitor of mutant p53 (Hassin and Oren, 2022; Levine, 2022), and this may contribute to its efficacy. Similarly, the quinolinol MMRi62 was shown to induce ferroptosis in pancreatic cancer cells by inducing ferritinophagy (see below), but also by mutp53 destabilization (Li et al., 2022). Thus, we speculate that mutant p53 can modulate the sensitivity of cancer cells to ferroptosis not only directly, e.g., controlling ferroptotic genes, but also indirectly, by facilitating cellular adaptation to cancer-related stress.

p53 and stress conditions triggering ferroptosis

Hypoxia

Hypoxia is chronic in most tumors, and this condition is often exploited by cancer cells to sustain proliferation, metabolism, tumor

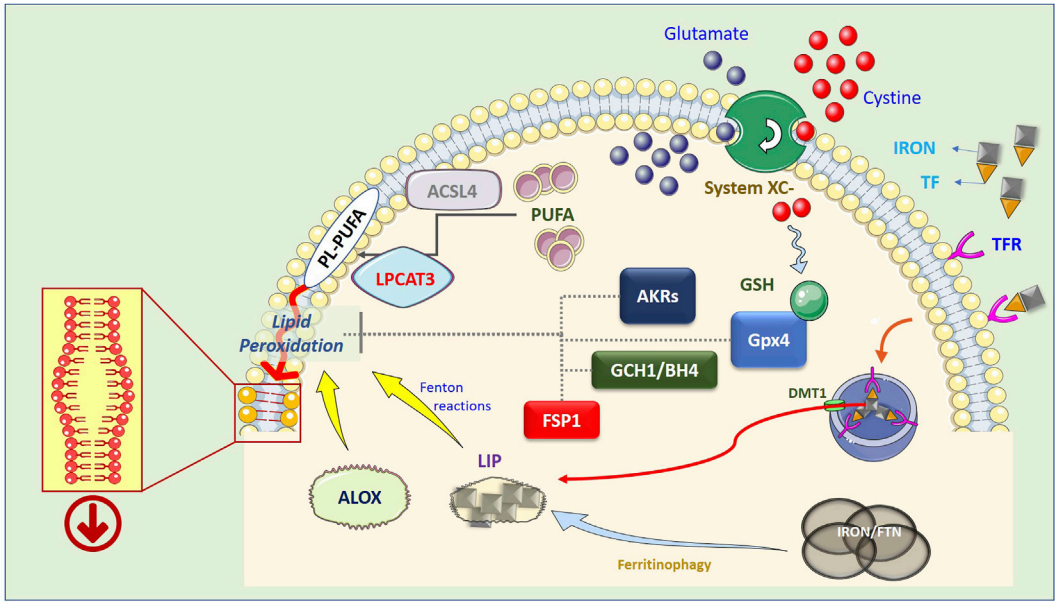

FIGURE 1
Schematic representation of the ferroptotic process. Lipid peroxidation resulting in the generation of Lipid-ROS is considered the point of no return in the execution of ferroptosis. However, the precise mechanism(s) by which these highly reactive molecules execute the cell death process is still not completely clear. The current hypothesis is that peroxidized PL-PUFAs destabilize the membrane thus compromising its barrier functions. PUFA are introduced into cell membranes, as PL-PUFA, through the combined activity of ACSL4 and LPCAT3, while lipid peroxidation is catalyzed by increased available iron (LIP) through Fenton reactions, or by lipoxygenases (ALOX), which use iron as a cofactor. In turn, LIP can be generated by both ferritinophagy, which degrades intracellular and ferritin-based iron stores, or increased uptake of extracellular iron, through the iron/TF interaction with membrane TFRs, endosomal release of iron, and DMT1-mediated relocation in the cytosol. On the other hand, Lipid-ROS can be actively degraded by the GSH-dependent activity of GPX4, by increased expression/activity of AKRs, or can be reduced by the FSP1- or GCH1/BH4- dependent cycles.

invasion, and metastasis (Yang et al., 2020). In this context, a key role is played by HIF1, a transcription factor activated by low oxygen and frequently overexpressed in cancer (Su et al., 2022). Interestingly, HIF1 inhibits ferroptosis by: i) upregulating SCD1 to increase MUFA synthesis; ii) inhibiting the expression of ACSL4 to reduce Lipid-ROS generation, and iii) inhibiting the degradation of SLC7A11 (Su et al., 2022). Therefore, the reduced efficacy of radiation or drug-based therapies in solid tumors has been, at least in part, associated with HIF1-mediated inhibition of ferroptosis (Wang et al., 2019; Su et al., 2022).

p53 is activated by hypoxia, driving a cellular response that also involves modulation of cell metabolism (Liu and Gu, 2022a). In particular, p53 has a complex relationship with HIF1α. The two proteins interact, and both wild-type and mutp53 potentiate the transcriptional activity of HIF1α (Sermeus and Michiels, 2011; Eriksson et al., 2019). Reciprocally, activated HIF1α stimulates p53 expression by binding to its promoter (Madan et al., 2019). Such positive feedback may be relevant for aberrant accumulation of highly stable mutp53 proteins in hypoxic cancer cells. In turn, mutp53 interacts with HIF1α, stabilizing it, and promoting its DNA binding, increasing expression of genes that contribute to hypoxia-induced cell growth and survival (Madan et al., 2019). Mutant p53 can enhance angiogenesis by HIF1/VEGF signaling, and many HIF1-target genes are also targets of NRF2, linking hypoxic response to redox homeostasis (Eriksson et al., 2019). It would be interesting to establish to what extent the interaction of mutp53 with HIF1α contributes to determine the sensitivity to ferroptosis of hypoxic cancer cells. Of note, mutp53/HIF1α complexes drive expression of miR-30d, that reshapes the structure of Golgi apparatus, promoting cancer cells secretory activity. This impacts on the tumor microenvironment, with implications for hormonal and mechanical signaling pathways (Capaci et al., 2020), but also affects ER homeostasis and UPR signaling that may affect ferroptosis (see below).

Oxidative stress

ROS production is associated with both physiological and pathological conditions. Proper ROS production contributes to differentiation, immunity, and cell signaling, but uncontrolled accumulation leads to damage of proteins, lipids, and nucleic acids, causing “oxidative stress”, involved in cardiovascular and neurodegenerative diseases, obesity, aging, and cancer (Pizzino et al., 2017; Szewczyk-Golec et al., 2018).

Oxidative DNA damage is one of the stimuli driving tumorigenesis (Pizzino et al., 2017), and was detected in cells dying through ferroptosis (Erlanson et al., 2019; Liu J. et al., 2021). Therefore, in addition to being an integral part of the molecular mechanism of ferroptotic death, oxidative stress might regulate the process itself (Liu J. et al., 2021).

p53 is activated by oxidative stress, and can reduce ROS to promote cell survival, or increase ROS to facilitate cell death, depending on its gene targets or binding partners (Eriksson et al.,

2019). The cellular response to oxidative stress is mainly regulated by NRF2, a transcription factor that controls expression of several antioxidant proteins (Rojo de la Vega et al., 2018). Notably, depending on cellular context, p53 can increase NRF2 levels by preventing its degradation, or reduce NRF2 levels by repressing its transcription (Eriksson et al., 2019; Liu and Gu, 2022a). Oncogenic mutp53 apparently has opposite effects. For instance, in lung and breast epithelial cells wt p53 suppressed NOX4 reducing ROS levels and cell migration, while mutp53 was shown to stimulate ROS production and metastasis (Boudreau et al., 2014). Mutp53 binds NRF2 on the SLC7A11 promoter, repressing transcription; this renders mutant p53 cells more sensitive to oxidative assaults and prone to ferroptosis (Liu et al., 2017). However, in breast cancer models, mutant p53 cooperates with NRF2 to transcribe proteasome components, alleviating proteotoxic stress and enhancing cell survival and cancer aggressiveness (Walerych et al., 2016; Lisek et al., 2018). Intriguingly, expression of transactivation-defective p53(3K), or ROS generation alone, could not induce ferroptosis, but their combination induced massive ferroptotic cell death (Jiang et al., 2015a; Jiang et al., 2015b); this indicates that p53-dependent ferroptosis may be a crucial tumor-suppressive response to oxidative stress. Similarly, the deacetylase SIRT3 represses p53-mediated ferroptosis in various cancer cells (Jin et al., 2021). SIRT3 expression is altered in several tumors (Chen et al., 2014; Ansari et al., 2017), and may cooperate with p53 mutation to increase cancer cell resistance to ferroptosis upon oxidative stress.

Oxidative stress can also trigger ferroptosis by enhancing peroxidation of membrane lipids. Interestingly, p53 can upregulate iPLA2β, a Ca-independent phospholipase that cleaves oxidized fatty acids, promoting their cytosolic detoxification, and thus limiting ferroptosis. Notably, p53 upregulates iPLA2β only under conditions of moderate lipid damage, facilitating adaptation to oxidative stress (Chen et al., 2021; Liu and Gu, 2022b). Loss of p53 function would cut this modulatory feedback, sensitizing p53-null cancer cells to ROS-induced lipid damage. Cells with oncogenic p53 mutations also lack this adaptive circuit, but may compensate with enhanced activity of NRF2 (see above).

Endoplasmic reticulum stress

Nutrient deprivation, proteasome dysfunction, sustained secretory activity, and somatic mutations in ER client proteins cause dysregulated proteostasis in proliferating tumor cells, thus triggering activation of the unfolded protein response (UPR) (Corazzari et al., 2017; Chen and Cubillos-Ruiz, 2021). Accumulation of unfolded/misfolded proteins in the ER is sensed by the receptors PERK, IRE1, and ATF6, that trigger activation/upregulation of transcription factors: ATF4, induced by PERK activation, XBP1s, produced by IRE1-dependent cytoplasmic splicing of XBP1 mRNA, and ATF6f, generated by proteolytic cleavage of activated ATF6. These factors orchestrate a transcriptional response aimed to: i) increase ER folding capacity; ii) inhibit cap-dependent translation; iii) degrade misfolded/unfolded ER client proteins (ERAD). Overall these activities sustain cell survival ("adaptation phase" of UPR), but acute or unresolved ER stress stimulates apoptosis ("cell death phase") (Pagliarini et al., 2015; Corazzari et al., 2017). A potential link between ER stress and ferroptosis has been proposed due to the identification of CHAC1 as a ferroptotic marker (Dixon et al., 2014); indeed CHAC1 is upregulated upon ER stress and contributes to GSH degradation (Galluzzi et al., 2012), thus connecting the two pathways (Dixon et al., 2014). However, we observed that UPR is not required for ferroptosis in metastatic melanoma cells, despite a clear and early upregulation of CHAC1, that could be be abrogated by inhibiting NRF2, suggesting that CHAC1 is under control of both UPR and NRF2 (Gagliardi et al., 2019; Gagliardi et al., 2020). Clearly, further studies are required to unveil the real involvement of ER stress in ferroptosis.

Evidence linking wt p53 to ER stress is scarce, but various observations implicate mutant p53 in protein homeostasis. First, mutp53 cooperates with NRF2 to upregulate proteasome components, thus increasing protein turnover in cancer cells (Walerych et al., 2016; Lisek et al., 2018). This accelerates degradation of tumor-suppressors, promoting cell proliferation; at the same time it can help reduce or resolve ER stress, promoting cell survival. Second, mutp53 enhances expression of ENTPD5, an ER enzyme involved in folding of N-glycosylated proteins (Vogiatzi et al., 2016). This facilitates the maturation and secretion of growth-factor receptors, promoting cell proliferation; it may also alleviate ER stress by enhancing protein folding. Third, mutp53 induces Golgi remodeling and increases protein secretion; this could alter ER protein homeostasis and favor adaptation to ER stress (Capaci et al., 2020). Finally, we found that mutp53 protects cancer cells from drug-induced ER stress by modulating the UPR, in particular by enhancing activation of ATF6 (Sicari et al., 2019). Although the impact of ER stress in ferroptosis remains to be defined, it is conceivable that alterations in p53 function may affect sensitivity to ferroptosis at least in part by modulating protein homeostasis and the UPR.

Nutrient deprivation and autophagy

Autophagy is an evolutionarily-conserved process responsible for lysosomal degradation of intracellular cargoes, sustaining cell survival under nutrient shortage conditions (Corazzari et al., 2013). Autophagy plays a paradoxical role in tumorigenesis, depending on the stage of tumor development; it is suppressive in early stages, mainly through degradation of potentially oncogenic molecules, but becomes oncogenic in advanced stages, promoting cell survival and ameliorating stress in the microenvironment (Galluzzi et al., 2015). Evidence of autophagy has been detected in cancer cells dying by ferroptosis, suggesting a potential connection between the two pathways (Liu L. et al., 2021). Indeed, NCOA4 mediates autophagy-dependent degradation of FTH, thus releasing iron (ferritinophagy) and triggering lipid peroxidation and ferroptosis (Mancias et al., 2014). Recently, other factors linking ferroptosis to specific autophagic processes have been identified, in particular affecting Lipid-ROS generation: for instance RAB7A (lipophagy) (Bai et al., 2019), ARNTL (clockophagy) (Yang et al., 2019), and HSP90/HSC70 (CMA) (Wu et al., 2019). In fact, it has been suggested that ferroptosis may be considered an autophagy-based type of cell death (Zhou et al., 2020), although this concept is still debated.

Wild-type p53 modulates autophagy both directly and indirectly (Maiuri et al., 2010; D'Orazi and Cirone, 2019; Liu and Gu, 2022a). When activated by DNA-damage, nuclear p53 upregulates

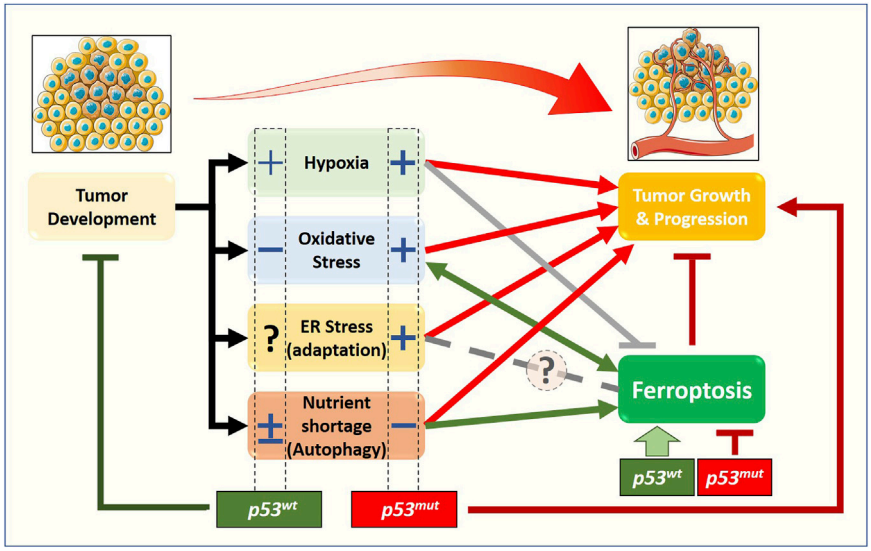

FIGURE 2
Relationship between stress-related signaling pathways, ferroptosis, and tumor growth, from a p53 status-centered (wt or mut) perspective. In the early stages of solid tumor development, cancer cells are subjected to oxygen and nutrient shortage, oxidative stress, and dysfunctional proteostasis. The molecular pathways activated in response to those stimuli will define the fate of the early tumor: survival (red arrows) or death. Beyond apoptosis, very recently, the new form of cell death named ferroptosis has been described to have a role in preventing/limiting the early tumor formation and growth, although the precise molecular mechanisms are still elusive. Emerging data show that stress-related signaling pathways have a non-negligible impact on ferroptosis induction/execution, with some of them stimulating (green arrows) while other preventing or inhibiting (gray lines) the process, thus enhancing or weakening the impact of ferroptotic cell death on tumor growth and progression. The complexity of this scenario is further amplified by the cellular response to stress-induced activation of the p53 tumor suppressor, and by the fact that a large fraction of tumors express gain-of-function p53 mutants. Accumulating data suggest that the p53 status (wt vs. mut) might have a significant impact on ferroptosis and tumor growth through a positive (+) or negative (–) effect on cancer-associated stress-related signaling pathways.

autophagy-associated genes, contributing to cancer cell death upon chemotherapy (Broz and Attardi, 2013). In contrast, cytoplasmic/mitochondrial p53 can suppress autophagy (Green and Kroemer, 2009). Additionally, p53 controls autophagy *via* interaction with key metabolic pathways, for instance positively modulating AMPK activity and negatively regulating AKT and mTOR (Mrakovcic and Fröhlich, 2018; Liu and Gu, 2022a).

Tumor-associated p53 mutants cannot transactivate autophagy genes and may acquire a suppressive role in autophagy (Cordani et al., 2017; Shi et al., 2021); especially mutp53 proteins with a pervasive cytoplasmic localization (Morselli et al., 2008). Mutp53 can also bind and inhibit AMPK (Zhou et al., 2014), and promote mTOR activation, indirectly suppressing autophagy (Liu and Gu, 2022a). So, although autophagy can help cancer cells overcome nutrient stress, evidence indicates that mutp53 inhibits autophagy to foster cancer aggressiveness. It is plausible that the p53 status may determine the sensitivity of cancer cells to ferroptosis also by modulating stress-induced autophagy.

Conclusion

Although cancer-related stress originates from a relatively small number of conditions—nutrient imbalance, hypoxia, reactive oxygen or nitrogen compounds, DNA damage, somatic mutations—the multiple pathways involved and the variable conditions that a tumor experiences during its clinical evolution generate an extremely complex scenario. Within this framework, the p53 pathway plays a central role in the response to stress, in particular determining whether cancer cells adapt or succumb to it *via* regulated cell death—including ferroptosis (Figure 2).

We suggest that mutant p53 can provide a selective advantage to tumors by facilitating adaptation to stress. This effect may not be evident under all conditions, but may become relevant under specific circumstances; for instance, at a given stage during cancer evolution, in response to a certain therapy, or in selected subpopulations of the tumor mass. Currently, there is a lack of experimental studies aimed to test this hypothesis, and we encourage research in this direction. Similarly, it may be important to define the specific stress conditions associated with a given tumor and/or chemotherapeutic drug; a better comprehension of this complexity may help predict the efficacy of treatments, in particular those inducing ferroptosis, in cancers with or without p53 mutation.

Research in the past decades led to development of several drugs that specifically target mutant p53, either by destabilizing the protein to reduce its levels, or by modulating its conformation to restore p53 tumor-suppressive functions (Dolma and Muller, 2022; Hassin and Oren, 2022; Levine, 2022). Such molecules are being tested for clinical use in combination with chemotherapy in p53 mutated cancers, with variable results. Many chemotherapeutic drugs can induce ferroptosis in addition to their primary action (e.g., cisplatin, gemcitabine, sorafenib); in preclinical cancer models their action is increased by co-treatment with ferroptosis inducers, such as drugs that inhibit System Xc^-, reduce

GSH, inhibit GPX4, or alter intracellular iron levels (Su et al., 2020; Wu et al., 2020; Lei et al., 2022). In this scenario, we hypothesize that targeting mutant p53 may increase the efficacy of pro-ferroptotic drugs under specific stress conditions, thus improving the clinical response of p53 mutated tumors.

Author contributions

Both authors conceptualized the paper and wrote the manuscript. MC conceived and drew the figures.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ansari, A., Rahman, M. S., Saha, S. K., Saikot, F. K., Deep, A., and Kim, K. H. (2017). Function of the SIRT3 mitochondrial deacetylase in cellular physiology, cancer, and neurodegenerative disease. *Aging Cell* 16 (1), 4–16. doi:10.1111/acel.12538

Bai, Y., Meng, L., Han, L., Jia, Y., Zhao, Y., Gao, H., et al. (2019). Lipid storage and lipophagy regulates ferroptosis. *Biochem. biophysical Res. Commun.* 508 (4), 997–1003. doi:10.1016/J.BBRC.2018.12.039

Bellazzo, A., Sicari, D., Valentino, E., Del Sal, G., and Collavin, L. (2018). Complexes formed by mutant p53 and their roles in breast cancer. *Breast Cancer (Dove Med. Press)* 10, 101–112. doi:10.2147/BCTT.S145826

Bersuker, K., Hendricks, J. M., Li, Z., Magtanong, L., Ford, B., Tang, P. H., et al. (2019). The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. *Nature* 575 (7784), 688–692. doi:10.1038/s41586-019-1705-2

Birsen, R., Larrue, C., Decroocq, J., Johnson, N., Guiraud, N., Gotanegre, M., et al. (2021). APR-246 induces early cell death by ferroptosis in acute myeloid leukemia. *Haematologica* 107 (2), 403–416. doi:10.3324/haematol.2020.259531

Boudreau, H. E., Casterline, B. W., Burke, D. J., and Leto, T. L. (2014). Wild-type and mutant p53 differentially regulate NADPH oxidase 4 in TGF-β-mediated migration of human lung and breast epithelial cells. *Br. J. Cancer* 110 (10), 2569–2582. doi:10.1038/bjc.2014.165

Broz, D. K., and Attardi, L. D. (2013). TRP53 activates a global autophagy program to promote tumor suppression. *Autophagy* 9 (9), 1440–1442. doi:10.4161/auto.25833

Capaci, V., Bascetta, L., Fantuz, M., Beznoussenko, G. V., Sommaggio, R., Cancila, V., et al. (2020). Mutant p53 induces Golgi tubulo-vesiculation driving a prometastatic secretome. *Nat. Commun.* 11 (1), 3945. doi:10.1038/s41467-020-17596-5

Chen, D., Chu, B., Yang, X., Liu, Z., Jin, Y., Kon, N., et al. (2021). iPLA2β-mediated lipid detoxification controls p53-driven ferroptosis independent of GPX4. *Nat. Commun.* 12 (1), 3644. doi:10.1038/s41467-021-23902-6

Chen, X., and Cubillos-Ruiz, J. R. (2021). Endoplasmic reticulum stress signals in the tumour and its microenvironment. *Nat. Rev. Cancer* 21 (2), 71–88. doi:10.1038/s41568-020-00312-2

Chen, Y., Fu, L. L., Wen, X., Wang, X. Y., Liu, J., Cheng, Y., et al. (2014). Sirtuin-3 (SIRT3), a therapeutic target with oncogenic and tumor-suppressive function in cancer. *Cell Death Dis.* 5 (2), e1047. doi:10.1038/cddis.2014.14

Corazzari, M., Fimia, G. M., Lovat, P., and Piacentini, M. (2013). Why is autophagy important for melanoma? Molecular mechanisms and therapeutic implications. *Semin. Cancer Biol.* 23 (5), 337–343. doi:10.1016/j.semcancer.2013.07.001

Corazzari, M., Gagliardi, M., Fimia, G. M., and Piacentini, M. (2017). Endoplasmic reticulum stress, unfolded protein response, and cancer cell fate. *Front. Oncol.* 7 (APR), 78. doi:10.3389/fonc.2017.00078

Cordani, M., Butera, G., Pacchiana, R., and Donadelli, M. (2017). Molecular interplay between mutant p53 proteins and autophagy in cancer cells. *Biochimica biophysica acta* 1867 (1), 19–28. doi:10.1016/j.bbcan.2016.11.003

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell* 149 (5), 1060–1072. doi:10.1016/j.cell.2012.03.042

Dixon, S. J., Patel, D. N., Welsch, M., Skouta, R., Lee, E. D., Hayano, M., et al. (2014). Pharmacological inhibition of cystine-glutamate exchange induces endoplasmic reticulum stress and ferroptosis. *eLife* 3, e02523. doi:10.7554/eLife.02523

Doll, S., Freitas, F. P., Shah, R., Aldrovandi, M., da Silva, M. C., Ingold, I., et al. (2019). FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* 575 (7784), 693–698. doi:10.1038/s41586-019-1707-0

Dolma, L., and Muller, P. A. J. (2022). GOF mutant p53 in cancers: A therapeutic challenge. *Cancers* 14 (20), 5091. doi:10.3390/cancers14205091

D'Orazi, G., and Cirone, M. (2019). Mutant p53 and cellular stress pathways: A criminal alliance that promotes cancer progression. *Cancers* 11 (5), 614. doi:10.3390/cancers11050614

Eriksson, S. E., Ceder, S., Bykov, V. J. N., and Wiman, K. G. (2019). p53 as a hub in cellular redox regulation and therapeutic target in cancer. *J. Mol. Cell Biol.* 11 (4), 330–341. doi:10.1093/jmcb/mjz005

Erlanson, D. A., Davis, B. J., and Jahnke, W. (2019). Fragment-based drug discovery: Advancing fragments in the absence of crystal structures. *Cell Chem. Biol.* 26 (1), 9–15. doi:10.1016/J.CHEMBIOL.2018.10.001

Friedmann Angeli, J. P., Krysko, D. V., and Conrad, M. (2019). Ferroptosis at the crossroads of cancer-acquired drug resistance and immune evasion. *Nat. Rev. Cancer* 19 (7), 405–414. doi:10.1038/S41568-019-0149-1

Fujihara, K. M., Zhang, B. Z., Jackson, T. D., Ogunkola, M. O., Nijagal, B., Milne, J. V., et al. (2022). Eprenetapopt triggers ferroptosis, inhibits NFS1 cysteine desulfurase, and synergizes with serine and glycine dietary restriction. *Sci. Adv.* 8 (37), eabm9427. doi:10.1126/sciadv.abm9427

Gagliardi, M., Cotella, D., Santoro, C., Cora, D., Barlev, N. A., Piacentini, M., et al. (2019). Aldo-keto reductases protect metastatic melanoma from ER stress-independent ferroptosis. *Cell Death Dis.* 10 (12), 902. doi:10.1038/s41419-019-2143-7

Gagliardi, M., Saverio, V., Monzani, R., Ferrari, E., Piacentini, M., and Corazzari, M. (2020). Ferroptosis: A new unexpected chance to treat metastatic melanoma? *Cell cycleGeorget. Tex.)* 19 (19), 2411–2425. doi:10.1080/15384101.2020.1806426

Galluzzi, L., de Santi, M., Crinelli, R., de Marco, C., Zaffaroni, N., Duranti, A., et al. (2012). Induction of endoplasmic reticulum stress response by the indole-3-carbinol cyclic tetrameric derivative CTet in human breast cancer cell lines. *PLoS ONE* 7 (8), e43249. doi:10.1371/JOURNAL.PONE.0043249

Galluzzi, L., Pietrocola, F., Bravo-San Pedro, J. M., Amaravadi, R. K., Baehrecke, E. H., Cecconi, F., et al. (2015). Autophagy in malignant transformation and cancer progression. *EMBO J.* 34 (7), 856–880. doi:10.15252/embj.201490784

Gnanapradeepan, K., Basu, S., Barnoud, T., Budina-Kolomets, A., Kung, C.-P., and Murphy, M. E. (2018). The p53 tumor suppressor in the control of metabolism and ferroptosis. *Front. Endocrinol.* 9, 124. doi:10.3389/fendo.2018.00124

Green, D. R., and Kroemer, G. (2009). Cytoplasmic functions of the tumour suppressor p53. *Nature* 458 (7242), 1127–1130. doi:10.1038/nature07986

Hassin, O., and Oren, M. (2022). Drugging p53 in cancer: One protein, many targets. *Nat. Rev. Drug Discov.* 22, 127–144. doi:10.1038/s41573-022-00571-8

Hong, Y., Ren, T., Wang, X., Liu, X., Fei, Y., Meng, S., et al. (2022). APR-246 triggers ferritinophagy and ferroptosis of diffuse large B-cell lymphoma cells with distinct TP53 mutations. *Leukemia* 36 (9), 2269–2280. doi:10.1038/s41375-022-01634-w

Hou, W., Xie, Y., Song, X., Sun, X., Lotze, M. T., Zeh, H. J., et al. (2016). Autophagy promotes ferroptosis by degradation of ferritin. *Autophagy* 12 (8), 1425–1428. doi:10.1080/15548627.2016.1187366

Jiang, L., Hickman, J. H., Wang, S.-J., and Gu, W. (2015a). Dynamic roles of p53-mediated metabolic activities in ROS-induced stress responses. *Cell Cycle* 14 (18), 2881–2885. doi:10.1080/15384101.2015.1068479

Jiang, L., Kon, N., Li, T., Wang, S.-J., Su, T., Hibshoosh, H., et al. (2015b). Ferroptosis as a p53-mediated activity during tumour suppression. *Nature* 520 (7545), 57–62. doi:10.1038/nature14344

Jiang, X., Stockwell, B. R., and Conrad, M. (2021). Ferroptosis: Mechanisms, biology and role in disease. *Nat. Rev. Mol. Cell Biol.* 22 (4), 266–282. doi:10.1038/S41580-020-00324-8

Jin, Y., Gu, W., and Chen, W. (2021). Sirt3 is critical for p53-mediated ferroptosis upon ROS-induced stress. *J. Mol. Cell Biol.* 13 (2), 151–154. doi:10.1093/jmcb/mjaa074

Kandoth, C., McLellan, M. D., Vandin, F., Ye, K., Niu, B., Lu, C., et al. (2013). Mutational landscape and significance across 12 major cancer types. *Nature* 502 (7471), 333–339. doi:10.1038/nature12634

Kennedy, M. C., and Lowe, S. W. (2022). Mutant p53: it's not all one and the same. *Cell Death Differ.* 29 (5), 983–987. doi:10.1038/s41418-022-00989-y

Kim, M. P., and Lozano, G. (2018). Mutant p53 partners in crime. *Cell death Differ.* 25, 161–168. doi:10.1038/cdd.2017.185

Kraft, V. A. N., Bezjian, C. T., Pfeiffer, S., Ringelstetter, L., Müller, C., Zandkarimi, F., et al. (2020). GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. *ACS Central Sci.* 6 (1), 41–53. doi:10.1021/acscentsci.9b01063

Lei, G., Zhuang, L., and Gan, B. (2022). Targeting ferroptosis as a vulnerability in cancer. *Nat. Rev. Cancer* 22 (7), 381–396. doi:10.1038/S41568-022-00459-0

Levine, A. J. (2020). p53: 800 million years of evolution and 40 years of discovery. *Nat. Rev. Cancer* 20 (8), 471–480. doi:10.1038/s41568-020-0262-1

Levine, A. J. (2022). Targeting the P53 protein for cancer therapies: The translational impact of P53 research. *Cancer Res.* 82 (3), 362–364. doi:10.1158/0008-5472.can-21-2709

Li, J., Lama, R., Galster, S. L., Inigo, J. R., Wu, J., Chandra, D., et al. (2022). Small molecule MMRi62 induces ferroptosis and inhibits metastasis in pancreatic cancer via degradation of ferritin heavy chain and mutant p53. *Mol. Cancer Ther.* 21 (4), 535–545. doi:10.1158/1535-7163.mct-21-0728

Limia, C. M., Sauzay, C., Urra, H., Hetz, C., Chevet, E., and Avril, T. (2019). Emerging roles of the endoplasmic reticulum associated unfolded protein response in cancer cell migration and invasion. *Cancers (Basel)* 11 (5), 631. doi:10.3390/cancers11050631

Lisek, K., Campaner, E., Ciani, Y., Walerych, D., and Del Sal, G. (2018). Mutant p53 tunes the NRF2-dependent antioxidant response to support survival of cancer cells. *Oncotarget* 9 (29), 20508–20523. doi:10.18632/oncotarget.24974

Liu, D. S., Duong, C. P., Haupt, S., Montgomery, K. G., House, C. M., Azar, W. J., et al. (2017). Inhibiting the system xC(-)/glutathione axis selectively targets cancers with mutant-p53 accumulation. *Nat. Commun.* 8, 14844. doi:10.1038/ncomms14844

Liu, J., Kang, R., and Tang, D. (2021a). Signaling pathways and defense mechanisms of ferroptosis. *FEBS J.* 289, 7038–7050. doi:10.1111/FEBS.16059

Liu, J., Zhang, C., Wang, J., Hu, W., and Feng, Z. (2020). The regulation of ferroptosis by tumor suppressor p53 and its pathway. *Int. J. Mol. Sci.* 21 (21), 8387. doi:10.3390/ijms21218387

Liu, L., Li, L., Li, M., and Luo, Z. (2021b). Autophagy-dependent ferroptosis as a therapeutic target in cancer. *ChemMedChem* 16 (19), 2942–2950. doi:10.1002/CMDC.202100334

Liu, Y., and Gu, W. (2022b). p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell Death Differ.* 29 (5), 895–910. doi:10.1038/s41418-022-00943-y

Liu, Y., and Gu, W. (2022a). The complexity of p53-mediated metabolic regulation in tumor suppression. *Seminars Cancer Biol.* 85, 4–32. doi:10.1016/j.semcancer.2021.03.010

Madan, E., Parker, T. M., Pelham, C. J., Palma, A. M., Peixoto, M. L., Nagane, M., et al. (2019). HIF-transcribed p53 chaperones HIF-1α. *Nucleic Acids Res.* 47 (19), 10212–10234. doi:10.1093/nar/gkz766

Magri, J., Gasparetto, A., Conti, L., Calautti, E., Cossu, C., Ruiu, R., et al. (2021). Tumor-associated antigen xCT and mutant-p53 as molecular targets for new combinatorial antitumor strategies. *Cells* 10 (1), 108. doi:10.3390/cells10010108

Maiuri, M. C., Galluzzi, L., Morselli, E., Kepp, O., Malik, S. A., and Kroemer, G. (2010). Autophagy regulation by p53. *Curr. Opin. Cell Biol.* 22 (2), 181–185. doi:10.1016/j.ceb.2009.12.001

Mancias, J. D., Wang, X., Gygi, S. P., Harper, J. W., and Kimmelman, A. C. (2014). Quantitative proteomics identifies NCOA4 as the cargo receptor mediating ferritinophagy. *Nature* 509 (7498), 105–109. doi:10.1038/NATURE13148

Mantovani, F., Collavin, L., and Del Sal, G. (2019). Mutant p53 as a guardian of the cancer cell. *Cell Death Differ.* 26 (2), 199–212. doi:10.1038/s41418-018-0246-9

Mao, C., Liu, X., Zhang, Y., Lei, G., Yan, Y., Lee, H., et al. (2021). DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. *Nature* 593:(7860), 586–590. doi:10.1038/s41586-021-03539-7

Morselli, E., Tasdemir, E., Maiuri, M. C., Galluzzi, L., Kepp, O., Criollo, A., et al. (2008). Mutant p53 protein localized in the cytoplasm inhibits autophagy. *Cell cycleGeorget. Tex)* 7 (19), 3056–3061. *PMID - 18818522* 7. doi:10.4161/cc.7.19.6751

Mrakovcic, M., and Fröhlich, L. F. (2018). p53-Mediated molecular control of autophagy in tumor cells. *Biomolecules* 8 (2), 14. doi:10.3390/biom8020014

Pagliarini, V., Giglio, P., Bernardoni, P., De Zio, D., Fimia, G. M., Piacentini, M., et al. (2015). Downregulation of E2F1 during ER stress is required to induce apoptosis. *J. Cell Sci.* 128 (6), 1166–1179. doi:10.1242/jcs.164103

Pilley, S., Rodriguez, T. A., and Vousden, K. H. (2021). Mutant p53 in cell-cell interactions. *Genes and Dev.* 35 (7-8), 433–448. doi:10.1101/gad.347542.120

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., et al. (2017). Oxidative stress: Harms and benefits for human Health. *Oxidative Med. Cell. Longev.* 2017, 8416763. doi:10.1155/2017/8416763

Rodriguez, R., Schreiber, S. L., and Conrad, M. (2022). Persister cancer cells: Iron addiction and vulnerability to ferroptosis. *Mol. Cell* 82 (4), 728–740. doi:10.1016/J.MOLCEL.2021.12.001

Rojo de la Vega, M., Chapman, E., and Zhang, D. D. (2018). NRF2 and the hallmarks of cancer. *Cancer Cell* 34 (1), 21–43. doi:10.1016/j.ccell.2018.03.022

Seiler, A., Schneider, M., Förster, H., Roth, S., Wirth, E. K., Culmsee, C., et al. (2008). Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* 8 (3), 237–248. doi:10.1016/J.CMET.2008.07.005

Senft, D., and Ronai, Z. E. (2016). Adaptive stress responses during tumor metastasis and dormancy. *Trends Cancer* 2 (8), 429–442. doi:10.1016/j.trecan.2016.06.004

Sermeus, A., and Michiels, C. (2011). Reciprocal influence of the p53 and the hypoxic pathways. *Cell Death Dis.* 2 (5), e164. doi:10.1038/cddis.2011.48

Shah, R., Shchepinov, M. S., and Pratt, D. A. (2018). Resolving the role of lipoxygenases in the initiation and execution of ferroptosis. *ACS Central Sci.* 4 (3), 387–396. doi:10.1021/acscentsci.7b00589

Shi, Y., Norberg, E., and Vakifahmetoglu-Norberg, H. (2021). Mutant p53 as a regulator and target of autophagy. *Front. Oncol.* 10, 607149. doi:10.3389/fonc.2020.607149

Sicari, D., Fantuz, M., Bellazzo, A., Valentino, E., Apollonio, M., Pontisso, I., et al. (2019). Mutant p53 improves cancer cells' resistance to endoplasmic reticulum stress by sustaining activation of the UPR regulator ATF6. *Oncogene* 38 (34), 6184–6195. doi:10.1038/s41388-019-0878-3

Stockwell, B. R. (2022). Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. *Cell* 185 (14), 2401–2421. doi:10.1016/J.CELL.2022.06.003

Su, X., Xie, Y., Zhang, J., Li, M., Zhang, Q., Jin, G., et al. (2022). HIF-α activation by the prolyl hydroxylase inhibitor roxadustat suppresses chemoresistant glioblastoma growth by inducing ferroptosis. *Cell death Dis.* 13 (10), 861. doi:10.1038/S41419-022-05304-8

Su, Y., Zhao, B., Zhou, L., Zhang, Z., Shen, Y., Lv, H., et al. (2020). Ferroptosis, a novel pharmacological mechanism of anti-cancer drugs. *Cancer Lett.* 483, 127–136. doi:10.1016/j.canlet.2020.02.015

Szewczyk-Golec, K., Czuczejko, J., Tylzanowski, P., and Lecka, J. (2018). Strategies for modulating oxidative stress under diverse physiological and pathological conditions. *Oxidative Med. Cell. Longev.* 2018, 3987941. doi:10.1155/2018/3987941

Vogiatzi, F., Brandt, D. T., Schneikert, J., Fuchs, J., Grikscheit, K., Wanzel, M., et al. (2016). Mutant p53 promotes tumor progression and metastasis by the endoplasmic reticulum UDPase ENTPD5. *Proc. Natl. Acad. Sci.* 113 (52), E8433–E8442. doi:10.1073/pnas.1612711114

Walerych, D., Lisek, K., Sommaggio, R., Piazza, S., Ciani, Y., Dalla, E., et al. (2016). Proteasome machinery is instrumental in a common gain-of-function program of the p53 missense mutants in cancer. *Nat. Cell Biol.* 18 (8), 897–909. doi:10.1038/ncb3380

Wang, H., Jiang, H., Van De Gucht, M., and De Ridder, M. (2019). Hypoxic radioresistance: Can ROS Be the key to overcome it? *Cancers* 11 (1), 112. doi:10.3390/CANCERS11010112

Wang, S. J., Li, D., Ou, Y., Jiang, L., Chen, Y., Zhao, Y., et al. (2016). Acetylation is crucial for p53-mediated ferroptosis and tumor suppression. *Cell Rep.* 17 (2), 366–373. doi:10.1016/j.celrep.2016.09.022

Wang, Z., Strasser, A., and Kelly, G. L. (2022). Should mutant TP53 be targeted for cancer therapy? *Cell Death Differ.* 29 (5), 911–920. doi:10.1038/s41418-022-00962-9

Wu, Y., Yu, C., Luo, M., Cen, C., Qiu, J., Zhang, S., et al. (2020). Ferroptosis in cancer treatment: Another way to rome. *Front. Oncol.* 10, 571127. doi:10.3389/fonc.2020.571127

Wu, Z., Geng, Y., Lu, X., Shi, Y., Wu, G., Zhang, M., et al. (2019). Chaperone-mediated autophagy is involved in the execution of ferroptosis. *Proc. Natl. Acad. Sci. U. S. A.* 116 (8), 2996–3005. doi:10.1073/PNAS.1819728116

Yang, G., Shi, R., and Zhang, Q. (2020). Hypoxia and oxygen-sensing signaling in gene regulation and cancer progression. *Int. J. Mol. Sci.* 21 (21), 8162–8224. doi:10.3390/IJMS21218162

Yang, M., Chen, P., Liu, J., Zhu, S., Kroemer, G., Klionsky, D. J., et al. (2019). Clockophagy is a novel selective autophagy process favoring ferroptosis. *Sci. Adv.* 5 (7), eaaw2238. doi:10.1126/SCIADV.AAW2238

Yang, W. S., and Stockwell, B. R. (2016). Ferroptosis: Death by lipid peroxidation. *Trends Cell Biol.* 26 (3), 165–176. doi:10.1016/j.tcb.2015.10.014

Zhou, B., Liu, J., Kang, R., Klionsky, D. J., Kroemer, G., and Tang, D. (2020). Ferroptosis is a type of autophagy-dependent cell death. *Seminars cancer Biol.* 66, 89–100. doi:10.1016/J.SEMCANCER.2019.03.002

Zhou, G., Wang, J., Zhao, M., Xie, T.-X., Tanaka, N., Sano, D., et al. (2014). Gain-of-Function mutant p53 promotes cell growth and cancer cell metabolism via inhibition of AMPK activation. *Mol. Cell* 54, 960–974. doi:10.1016/j.molcel.2014.04.024

Zou, Y., Li, H., Graham, E. T., Deik, A. A., Eaton, J. K., Wang, W., et al. (2020). Cytochrome P450 oxidoreductase contributes to phospholipid peroxidation in ferroptosis. *Nat. Chem. Biol.* 16, (3), 302–309. doi:10.1038/s41589-020-0472-6

Glossary

ACSL4 acyl-CoA synthetase long-chain family member 4
AKRs aldo-keto reductases
ALOXs arachidonate lipoxygenases
ARNTL aryl hydrocarbon receptor nuclear translocator-like
ATF6 Activating Transcription Factor 6
ATF4 Activating Transcription Factor 4
CHAC1 ChaC Glutathione Specific Gamma-Glutamylcyclotransferase 1
CMA chaperone-mediated autophagy
DHODH dihydroorotate dehydrogenase
ER endoplasmic reticulum
ERAD endoplasmic-reticulum-associated protein degradation
FTH ferritin heavy chain
FSP1 Ferroptosis suppressor protein 1, or AIFM2 or AMID
GCHI/BH4 GTP cyclohydrolase 1/tetrahydrobiopterin
GPX4 Glutathione peroxidase 4
GSH Glutathione
HIF1 Hypoxia Inducible Factor 1
Hsp90 heat shock protein 90
IRE1 Inositol-Requiring Enzyme 1
Lipid-ROS lipid peroxides
MUFA monounsaturated fatty acids
NCOA4 nuclear receptor coactivator 4
NOX4 NADPH oxidase 4
NRF2 nuclear factor erythroid 2-related factor 2
PERK PKR-Like ER Kinase
PUFA Polyunsaturated fatty acids
PL-PUFA PUFA-containing membrane-bound phospholipids
POR cytochrome P450 oxidoreductase
RAB7A member RAS oncogene family
RSL3 RAS-selective-lethal-3
ROS reactive oxygen species
System Xc- cystine/glutamate antiporter system
SLC7A11 solute carrier family 7 member 11
SCD1 stearoyl-CoA desaturase 1
SIRT3 Sirtuin 3
TFR Transferrin Receptor
UPR unfolded protein response
XBP1 X-Box Binding Protein 1

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The regulatory effects of p53 on the typical and atypical ferroptosis in the pathogenesis of osteosarcoma: A systematic review

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Study background: As a rare condition, osteosarcoma affects approximately 3% of all cancer patients. Its exact pathogenesis remains largely unclear. The role of p53 in up- and down-regulating atypical and typical ferroptosis in osteosarcoma remains unclear. The primary objective of the present study is investigating the role of p53 in regulating typical and atypical ferroptosis in osteosarcoma.

Methods: The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) and the Patient, Intervention, Comparison, Outcome, and Studies (PICOS) protocol were used in the initial search. The literature search was performed in six electronic databases, including EMBASE, Cochrane library of trials, Web of Science, PubMed, Google Scholar, and Scopus Review, using keywords connected by Boolean operators. We focused on studies that adequately defined patient profiles described by PICOS.

Results and discussion: We found that p53 played fundamental up- and down-regulatory roles in typical and atypical ferroptosis, resulting in either advancement or suppression of tumorigenesis, respectively. Direct and indirect activation or inactivation of p53 downregulated its regulatory roles in ferroptosis in osteosarcoma. Enhanced tumorigenesis was attributed to the expression of genes associated with osteosarcoma development. Modulation of target genes and protein interactions, especially SLC7A11, resulted in enhanced tumorigenesis.

Conclusion: Typical and atypical ferroptosis in osteosarcoma were regulatory functions of p53. The activation of MDM2 inactivated p53, leading to the downregulation of atypical ferroptosis, whereas activation of p53 upregulated typical ferroptosis. Further studies should be performed on the regulatory roles of p53 to unmask its possible clinical applications in the management of osteosarcoma.

KEYWORDS

osteosarcoma, typical ferroptosis, p53 ferroptosis, atypical ferroptosis, lipid peroxidation, SLC7A11, MDM2 activation

Introduction

As a bone tumor, osteosarcoma affects the origin of the mesenchyme and mainly occurs during the growth phase of long bones (Prater and McKeon, 2020; Liu et al., 2022). The tumors grow around the epiphyseal growth plates of the tibia of the femur. In most cases, osteosarcoma has been associated with gene disorganization, dysregulation of genes suppressing tumors and cell cycle, and chromosomal alterations in aneuploidy, alongside inadequate repair of the deoxyribonucleic acid. Osteosarcoma is either inherited or acquired at birth (Hameed and Mandelker, 2018). However, most cases occur due to gene mutation. In addition, ferroptosis plays a crucial role in developing osteosarcoma, like other forms of cancer. Ferroptosis unfolds *via* phospholipid damage and the over-production of reactive oxygen species (ROS) (Zou and Schreiber, 2020).

Even though osteosarcoma remains uncommon worldwide, it raises serious health concerns as it leads to loss of bones, pain, and physical support for body structures among children aged 13 to 16 (Petriceks and Salmi, 2019). According to Petriceks et al., osteosarcoma accounts for approximately 3% of childhood cancer and less than 1% of cancers diagnosed in the United States of America in a year. Despite the small number of people affected, it is still necessary to understand the pathogenesis of osteosarcoma, which may be beneficial for the management of clinical treatment guidelines.

The genetic material is also associated with the atypical and typical ferroptosis in osteosarcoma. However, genetic outplay concerns target genes and mutations. For example, genes modulated by p53 or p53 target genes have been reported to play a significant role in the course of osteosarcoma *via* typical and atypical mechanisms (Rickel et al., 2017; Czarnecka et al., 2020).

The exact cause of osteosarcoma remains unknown. However, various biological processes and events have been associated with its pathogenesis. Of the biological elements attributed to osteosarcomas, p53 has been linked to different cellular processes and events. Previous literature has shown the role of p53 in regulating atypical and typical ferroptosis, where iron-dependent cell lysis is commanded by oxidative phospholipid damages (Zou and Schreiber, 2020). Cell death mechanisms stand out in understanding the role of p53 in osteosarcoma-related ferroptosis. Figure 1 illustrates the roles of p53 in atypical and typical ferroptosis in the course of osteosarcoma. TP53 mediates the expression of SLC7A11C in human cancers, and Liu et al. have clarified crucial elements concerning the roles of p53 in regulating atypical and typical ferroptosis in osteosarcoma (Liu et al., 2020). Moreover, previous literature has reported and emphasized the role of p53 in ferroptosis: up- and downregulation of atypical and typical ferroptosis.

In the present study, we focused on a systematic review of studies reporting the regulatory roles of p53 in atypical and typical ferroptosis in the tumorigenesis of osteosarcoma. Up and down-regulatory functions as discussed with a focus on how either of the two aspects influence osteosarcoma. In addition, this review investigated up- and down-regulatory functions performed by p53 in osteosarcoma (Zhao et al., 2021). Even though existing literature has demonstrated the regulatory roles of p53 (see Figure 1; Figure 2), more knowledge is required to expand an understanding of p53's roles in ferroptosis in osteosarcoma.

Typical and atypical ferroptosis in osteosarcoma are complex biological processes (Figure 2). In the present study, we aimed to review and simplify evidence to enhance comprehension. Additionally, facts and evidence found in the present study were of great clinical significance. The target genes and interactions are potential clinical information for interventions against the disease and management (Giaccone, 2019; Chen et al., 2022). Robust outcomes will improve clinical outcomes of osteosarcoma management as better intervention approaches are developed.

Methods

Study design and database search

The initial literature search was performed in six electronic databases, such as EMBASE, Cochrane library of trials, Web of

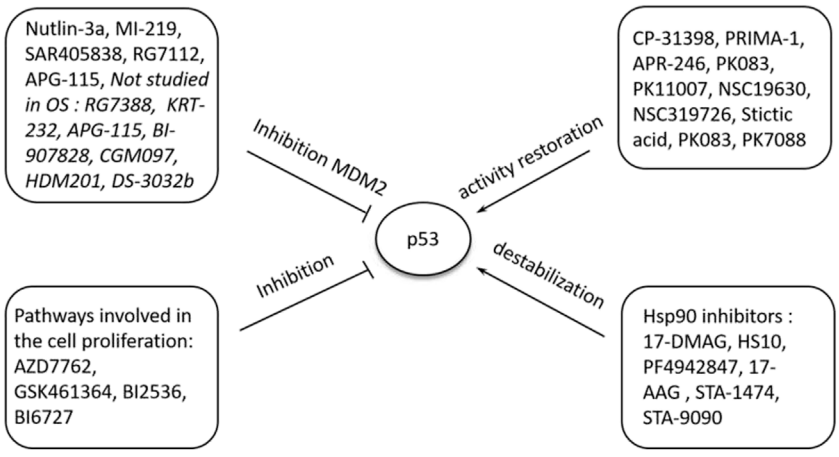

FIGURE 1
A summary of the regulation and occurrence of ferroptosis in osteosarcoma. A four-fold manifestation of p53 shows how osteosarcoma outcomes unfolds: inhibition, destabilization, activity restoration and inhibition via MDM2. Each of these pathways down- and up-regulate p53's inhibitory and excitatory functions in osteosarcoma development.

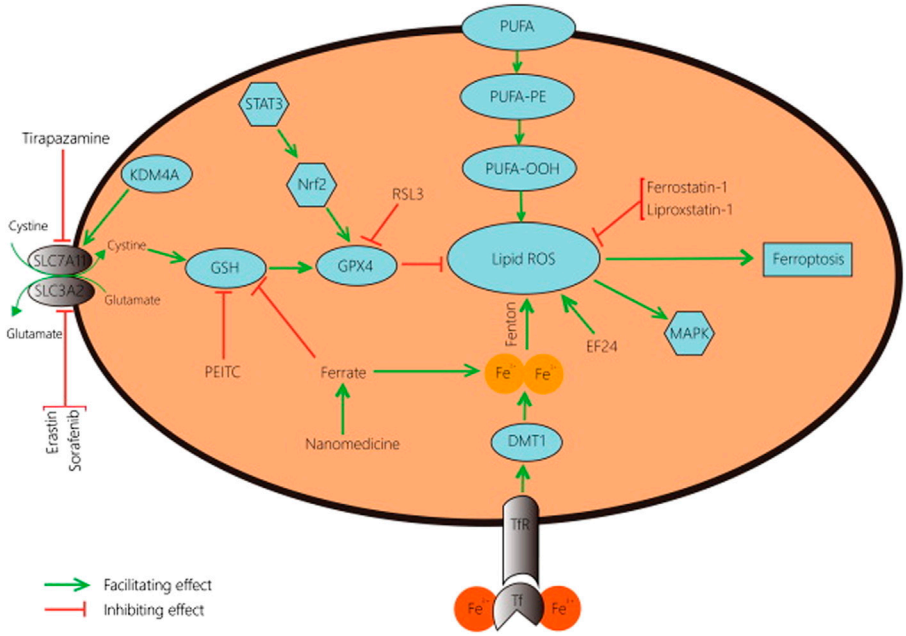

FIGURE 2
A schematic representation of typical ferroptosis mediated by P53. The outcome of p53-mediated ferroptosis is apoptosis; cell death mechanisms characterizing osteosarcoma.

TABLE 1 Search strategy: Electronic databases where literature search was done and the keywords.

Electronic database	Keywords	Regulatory effects on atypical or typical ferroptosis
EMBASE	Typical ferroptosis, atypical ferroptosis, p53	Upregulation
Cochrane Library of trials	P53, osteosarcoma, tumorigenesis, lipid peroxidation	Upregulation
Web of Science	P53 inactivation, p53 activation, typical ferroptosis	Dow-regulation
PubMed	P53, osteosarcoma, tumorigenesis, SLC7A11	Downregulation
Google Scholar	Mutant-p53, osteosarcoma, p53 target genes	Upregulation, downregulation
Sopus review	Mutant-p53, typical ferroptosis, atypical ferroptosis	Upregulation, downregulation

Science, PubMed, Google Scholar, and Scopus Review, following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) protocols (Page et al., 2021; Rethlefsen et al., 2021). Eligible studies were collected in the initial literature search. A specific approach was used to obtain the most appropriate studies matching the profile of the required studies. Eligible studies were identified by a rough view of the abstracts and titles.

Search strategy

Keywords and Boolean operators were the primary tools used to identify eligible studies. Different keywords were used to locate the articles in the electronic databases. As mentioned, the initial literature search was performed in six electronic databases, and the keywords were indiscriminately applied to identify available material. The Boolean operators "AND" and "OR" were used to combine keywords (Table 1). Furthermore, the Boolean operator "AND" was used to combine keywords with distinct meanings, whereas the Boolean operator "OR" was used to combine keywords with similar meanings. Table 1 summarizes the keywords used to locate eligible literature in the present study.

Eligibility criteria

Study eligibility was determined by the Cochrane collaboration framework of inclusion and exclusion criteria (Cumpston et al., 2019; Campbell et al., 2020). PICOS protocols were used to assess eligible studies for inclusion in the review. However, since the present study did not rely on intervention and the outcomes, we only focused on patient features because these features would describe the role of p53 in ferroptosis in osteosarcoma.

Inclusion criteria

1. Studies meeting the following criteria were included in the present review.
2. Patients: The patient population was adequately defined, per the requirements of the Cochrane Collaboration of systematic reviews. The present review included osteosarcoma patients only. They could be adults or children.
3. Intervention: Not applicable
4. Comparison: Not applicable
5. Outcomes: We focused on the role of p53 in typical and atypical ferroptosis among osteosarcoma patients. All study outcomes pertaining to these outcomes were observed and recorded. Studies reporting these outcomes were included in the present work.
6. Studies: All the studies included in the present review were PICO studies: studies reporting the role of p53 in ferroptosis among osteosarcoma patients.

Exclusion criteria

Studies were excluded in the present review if they met the following exclusion criteria.

1 Studies reporting outcomes irrelevant to the present topic.
2 Non-PICO studies not reporting the regulatory roles of p53 in typical and atypical ferroptosis in osteosarcoma.
3 Ongoing studies.
4 Studies involving interventions that could distort or influence the outcomes negatively.

Evaluation of quality of literature

The quality of the literature set for inclusion was assessed using Version 2 of the Cochrane risk of bias tool (Cochrane, 2011). The five domains of risk of bias were assessed: random sequence generation, allocation concealment, blinding of participants and personnel, incomplete outcome data, selective reporting, and other forms of bias, and the overall outcomes were reported as low risk of bias, high risk of bias, and unclear risk of bias. This quality assessment provided an image of the overall status of the quality of included literature. Furthermore, the quality of individual studies was assessed.

Data selection and extraction

Data selection and extraction in the present review were performed by two independent reviewers. A systematic approach was deployed where the independent reviewers would examine the studies for potential information or data pertinent to the current topic. The data selection and extraction process were governed by discussion, as the independent reviewers occasionally disagreed on the type of data or particular information to be included in the present study. Disputes would be amicably solved through discussion, and data selection and extraction on studies reporting the role of p53 in atypical and typical ferroptosis in osteosarcoma were limited by the independent reviewers. These study outcomes would be reviewed to reveal the overall effects of p53 on atypical and typical ferroptosis in osteosarcoma.

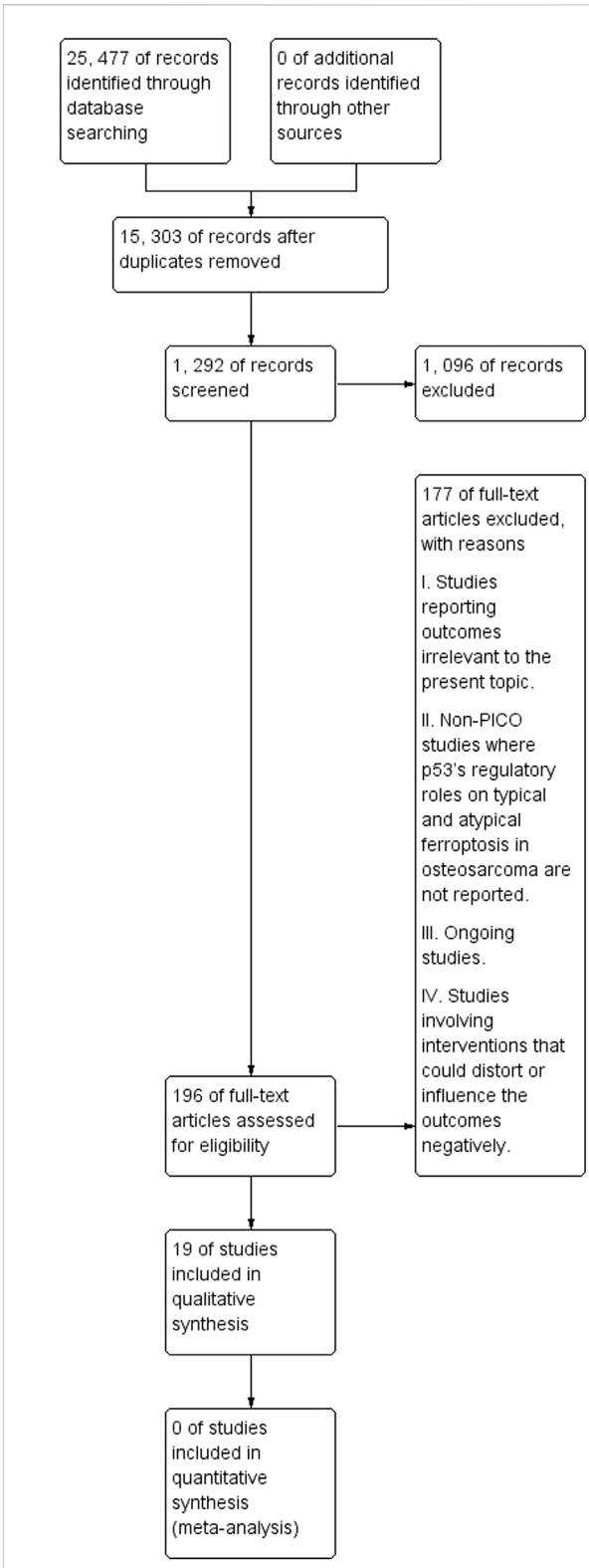

FIGURE 3
A PRISMA flowchart of the initial literature search.

TABLE 2 Basic features of included literature.

Study	Effects of ferroptosis in osteosarcoma	Regulatory mechanism of p53	Typical ferroptosis	Atypical ferroptosis
Ji et al. (2022)	Enhanced tumor progression	Enhancing cellular sensitivity to ferroptosis	Induced	NA
	Reduced tumor progression	Delaying the generation of cells for ferroptosis	Induced	NA
Lu et al. (2020)	Induction and suppression of osteosarcoma	Enhanced interaction between p53 and SLC7A11	Up and downregulation	NA
Luo et al. (2018)	The wild-type p53 mutant is downregulated	Up or downregulation of ferroptosis	Up and downregulated	NA
Velletri et al. (2016)	Enhances tumor progression	Amplified apoptosis, metabolism, and regulation of cell cycle	Upregulation	NA
	Establishment of bone tumor microenvironment components	Mutations in p53 result in aberrant transformations of mesenchymal stem cells	Upregulation of osteosarcoma	NA
Sciot (2021)	Differentiation of tumor cells	MDM2 overexpression blocks p53's regulatory mechanisms	Upregulation of osteosarcoma	NA
Usman et al. (2020)	Promotion of cancer progression through autophagy	Drug resistance and mutations	Upregulation	NA
Magri et al. (2021)	Promotes tumorigenesis	The wild-type p53 mutant indirectly modulates (an inhibitory mechanism) xCT expression	Upregulation	NA
Hatina et al. (2019)	Abrogation of tumor suppression processes	Modification of transcriptome and alteration of epigenetic regulation	Down and upregulation	NA
Bouchalova et al. (2014)	Enhanced cell proliferation	Induction of p53-oncogenic functions	NA	Induced
Su et al. (2022)	Cell death	Modulating programmed cell death	NA	Induced
Babamohamadi, et al. (2022)	Increased apoptosis	Abnormal signaling pathways	NA	Induced
Gnanapradeepan et al. (2018)	Lipid and iron-dependent cell death	Enhanced ferroptosis: high production of ROS and initiation of the lipid peroxidation	NA	Induced
Xie et al. (2016)	Cell death	Production of lethal reactive oxygen species and lipid peroxidation	NA	Induced
Leroy et al. (2017)	Tumor progression and activation of tumor suppressor TP53	Activation of p53 and the initiation of tumor suppressor TP53	NA	Induced
Yang and Zhang (2013)	Antagonistic targeting of osteosarcoma target sites and downregulation of tumorigenesis	Genetic aberrations	NA	Induced
Saraf et al. (2018)	Activation of antitumor processes	Inactivation of p53	NA	Induced
Pang et al. (2020)	Enhance antitumor functions	Activation of TP53 antitumor activities	NA	Induced
Van Maerken et al. (2014)	Loss of tumor suppressor functions of the p53	Loss-of-functions mutations in TP53	NA	Induced
Komori (2016)	Cell death	Deletion of p53 and inactivation	NA	Induced

Results

Study selection

Again, the study selection procedure was systematically performed by the independent reviewers. First, the independent reviewers checked the titles and abstracts of studies for eligibility. Studies with topics and abstracts unrelated to the present topic were not subjected to further analysis. Abstract screening seconded title screening as the latter was a key indicator of potential studies. Finally, eligible studies were subjected to full-text screening to establish accounts of data pertinent to the present topic. Figure 3 illustrates the flowchart diagram of the initial literature search. The initial literature search identified 25,477 records from the six electronic databases. After removing duplicated studies, 15,303 records remained, out of which 1,292 were screened. Such screening led to removing 1,096 records, with reasons previously indicated.

Baseline features of included literature

Table 2 summarizes the basic features of the included literature. Focusing on the regulatory effects of p53 on atypical and typical apoptosis in osteosarcoma-related ferroptosis, we investigated

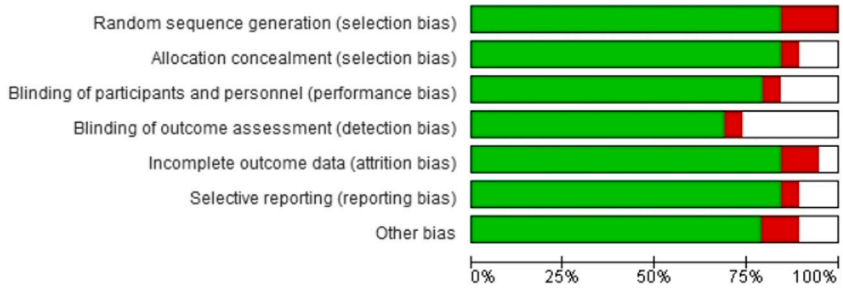

FIGURE 4
The overall risk of bias in included studies.

studies reporting such outcomes by assessing the effects of ferroptosis and regulatory mechanisms of p53. The regulatory mechanisms indicate whether a regulatory effect is atypical or typical. The summary included two distinct pieces of information obtained from the studies; regulatory effects of p53 on atypical ferroptosis or typical ferroptosis. Included literature could be identified through author ID and the year of publication. We did not find a study reporting the regulatory effects of p53 on typical and atypical ferroptosis.

Quality appraisal

As indicated earlier, we summarized the overall risk of bias outcomes obtained from the Cochrane collaboration of systematic reviews (Version 2). Figure 4 summarizes the risk of bias in the seven domains, including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other forms of bias. Color coding was used to represent the overall risk of bias in each domain. The green color represented a low risk of bias, whereas red and white color coding represented a high and unclear risk of bias, respectively. A visual inspection of Figure 4 showed that most studies had a low risk of bias.

Quality assessments

We investigated the quality of individuals to ascertain eligibility. Figure 5 represents the quality assessment outcomes of the studies in every domain. The color coding used in the figure above was applied in the present study. Color coding was used to represent the overall risk of bias in each domain. The green color represented a low risk of bias, whereas red and white color coding represented a high and unclear risk of bias, respectively.

The regulatory effects of p53 on typical ferroptosis in osteosarcoma

We reviewed 10 studies to unmask the regulatory effects of p53 on typical ferroptosis in osteosarcoma and found different mechanisms and biological processes. Several studies (Velletri et al., 2016; Luo et al., 2018; Sciot, 2021; Hatina et al., 2019; Lu et al., 2020; Usman et al., 2020; Magri et al., 2021; Ji et al., 2022) have shown that p53 exerts different regulatory effects on typical ferroptosis, including enhancing cellular sensitivity to ferroptosis, delaying the generation of cells for apoptotic process, enhancing p53-SLC7A11 interaction, amplification of apoptosis, metabolism, and regulation of cell cycle, induction of aberrant mesenchymal stem cells (MSCs) through p53 mutations, MDM2 overexpression, drug resistance, indirect inhibitory modulation of xCT expression, induction of p53's oncogenic functions, and transcriptome modification. The overall effect of these functions is up-regulating ferroptosis as they enhance p53's functions in enhancing ferroptosis.

All these observations indicated that p53 modulated the pathway for typical ferroptosis differently. The mechanisms listed above emphasized the differences in regulatory measures reported by the studies. Therefore, the genetic regulatory role of p53 was crucial in the present review. Luo et al. (2018) have reported that the wild-type p53 exploits microRNAs to inhibit cancer development, and the gain-of-function mutant of p53 triggers oncogenic properties. Ji et al. have articulated the oncogenic properties and the pathways through which p53 regulates typical ferroptosis. According to Ji et al., p53 enhances cellular sensitivity to ferroptosis and functions like a rheostat, leading to the up- and downregulation of sensitivity. The mutation modifies p53, leading to loss of antitumor functions (Liu et al., 2019); P53 modification is the parent of the wild-type p53 protein and the loss of antitumor function. This is the mechanism through which ferroptosis is enhance.

The genetic approach to regulatory frameworks of p53 involves cellular susceptibility to ferroptosis. By enhancing the susceptibility of bone cells to ferroptosis, p53 inhibits cancer and the accumulation of mutations associated with osteosarcoma (Zhang et al., 2022a). Genetic mutations are at the center of p53-induced ferroptosis as genetic mutations affect the expression of cells, protein interactions, and the resulting outcomes. Figure 6 is a schematic representation of genetic modifications altering p53-PVT1 interaction and the effects on biological processes. The genetic modifications delay the generation of cells for ferroptosis (Ji et al., 2022), modulate SLC7A11-p53 interaction (Lu et al., 2020), cause up- and

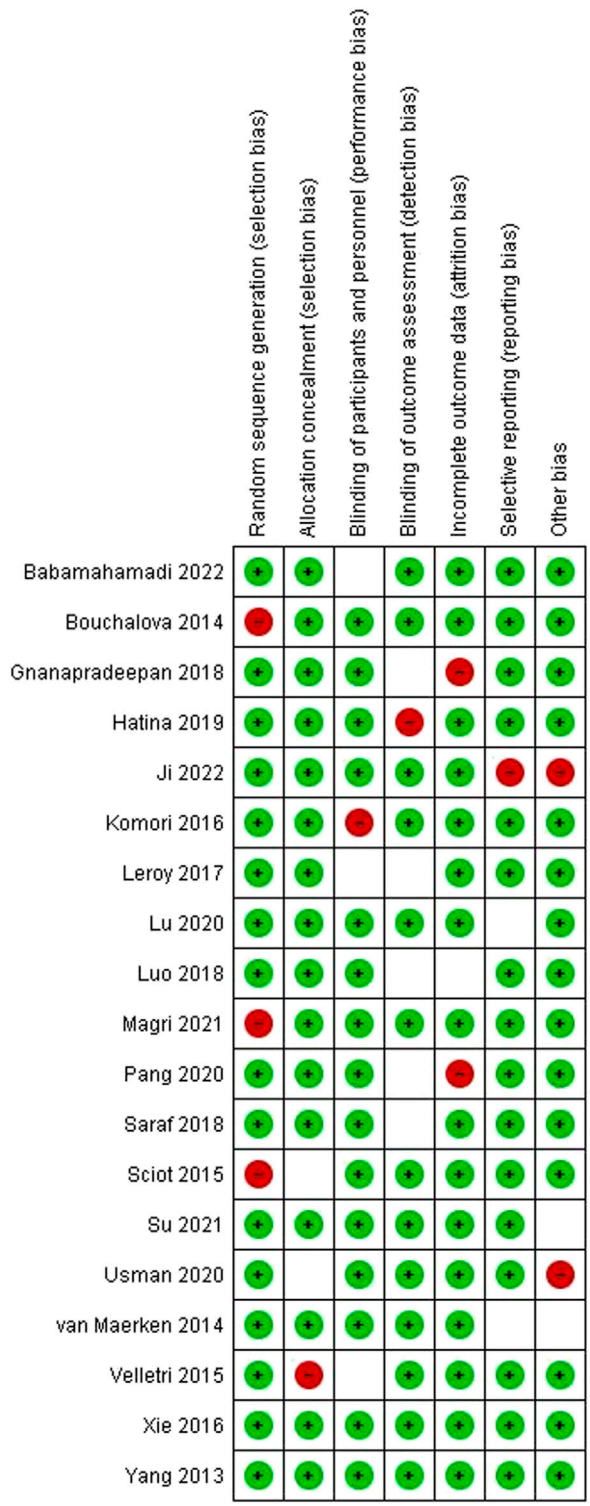

FIGURE 5
Risk of bias assessment outcomes on individual studies.

downregulation of ferroptosis (Luo et al., 2018), and amplify the transformation of MSCs and apoptosis (Velletri et al., 2016). Table 3 summarizes the roles of p53 in cell death characterizing ferroptosis in osteosarcoma.

The regulatory effects of p53 on atypical ferroptosis in osteosarcoma

As the atypical tumor suppressor, p53 is produced under the instructions issued by TP53. We found that p53 regulated cell division through proliferation, which is rapid cell division and inhibiting uncontrollable cell growth (Kang et al., 2019). Gene targeting defines the regulatory effects of p53 on atypical ferroptosis in osteosarcoma. Liu et al. (2022) have reported that the upregulation of p53 directly inhibits the activity of SLC7A11, promoting ferroptosis. Therefore, we sought evidence of up- and downregulation of atypical ferroptosis and compared the outcomes. The role of p53 in regulating atypical ferroptosis could reduce the severity of the disease or implicate them by exacerbating tumor progression.

Upregulation and downregulation of p53 enhanced and reduced ferroptosis, respectively. Different studies have shown that atypical ferroptosis is either increased or decreased in osteosarcoma. Six studies (Bouchalova et al., 2014; Xie et al., 2016; Gnanapradeepan et al., 2018; Su et al., 2022; Babamohamadi et al., 2022) have reported that atypical ferroptosis is enhanced in osteosarcoma. Table 4 summarizes the regulatory mechanisms of p53 on atypical ferroptosis. We found that upregulation of p53 enhanced the production of ROS, medicated lipid and iron-mediated cell death, and increased proliferation. We compared these outcomes with p53 inactivation and the resulting status regarding osteosarcoma-related ferroptosis (Table 4).

Comparing the outcomes reported in Table 4 and the pathway illustrated in Figure 7, we could deduce two possibilities from the regulatory outcomes of p53: enhanced ferroptosis or inhibited ferroptosis (de Azevedo et al., 2019). Several studies (Bouchalova et al., 2014; Xie et al., 2016; Gnanapradeepan et al., 2018; Su et al., 2022; Babamohamadi et al., 2022) have indicated that upregulation of p53 enhances atypical ferroptosis in the course of osteosarcoma, whereas other studies (Komori, 2016; Van Maerken et al., 2014; Pang et al., 2020; Saraf et al., 2018; Yang and Zhang, 2013; Leroy et al., 2017) have reported that inactivation of p53 downregulates atypical ferroptosis in osteosarcoma.

p53 and cell proliferation in osteosarcoma

We reviewed six studies reporting p53 inactivation and the resulting cell proliferation to illustrate the regulatory mechanisms of p53 in atypical ferroptosis in osteosarcoma. Several studies (Yang and Zhang, 2013; Van Maerken et al., 2014; Komori, 2016; Saraf et al., 2018; Pang et al., 2020;; Leroy et al., 2017) have reported the regulatory mechanisms of p53 on atypical ferroptosis in the course of osteosarcoma, while inactivation of the gene inhibits cell proliferation. These studies have shown that p53 downregulates atypical ferroptosis in osteosarcoma by inhibiting cell proliferation. In addition, Pang et al. (2020) have reported that 95% of osteosarcoma cases are associated with p53 inactivation.

However, we found unique and distinct mechanisms through which p53 inactivation implicated inhibitory effects on cell proliferation. Eventually, the inactivation of p53 inhibited the expression of SLC7A11 as the interaction between SLC7A11 and p53 was limited. We found that p53 inactivation resulted from

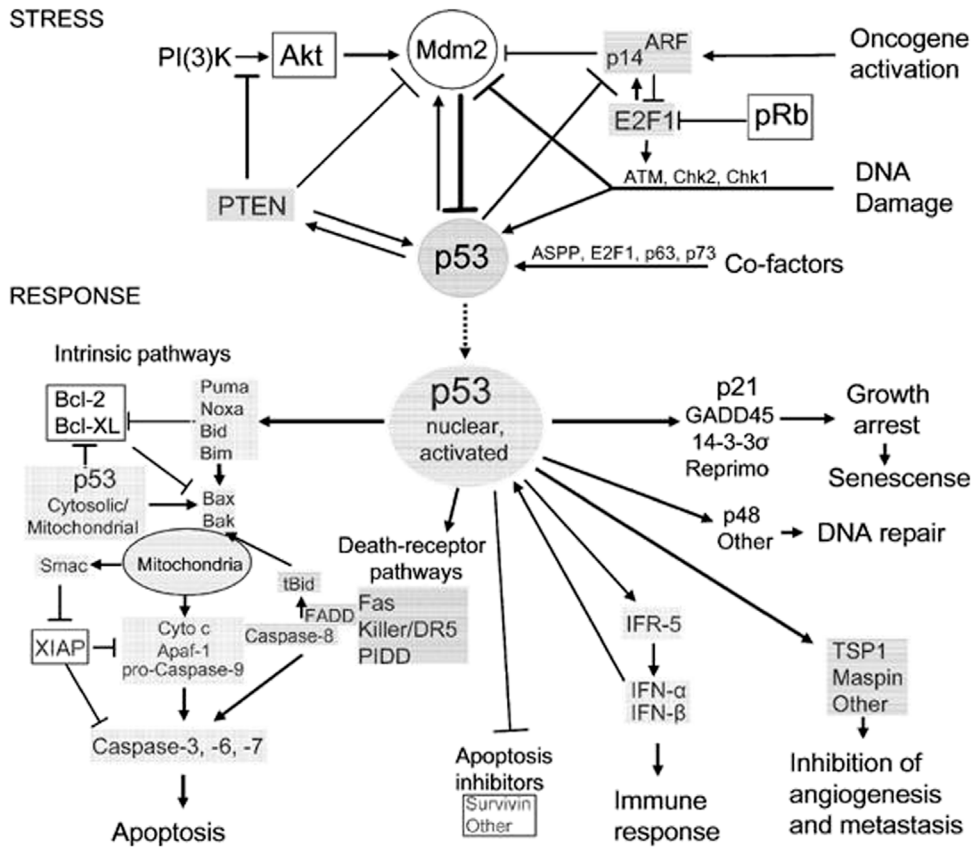

FIGURE 6
A schematic representation of p53's role in modulation of typical ferroptosis in osteosarcoma. The resulting effects of the biological processes are based on molecular responses to stress. Nuclear activation related to p53 protein are generated from the stress induced by tumorigenesis.

TABLE 3 A summary of roles of p53 in different processes involved in osteosarcoma. Even though some of these processes do not unfold in typical and atypical ferroptosis, they are vital in explaining the roles of p53 in osteosarcoma.

Regulation of metabolism	DNA damage through oxidation	Cellular reprogramming
TIGAR, SCO^2	ATR/CHK1 and ATM/CHK2	MMP-9
GLUT1 and GLUT4	SAPK, JNK and p38MAPK	CXC chemokines
PFKFB3 and PFKFB4		

TABLE 4 A comparison of p53's up-and down-regulatory mechanisms in osteosarcoma-related ferroptosis.

Upregulation of p53		Downregulation of p53
Increased cell death	High production of lethal reactive oxygen species	Balancing the production of antioxidant cellular products to counter excess reactive oxygen species
Increased cell metabolism and cell death	Lipid and iron-mediated cell death	Down-regulating lipid peroxidation
Induction of p53 oncogenic functions	Enhanced cell proliferation	Activating p53's antitumor functions and actibities

multiple and different cell processes. Table 5 summarizes the different mechanisms reported by individual studies. The inactivation of p53 promotes the survival of tumor cells (Van Maerken et al., 2014). Leroy et al. (2017) and Pang et al. (2020) have shown that a high incidence of osteosarcoma is associated with TP53 mutation.

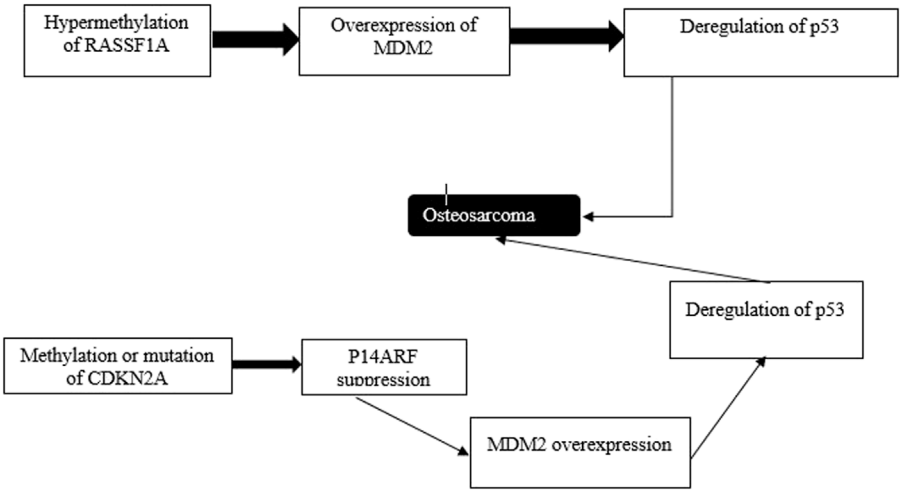

FIGURE 7
Atypical ferroptosis results from p53 deregulation. Epigenetic events contribute to the progression of induction of oxidation. Genes whose activities are modulated by p53 or p53's target genes, including RSSF1A and CDKN2A are silenced *via* methylation.

TABLE 5 A summary of mechanisms of p53 inactivation in the course of osteosarcoma.

Study	Reported mechanism of cell proliferation inhibition
Komori (2016)	Inhibition of cell cycle, induced cell death, inhibition of DNA repair, and antagonism of osteoblast proliferation
Van Maerken et al. (2014)	P53 regulation by MDM2. MDM2 activation inactivates p53, resulting in a decrease in p53 activation
Pang et al. (2020)	TP53 mutation
Saraf et al. (2018)	P53 mutation
Yang and Zhang (2013)	Apoptosis and tumor proliferation
Leroy et al. (2017)	TP53 alterations

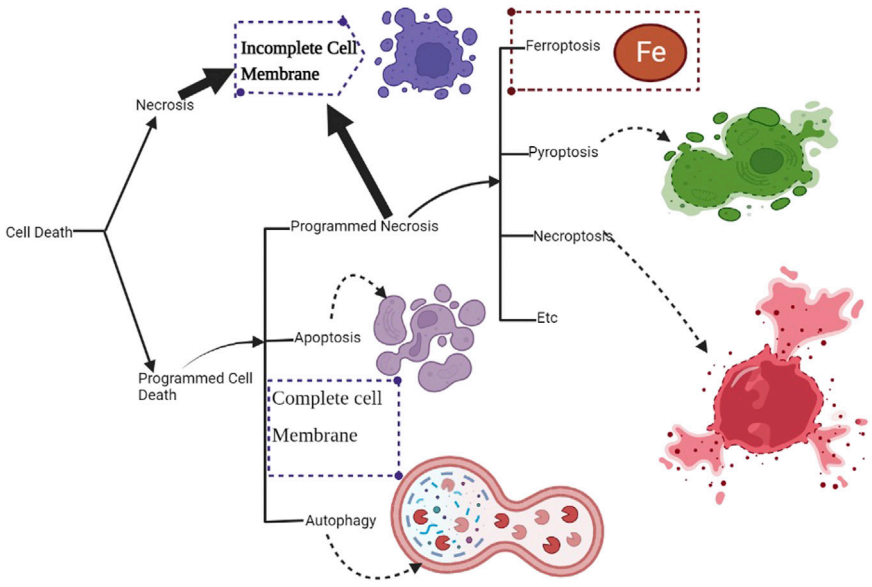

FIGURE 8
An illustration of p53's role in ferroptosis. Cell death mediated by p53 is largely dependent on programmed necrosis. Programmed cell death mediates iron-dependent cell death, ferroptosis.

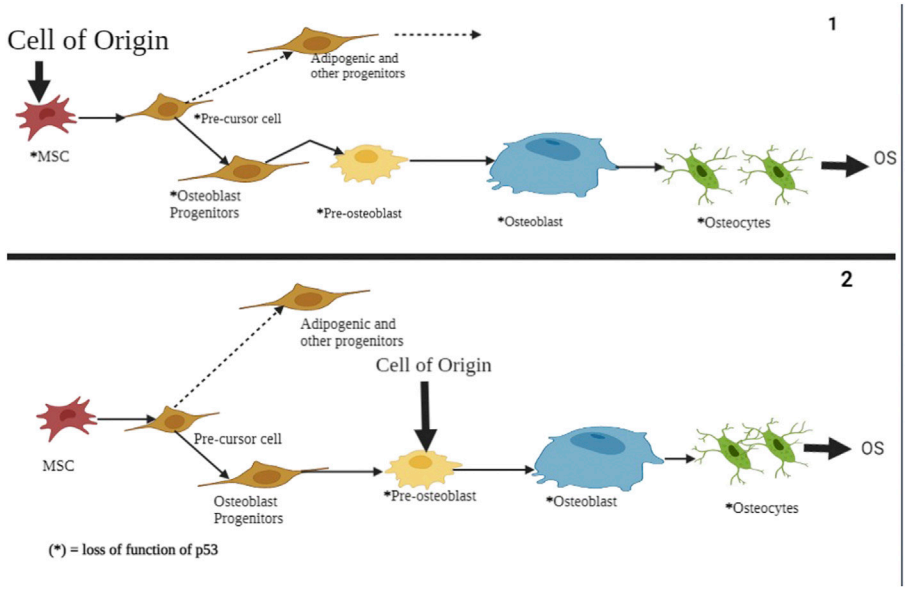

FIGURE 9
A schematic representation of p53's role in atypical and typical ferroptosis in osteosarcoma. In both cases, the functional loss of p53 is the origin of osteosarcoma and undifferentiated MSCs. The regulatory mechanisms affect osteoblasts, pre-osteoblasts, and undifferentiated MSCs. Mutation of the p53 tumor suppressor genes results in aberration of pre-osteoblast cells and mesenchymal progenitor cells, subsequently showing proliferation and compromised growth alongside terminal differentiation.

Van Maerken et al. (2013) have postulated that p53 is inactivated when MDM2 is activated. Saraf et al. have cited p53 mutation as the regulation of atypical ferroptosis, whereas (Komori, 2016) have indicated that interference with the morphology of p53 disrupts normal cell physiology and function. Impaired cell physiology undermines the interaction between SLC7A11 and p53, leading to enhanced tumor development. Additionally, a review of the outcomes reported by the two studies has indicated possible up- and downregulation of atypical ferroptosis in osteosarcoma. Moreover, p53 inactivation, as reported by the studies, undermines the formation of the SLC7A11-p53 complex, promoting osteosarcoma. The converse of this phenomenon is true, as p53 activation would not promote tumorigenesis. Figure 8 is a schematic representation of the floated p53 activation; Table 5 corroborates the scope provided in the Figure 8 by indicating that cell death is downregulated when osteoblast proliferation is initiated. The latter reflects p53's down-regulatory function achieved when the processes enhancing proliferation are down-scaled. In addition, the upregulation of p53 directly antagonizes the activity of SLC7A11 (Liu et al., 2022). The direct antagonism of SLC7A11 enhances ferroptosis in osteosarcoma (Table 6).

Discussion

The regulatory role of p53 in atypical and typical ferroptosis in osteosarcoma remains unknown. However, the regulatory effects of p53 are rapidly evolving, and many scholars have studied its role. In the present study, we aimed to unmask the role of p53 in ferroptosis. Several findings arose in the present study, implicating clinical significance. They included the up- and downregulation of ferroptosis in both atypical and typical ferroptosis in osteosarcoma. In addition, we found that either of the two regulatory roles enhanced ferroptosis, resulting in tumor progression.

Since osteosarcoma is a severe health issue for adults and children, it is urgently necessary to comprehensively understand various biological processes underpinning its occurrence. Among the multiple biological agents, the regulatory role of p53 is involved in ferroptosis associated with osteosarcoma. The literature on osteosarcoma suggests that p53 facilitates up- and downregulation of ferroptosis, as shown in Figure 9. Interestingly, up- and downregulation of ferroptosis implicate outcomes of great clinical significance.

Typical ferroptosis in osteosarcoma remains a function of different elements and biological processes within the cells. After evaluating evidence from 10 studies (Velletri et al., 2016; Luo et al., 2018; Hatina et al., 2019; Lu et al., 2020; Usman et al., 2020; Sciot, 2021; Magri et al., 2021; Ji et al., 2022), we found strong evidence on different regulatory frameworks of p53. Genetic regulatory outcomes stood out in the review as Ji et al., Luo et al., 2018, Lu et al., 2020, Velletri et al., 2016, Sciot, 2021, and Gnanapradeepan et al. have reported strong genetic evidence in regulatory processes of p53. A general observation of the outcomes reported on typical ferroptosis indicates strong up- and downregulation of ferroptosis.

According to Ji et al., the target genes of p53 are fundamental in the regulation of typical ferroptosis in the development of osteosarcoma. Furthermore, tumor suppression or progression is a function of p53 to select cells upon stimulation or inhibition through protein or gene expression. This finding is supported by several studies (Hatina et al., 2019; Usman et al., 2020; Magri et al., 2021), where a direct modulation of target cells involved in or modulating tumor cells has been reported.

TABLE 6 A summary of p53's roles in typical and atypical ferroptosis in osteosarcoma.

P53 regulatory roles in typical ferroptosis	P53's regulatory roles in atypical ferroptosis
Protein-protein interaction with SLC7A11	Inactivation of p53 through MDM2 activation
Modulation of target genes	Genetic mutations that alter cellular functions
Down- or upregulation regulation of tumorigenesis	Production of reactive oxygen species
Initiation of p53's antitumor functions	Regulatory effects of mutant-p53

Lu et al. (2020) have mentioned that PVT1 and miRNA-214 are critical players in the regulatory effects of p53 on osteosarcoma-related ferroptosis. PVT1 inhibits miRNA-214 overexpression, which leads to minimal or poor miRNA-214-p53 binding. The latter reduces ferroptosis as the functions of p53 are significantly reduced. A somewhat similar phenomenon unfolds through p53 activation, where the gain-of-function mutant implicates oncogenic functions. The p53 mutant is an agent of altered functions in ferroptosis events as it reverses the normal biological effects, increasing the gene expression and enhancing consequential ferroptosis.

Usman et al. and Magri et al. have reported that the p53 mutant mediates the down-modulation of xCT, a tumor-linked antigen that protects cells against ferroptosis and oxidative stress. The additional functions of xCT, such as metabolic reprogramming, chemoresistance, and enhanced tumor progression, unmasks that p53 plays a role in regulating ferroptosis in osteosarcoma. Magri et al. have reported that p53 downregulates xCT, resulting in enhanced tumor progression and metabolic reprogramming. These outcomes represent adverse outcomes of p53 and regulatory effects of ferroptosis in osteosarcoma. The literature on genetic mutations and ferroptosis reveals the alteration of genes associated with osteosarcoma. The resulting abnormal cell growth and progression are attributed to the generated cellular defects. Figure 6 illustrates alterations in the deoxyribonucleic acid, suggesting altered cell functions. Theoretically, altered cell functions are central to ferroptosis through mechanisms like the production of ROS.

However, the pathogenesis of osteosarcoma remains unclear. The tumor suppressor, p53, has been associated with the prevention of tumorigenesis. Inactivation of this tumor suppressor enhances drug resistance and promotes the development of osteosarcoma (Chen et al., 2021). In addition, p53 has a context-dependent function in the modulation of lipid peroxidation in ferroptosis in three ways: inhibiting SLC7A11 expression, alongside promoting GLS2 and SAT1 expression (Kang et al., 2019). Lipid peroxidation and ROS production in amounts exceeding the detoxification rate are vital processes responsible for ferroptosis in many cells. The ROS interacts with essential macromolecules like proteins and lipids, resulting in cell death. In addition, lipid peroxidation produces unstable compounds (Liu and Gu, 2022a). The regulatory effects of p53 on typical ferroptosis in osteosarcoma are associated with protein-protein interaction where MDM2-p53 interaction emerges. Table 5 summarizes the mechanisms through which p53 inactivation is achieved, including MDM2-p53 interaction. Our findings were consistent with the existing literature (Neochoritis et al., 2014). This regulatory mechanism is a two-sided phenomenon, as p53 can be activated when MDM2 is inactivated. However, MDM2 inactivation suppresses the p53-MDM2 interaction, which does not result in cell proliferation. Figure 1 and Table 4 and Table 5 represents MDM2's fuantagonistic effects on p53 protein. Upregulation of either typical or atypical ferroptosis is subject to MDM2's influence on the protein. Activation by MDM2 enhances p53's up-regulatory functions, and the converse is true.

Pang et al. (2020) have contested that TP53 mutation is the driver of the high incidence of osteosarcoma, indicating the critical subject in p53's regulatory effects on atypical ferroptosis in the disease. The literature on p53 biology and osteosarcoma treatment emphasizes the oncogenic functions of PT53: maintenance of the proliferation of tumor cells and promoting tumor growth (Synoradzki et al., 2021). Since TP53 gives instructions for p53 production, its mutation threatens the latter process, implying decreased ferroptosis. This phenomenon provides the connection between p53 and ferroptosis, as suggested by previous studies (Liu et al., 2022). As summarized in Table 5, genetic mutation, TP53, and p53 alterations are critical factors in the progression of osteosarcoma. Mutated forms of these genes do not interact with their target sites, causing tumor progression. Our findings were consistent with a previous study in which p53 alterations fuel the progression and spread of tumors (Magri et al., 2021). Additionally, we found that MDM2 played a significant role in the inactivation of p53, and the activation of the former activates the latter, resulting in tumor progression.

Even so, the primary mechanism of p53 is binding to SLC7A11, a promoter of tumorigenesis, and inhibiting its expression. This process modulates the sensitivity and metabolism of cancer cells to ferroptosis (Zhang et al., 2022b). Even though the authors report atypical regulatory accounts, events leading to ferroptosis in the course of osteosarcoma are key. Several studies (Yang and Zhang, 2013; Van Maerken et al., 2014; Komori, 2016; Leroy et al., 2017; Saraf et al., 2018; Pang et al., 2020) have reported a downregulated atypical ferroptosis through inactivation. The theoretical perspectives explain this phenomenon by indicating that the inactivation of p53 hinders its binding and interaction with SLC7A11, resulting in the inhibition of SLC7A11 (explanation for Table 6). As a promoter of tumorigenesis, inhibition of SLC7A11 downregulates the progression of osteosarcoma (Zhang et al., 2018). The above-stated regulatory mechanisms on atypical ferroptosis can be reversed when p53 is activated. The activated version of p53 represses SLC7A11 and subsequently enhances ferroptosis. This postulation is consistent with Liu et al. that the upregulation of p53 enhances ferroptosis by inhibiting SLC7A11 functions (de Azevedo et al., 2019). Upregulation of ferroptosis is detrimental in osteosarcoma as signs will appear through bone fractures, inability to support the body posture, and maintenance of balance.

We found evidence opposing the outcomes of p53 inactivation in ferroptosis in the course of osteosarcoma. Several studies (Bouchalova et al., 2014; Xie et al., 2016; Gnanapradeepan et al., 2018;

Babamohamadi et al., 2022; Su et al., 2022) have contended that p53 plays a central role in up-regulating atypical ferroptosis in the course of osteosarcoma through increasing the production of ROS, lipid and iron-mediated cell death, and cell proliferation (Table 4) (Liu and Gu, 2022b). Upregulation of atypical ferroptosis unfolds through increased production of ROS, lipid peroxidation and enhanced cell proliferation. High lipid peroxidation and ROS production intoxicates cells and disrupts cell physiology, leading to mass cell death. Subsequently, impaired cell physiology interferes with cell growth processes, including proliferation. Metastasis, alongside other complications, such as hypercalcemia, cancer cachexia, bone pain induced by cancer, metastasis of the epidural spinal cord compression, and pathological bone fracture, stand out as common complications (Brown et al., 2017; Eaton et al., 2021). The clinical significance of this regulatory role concerns the management of osteosarcoma. Management strategies seek ways of down-regulating p53 or inactivation to prevent tumor progression.

Conclusion

Even though osteosarcoma is an uncommon type of cancer and accounts for approximately 3% of diagnosed cancers, the severe outcomes due to its exacerbation and progression in a considerable population raise the alarm. Furthermore, the origin and cause of osteosarcoma remain unknown, making the development of an effective treatment or intervention an uphill task. Therefore, we systematically reviewed studies reporting the role of p53 in atypical and typical ferroptosis to unmask the biological processes and events characterizing osteosarcoma. Our findings provided valuable insights into clinical practice and drug development for effective medication.

We found that typical and atypical ferroptosis enhanced tumor progression in osteosarcoma, and p53 played fundamental modulatory roles. Furthermore, biological processes underscored the regulatory functions of p53: multiple events and biological processes characterized the outcomes of osteosarcoma. Figure 1 summarizes the biological processes underscoring p53's functions; upregulation of the protein enhances typical and atypical ferroptosis. Activation by MDM2 up scalesferroptosis.

Protein interaction and its influence on target genes was a fundamental observation in the present review. In addition, p53 inactivation was a significant inhibitor of atypical ferroptosis as all the processes triggered or stimulated by the protein were hindered. Likewise, p53-associated processes that downregulated tumorigenesis were negatively affected by the activation of the protein. The SLC7A11-p53 interaction, which downregulated osteosarcoma, was negatively affected by p53 inactivation, and the converse was true. Liu et al. have contended that upregulation of p53 promotes ferroptosis through inhibiting SLC7A11.

The review reported the effects of MDM2 on p53-associated ferroptosis in osteosarcoma. We found that the interaction between MDM2 and p53 inhibited cell proliferation, slowing down tumorigenesis. MDM2-p53 interaction was of clinical significance as it could be used as an intervention against osteosarcoma. Table 5 summarizes p53 inactivation following MDM2 activation. Interaction between p53 and genes promotes typical or atypical ferroptosis to up- or downregulate osteosarcoma. We did not find up- and downregulation of the tumors occurring concomitantly.

Mutation and the effects of the mutant gene play a fundamental role in typical and atypical ferroptosis. The target genes and genetic expression underpinned the regulatory effects of p53 in osteosarcoma. Whichever direction is taken by the genetic and expression activities, osteosarcoma either increases or decreases. Several studies (Velletri et al., 2016; Luo et al., 2018; Hatina et al., 2019; Lu et al., 2020; Usman et al., 2020; Sciot, 2021; Magri et al., 2021) have reported the direct modulation of target genes and the effects of mutant-p53 in the tumor development process. We could take away that genetic mutations enhanced typical ferroptosis in osteosarcoma due to resulting changes in the deoxyribonucleic acid and cellular activities. The major takeaway from the mutant p53 gene concerns osteosarcoma progression based on the protein involvement. The genes are tailored to initiate tumorigenesis.

Additionally, we found that p53 complicated osteosarcoma management by inducing chemoresistance through the genetic pathways and processes. Genetic mutations, especially the mutant-p53, altered cell structures and functions, resulting in chemoresistance and enhancing cellular susceptibility to ferroptosis. With chemoresistance, interventions against osteosarcoma will be repelled, giving room to the progression of tumors and spreading to other cells. This phe nomenon bears much weight concerning the management of ferroptosis in osteosarcoma. The latter was a significant event leading to enhanced tumorigenesis. However, activation of p53 downregulated tumorigenesis and improved the health of osteosarcoma patients.

Cell proliferation and inhibition of cell division are mechanistic models through which p53 downregulates ferroptosis. Several studies (Yang and Zhang, 2013; Van Maerken et al., 2014; Komori, 2016; Leroy et al., 2017; Saraf et al., 2018; Schiavone et al., 2019; Pang et al., 2020) have reported that the inactivation of p53 inhibits cell proliferation and subsequently downregulates atypical ferroptosis in osteosarcoma. Pang et al. have indicated that approximately 95% of osteosarcoma cases result from p53 inactivation.

Author contributions

SP and LW.conceived the paper. SP wrote the first version of the manuscript and designed the display items with constant input from LW and SP integrated comments from the reviewers. Both authors approved the final version of the article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Babamohamadi, M., Babaei, E., Ahmed Salih, B., Babamohammadi, M., Jalal Azeez, H., and Othman, G. (2022). Recent findings on the role of wild-type and mutant p53 in cancer development and therapy. *Front. Mol. Biosci.* 9 (8), 903075. doi:10.3389/fmolb.2022.903075

Bouchalova, P., Nenutil, R., Muller, P., Hrstka, R., Appleyard, M. V., Murray, K., et al. (2014). Mutant p53 accumulation in human breast cancer is not an intrinsic property or dependent on structural or functional disruption but is regulated by exogenous stress and receptor status. *J. Pathology* 233 (3), 238–246. doi:10.1002/path.4356

Brown, H. K., Tellez-Gabriel, M., and Heymann, D. (2017). Cancer stem cells in osteosarcoma. *Cancer Lett.* 386 (21), 189–195. doi:10.1016/j.canlet.2016.11.019

Campbell, M., McKenzie, J. E., Sowden, A., Katikireddi, S. V., Brennan, S. E., Ellis, S., et al. (2020). Synthesis without meta-analysis (SWiM) in systematic reviews: Reporting guideline. *BMJ* 368 (1), l6890. doi:10.1136/bmj.l6890

Chen, X., Huang, J., Kudo, Y., and Chen, J. (2022). *Ferroptosis as new therapeutic targets in cancer: From molecular mechanisms to therapeutic opportunities*. Lausanne: Frontiers Media SA.

Chen, Y., Hao, Q., Wang, S., Cao, M., Huang, Y., Weng, X., et al. (2021). Inactivation of the tumor suppressor p53 by long noncoding RNA RMRP. *Proc. Natl. Acad. Sci.* 118 (29), e2026813118. doi:10.1073/pnas.2026813118

Cochrane (2011). RoB 2: A revised Cochrane risk-of-bias tool for randomized trials. Cochrane.org. Available at: https://methods.cochrane.org/bias/resources/rob-2-revised-cochrane-risk-bias-tool-randomized-trials (accessed Jan 22, 2023).

Cumpston, M., Li, T., Page, M. J., Chandler, J., Welch, V. A., Higgins, J. P., et al. (2019). Updated guidance for trusted systematic reviews: A new edition of the Cochrane handbook for systematic reviews of interventions. *Cochrane Database Syst. Rev.* 1 (123), ED000142. doi:10.1002/14651858.ed000142

Czarnecka, A. M., Synoradzki, K., Firlej, W., Bartnik, E., Sobczuk, P., Fiedorowicz, M., et al. (2020). Molecular biology of osteosarcoma. *Cancers* 12 (8), 2130. doi:10.3390/cancers12082130

de Azevedo, J., de Medeiros Fernandes, T. A. A., Fernandes, J. V., Lanza, D. C. F., Bezerra, C. M., Andrade, V. S., et al. (2019). Biology and pathogenesis of human osteosarcoma. *Oncol. Lett.* 7 (21), 1099–1116. doi:10.3892/ol.2019.11229

Eaton, B. R., Schwarz, R., Vatner, R., Yeh, B., Claude, L., Indelicato, D. J., et al. (2021). Osteosarcoma. *Pediatr. Blood Cancer* 68 (2), e28352. doi:10.1002/pbc.28352

Giaccone, G. (2019). *Frontiers in oncology: Quarterly highlights: Q1 2019. [Erscheinungsort nicht ermittelbar]*. Lausanne: Frontiers Media SA.

Gnanapradeepan, K., Basu, S., Barnoud, T., Budina-Kolomets, A., Kung, C.-P., and Murphy, M. E. (2018). The p53 tumor suppressor in the control of metabolism and ferroptosis. *Front. Endocrinol.* 9 (12), 124. doi:10.3389/fendo.2018.00124

Hatina, J., Kripnerova, M., Houfkova, K., Pesta, M., Kuncova, J., Sana, J., et al. (2019). Sarcoma stem cell heterogeneity. *Adv. Exp. Med. Biol.* 1123 (47), 95–118. doi:10.1007/978-3-030-11096-3_7

Ji, H., Wang, W., Li, X., Han, X., Zhang, X., Wang, J., et al. (2022). p53: A double-edged sword in tumor ferroptosis. *Pharmacol. Res.* 177 (7), 106013. doi:10.1016/j.phrs.2021.106013

Kang, R., Kroemer, G., and Tang, D. (2019). The tumor suppressor protein p53 and the ferroptosis network. *Free Radic. Biol. Med.* 133 (2), 162–168. doi:10.1016/j.freeradbiomed.2018.05.074

Komori, T. (2016). Cell death in chondrocytes, osteoblasts, and osteocytes. *Int. J. Mol. Sci.* 17 (12), 2045. doi:10.3390/ijms17122045

Leroy, B., Ballinger, M. L., Baran-Marszak, F., Bond, G. L., Braithwaite, A., Concin, N., et al. (2017). Recommended guidelines for validation, quality control, and reporting of TP53 variants in clinical practice. *Cancer Res.* 127 (21), 1250–1260. doi:10.1158/0008-5472.can-16-2179

Liu, J., Zhang, C., Wang, J., Hu, W., and Feng, Z. (2020). The regulation of ferroptosis by tumor suppressor p53 and its pathway. *Int. J. Mol. Sci.* 21 (21), 8387. doi:10.3390/ijms21218387

Liu, X., Du, S., Wang, S., and Ye, K. (2022). Ferroptosis in osteosarcoma: A promising future. *Front. Oncol.* 12 (3), 1031779. doi:10.3389/fonc.2022.1031779

Liu, Y., and Gu, W. (2022a). The complexity of p53-mediated metabolic regulation in tumor suppression. *Seminars cancer Biol.* 85, 4–32. doi:10.1016/j.semcancer.2021.03.010

Liu, Y., and Gu, W. (2022b). p53 in ferroptosis regulation: the new weapon for the old guardian. *Cell. Death Differ.* 29 (5), 895–910. doi:10.1038/s41418-022-00943-y

Liu, Y., Tavana, O., and Gu, W. (2019). p53 modifications: exquisite decorations of the powerful guardian. *J. Mol. Cell. Biol.* 11 (7), 564–577. doi:10.1093/jmcb/mjz060

Lu, J., Xu, F., and Lu, H. (2020). LncRNA PVT1 regulates ferroptosis through miR-214-mediated TFR1 and p53. *Life Sci.* 260 (27), 118305. doi:10.1016/j.lfs.2020.118305

Luo, Z., Cui, R., Tili, E., and Croce, C. (2018). Friend or foe: MicroRNAs in the p53 network. *Cancer Lett.* 419 (91), 96–102. doi:10.1016/j.canlet.2018.01.013

Magri, J., Gasparetto, A., Conti, L., Calautti, E., Cossu, C., Ruiu, R., et al. (2021). Tumor-associated antigen xCT and mutant-p53 as molecular targets for new combinatorial antitumor strategies. *Cells* 10 (1), 108. doi:10.3390/cells10010108

Hameed, M., and Mandelker, D. (2018). Tumor syndromes predisposing to osteosarcoma. *Adv. anatomic pathology* 25 (4), 217–222. doi:10.1097/PAP.0000000000000190

Neochoritis, C., Estrada-Ortiz, N., Khoury, K., and Dömling, A. (2014). Chapter twelve - p53–MDM2 and MDMX antagonists. *Annu. Rep. Med. Chem.* 49, 167–187. doi:10.1016/B978-0-12-800167-7.00012-2

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Syst. Rev.* 10 (1), 89. doi:10.1186/s13643-021-01626-4

Pang, L. K., Pena, M., Zhao, R., and Lee, D.-F. (2020). Modeling of osteosarcoma with induced pluripotent stem cells. *Stem Cell. Res.* 49 (12), 102006. doi:10.1016/j.scr.2020.102006

Petriceks, A. H., and Salmi, D. (2019). Educational case: Primary osteosarcoma. *Acad. Pathol.* 6 (32), 2374289518820337. doi:10.1177/2374289518820337

Prater, S., and McKeon, B. (2020). *Osteosarcoma*. Treasure Island: StatPearls Publishing.

Rethlefsen, M. L., Kirtley, S., Waffenschmidt, S., Ayala, A. P., Moher, D., Page, M. J., et al. (2021). PRISMA-S: An extension to the PRISMA statement for reporting literature searches in systematic reviews. *Syst. Rev.* 10 (1), 39. doi:10.1186/s13643-020-01542-z

Rickel, K., Fang, F., and Tao, J. (2017). Molecular genetics of osteosarcoma. *Bone* 102 (345), 69–79. doi:10.1016/j.bone.2016.10.017

Saraf, A. J., Fenger, J. M., and Roberts, R. D. (2018). Osteosarcoma: Accelerating progress makes for a hopeful future. *Front. Oncol.* 8 (12), 4. doi:10.3389/fonc.2018.00004

Schiavone, K., Garnier, D., Heymann, M. F., and Heymann, D. (2019). The heterogeneity of osteosarcoma: The role played by cancer stem cells. *Stem Cells Heterogeneity Cancer* 1139, 187–200. doi:10.1007/978-3-030-14366-4_11

Sciot, R. (2021). MDM2 amplified sarcomas: A literature review. *Diagnostics* 11 (3), 496. doi:10.3390/diagnostics11030496

Su, Y., Sai, Y., Zhou, L., Liu, Z., Du, P., Wu, J., et al. (2022). Current insights into the regulation of programmed cell death by TP53 mutation in cancer. *Front. Oncol.* 12 (51), 1023427. doi:10.3389/fonc.2022.1023427

Synoradzki, K. J., Bartnik, E., Czarnecka, A. M., Fiedorowicz, M., Firlej, W., Brodziak, A., et al. (2021). TP53 in biology and treatment of osteosarcoma. *Cancers* 13 (17), 4284. doi:10.3390/cancers13174284

Usman, R. M., Razzaq, F., Akbar, A., Farooqui, A. A., Iftikhar, A., Latif, A., et al. (2020). Role and mechanism of autophagy-regulating factors in tumorigenesis and drug resistance. *Asia-Pacific J. Clin. Oncol.* 17 (3), 193–208. doi:10.1111/ajco.13449

Van Maerken, T., Rihani, A., Van Goethem, A., De Paepe, A., Speleman, F., and Vandesompele, J. (2014). Pharmacologic activation of wild-type p53 by nutlin therapy in childhood cancer. *Cancer Lett.* 344 (2), 157–165. doi:10.1016/j.canlet.2013.11.002

Velletri, T., Xie, N., Wang, Y., Huang, Y., Yang, Q., Chen, X., et al. (2016). P53 functional abnormality in mesenchymal stem cells promotes osteosarcoma development. *Cell. Death Dis.* 7 (1), e2015. doi:10.1038/cddis.2015.367

Xie, Y., Hou, W., Song, X., Yu, Y., Huang, J., Sun, X., et al. (2016). Ferroptosis: Process and function. *Cell. Death Differ.* 23 (3), 369–379. doi:10.1038/cdd.2015.158

Yang, J., and Zhang, W. (2013). New molecular insights into osteosarcoma targeted therapy. *Curr. Opin. Oncol.* 25 (4), 398–406. doi:10.1097/cco.0b013e3283622c1b

Zhang, C., Yu, J., Yang, C., Shang, S., Lv, X., Cui, B., et al. (2022). Crosstalk between ferroptosis and stress—implications in cancer therapeutic responses. *Cancer Innov.* 1 (1), 92–113. doi:10.1002/cai2.7

Zhang, L., Hou, N., Chen, B., Kan, C., Han, F., Zhang, J., et al. (2022). Post-translational modifications of p53 in ferroptosis: Novel pharmacological targets for cancer therapy. *Front. Pharmacol.* 13 (24), 908772. doi:10.3389/fphar.2022.908772

Zhang, W., Gai, C., Ding, D., Wang, F., and Li, W. (2018). Targeted p53 on small-molecules-induced ferroptosis in cancers. *Front. Oncol.* 8 (31), 507. doi:10.3389/fonc.2018.00507

Zhao, J., Zhao, Y., Ma, X., Zhang, B., and Feng, H. (2021). Targeting ferroptosis in osteosarcoma. *J. Bone Oncol.* 30 (327), 100380. doi:10.1016/j.jbo.2021.100380

Zou, Y., and Schreiber, S. L. (2020). Progress in understanding ferroptosis and challenges in its targeting for therapeutic benefit. *Cell. Chem. Biol.* 27 (4), 463–471. doi:10.1016/j.chembiol.2020.03.015

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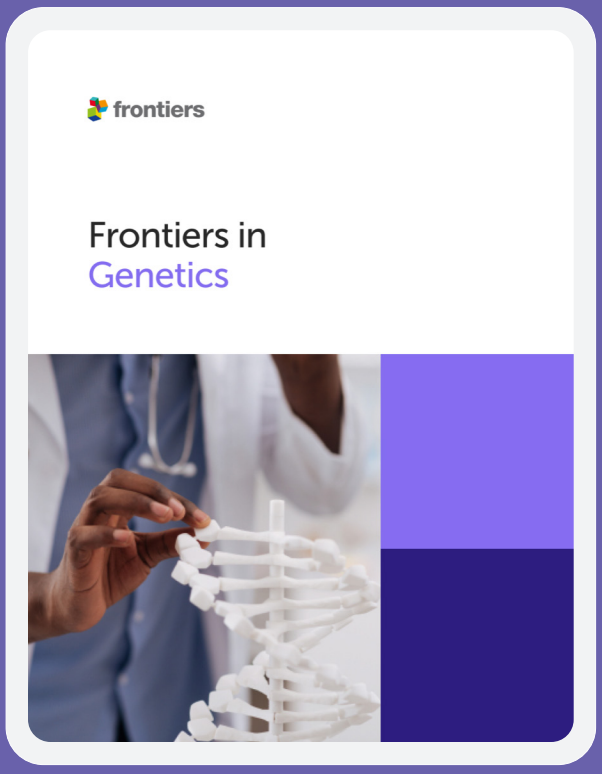

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