Mutant p53 – heat shock response oncogenic cooperation: a new mechanism of cancer cell survival

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The main tumor suppressor function of p53 as a "guardian of the genome" is to respond to cellular stress by transcriptional activation of apoptosis, growth arrest, or senescence in damaged cells. Not surprisingly, mutations in the p53 gene are the most frequent genetic alteration in human cancers. Importantly, mutant p53 (mutp53) proteins not only lose their wild-type tumor suppressor activity but also can actively promote tumor development. Two main mechanisms accounting for mutp53 proto-oncogenic activity are inhibition of the wild-type p53 in a dominant-negative fashion and gain of additional oncogenic activities known as gain-of-function (GOF). Here, we discuss a novel mechanism of mutp53 GOF, which relies on its oncogenic cooperation with the heat shock machinery. This coordinated adaptive mechanism renders cancer cells more resistant to proteotoxic stress and provides both, a strong survival advantage to cancer cells and a promising means for therapeutic intervention.

Keywords: mutant p53, GOF, heat shock response, HSF1, Her2, Neu, EGFR

Oncogenic Functions of Mutant p53

Mutations in the p53 tumor suppressor gene are the most frequent tumor-associated genetic alterations throughout the entire spectrum of human cancers (1). In contrast to other tumor suppressors that are commonly inactivated by frameshift and nonsense mutations resulting in loss-of-function, the majority of p53 alterations are missense mutations clustered in six "hot-spots" of the DNA-binding domain of p53 (2). Numerous mouse models, *in vitro* and clinical studies have demonstrated that in addition to simple loss of the tumor suppressor function of p53, many mutant p53 (mutp53) proteins gain neomorphic oncogenic activities, termed as gain-of-function (GOF) (2, 3). These GOF activities contribute to malignant transformation by enhancing cells proliferation, invasion, metastatic ability, and chemoresistance (2, 3). The concept of mutp53 GOF is strongly supported by human clinical studies on Li–Fraumeni syndrome (LFS) patients carrying germline p53 mutations and by GOF mouse models (4–7). Thus, several studies have found that the median age of cancer onset in LFS patients with mutp53 missense mutations is 9–20 years earlier than in LFS patients with loss of p53 expression (8, 9). Moreover, clinical evaluation of 1,794 breast cancer patients revealed that *somatic* p53 mutations are also associated with a shorter overall survival, independently of stage, grade, and hormone receptors status (10), similarly to other cancer types harboring mutp53 (1). This is fully confirmed by mouse mutp53 knock-in models, manifesting GOF by increased

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Abbreviations: EGFR, epidermal growth factor receptor; GOF, gain-of-function; HSE, heat shock elements; HSF1, heat shock factor 1; HSP, heat shock protein; LFS, Li–Fraumeni syndrome; mutp53, mutant p53; NF1, neurofibromatosis type 1; PDGFR, platelet-derived growth factor receptor.

metastases, broader tumor spectrum, more invasive tumor fronts, more malignant histology, and higher tumor bulk compared to p53-null tumors (4–7).

A broad spectrum of GOF activities has been described for mutp53 [reviewed in Ref. (2, 3)]. Among the most prominent ones is the ability of mutp53 to promote cells proliferation, invasion, and motility by stimulating signal transduction pathways downstream of growth factor receptors, such as TGFβ receptor (11), EGFR (4, 12, 13), MET (14), PDGFRβ (15), as well as ErbB2/Her2 (4, 13) discussed below. Also, a number of *in vitro* and *in vivo* studies have described a critical role of mutp53 in tumor initiation via enhanced generation and expansion of cell populations with stem cell/cancer stem cell properties (4, 5, 16, 17). In addition, recent reports indicate that mutp53 promotes the inflammatory response and inflammation-associated cancers by stimulating NF-κB activation (18, 19). Finally, a novel intriguing mutp53 GOF activity has been described in tumor-associated fibroblasts (20, 21), suggesting that mutp53 can play oncogenic roles not only in cancer cells but also in the tumor stromal cells.

Mechanistically, although the majority of mutp53 missense mutations map to its DNA-binding domain, mutp53 GOF activities are still largely attributed to transcriptional regulation of specific target genes, which differ from typical wild-type p53 targets [reviewed in Ref. (2)]. While mutp53-specific DNA consensus sites have not been identified, it appears that mutp53 is a potent modulator of other transcription factors and co-factors, via regulation of their DNA binding and transcriptional properties, such as p63 (11, 12, 22, 23), p73 (22), SP1 (24), SREBP (25), and others [reviewed in Ref. (2, 3)] including the master heat shock regulator HSF1 (13), discussed below.

A critical feature of most mutp53-harboring tumors is significantly increased mutp53 protein stability, manifested by massive mutp53 accumulation in tumors, but not in normal tissues (6, 26). We and others showed that cancer-specific accumulation of mutp53 is crucial for many aspects of tumorigenesis and is the key determinant of mutp53 GOF *in vitro* and *in vivo* (7, 11, 27, 28). Thus, acute downregulation of stabilized mutp53 by RNA interference (RNAi) strongly inhibits malignant phenotypes (11, 27, 28). For example, we found that stable and Tet-inducible knockdown of endogenous mutp53 in breast (MDA231) and colon (SW480) cancer cells by p53 RNAi dramatically inhibits growth of these human cancer cells *in vitro* and in xenografts and their invasive properties (28). These data are consistent with other reports showing that mutp53 downregulation by RNAi suppresses invasion (11, 28), restores normal mammary architecture in 3D culture of breast cancer cell lines (25), and inhibits metastasis *in vivo* (11, 15). Thus, cancer cells appear to be addicted to high levels of mutp53 for their survival and oncogenic properties.

Addiction of cancer cells to stabilized mutp53 underscores the translational significance of mutp53 as a promising therapeutic target. However, targeting mutp53 by conventional modalities is a very challenging task, since mutp53 is neither an enzyme nor a cell surface protein. Therefore, one promising alternative for abolishing mutp53 GOF in mutp53-harboring cancers could be its depletion/destabilization. We recently proposed that this could be achieved by exploiting mutp53 interdependence with the heat shock response machinery (13, 28, 29). Thus, we showed that mutp53 has a novel GOF activity as an essential regulator of protein homeostasis in cancer. Specifically, it augments the pro-survival heat shock response machinery via activating the master transcriptional regulator heat shock factor 1 (HSF1), which in a positive feed-forward loop further stabilizes mutp53 itself, along with other tumor-promoting clients (13). This novel oncogenic GOF activity of mutp53 may represent a unique adaptive mechanism for superior survival of mutp53-harboring cancer cells in the hostile tumor environment.

Mutant p53 and the Heat Shock Response

Inherent to malignant transformation is the constant proteotoxic stress due to aneuploidy, accumulation of reactive oxygen species (ROS), hypoxia, acidosis, and accumulation of mutated, conformationally aberrant proteins (30–32). To overcome these potentially deadly conditions for their survival, cancer cells heavily depend on molecular chaperones, heat shock proteins (HSPs), whose induction in cancer constitutes the powerful adaptive pro-survival mechanism known as the heat shock response (32). Under proteotoxic stress, induction of HSPs restores protein homeostasis by repairing and proper folding of damaged and mutated proteins with aberrant conformation. HSPs induction in cancer cells is triggered by the transcription factor HSF1 that binds to unique DNA sequence motifs known as heat shock elements (HSEs) in the promoters of HSPs, inducing their transcription (33). In unstressed cells, HSF1 is sequestered by HSP90 predominantly in the cytoplasm (30). However, proteotoxic stress induces HSF1 phosphorylation, liberation from the HSP90 inhibitory complex, trimerization, and translocation to the nucleus to activate HSPs expression (34). It has been shown that phosphorylation of HSF1 at serine 326 (pSer326) is pivotal to render HSF1 transcriptionally competent (30, 34). Also, HSF1 stabilization and activation may be induced by genetic changes such as loss of the neurofibromatosis type 1 (NF1) tumor suppressor gene (35).

The essential role of HSF1 in malignant transformation and progression is well documented in literature. Specifically, HSF1 induces a diverse array of HSP-mediated pro-survival mechanisms, including stabilization of oncogenic clients, altered glucose metabolism and signal transduction, and upregulation of protein translation (4, 32, 35, 36). Interestingly, a recent study by the Linquist group has shown that HSF1 has both distinct and overlapping activities in the maintenance of normal protein homeostasis vs. tumorigenesis (36). In cancer HSF1 orchestrates, a wide range of fundamental cellular processes that are not related to heat shock response but are critical for malignant transformation and maintenance, including cell-cycle control, ribosomal biogenesis, protein translation, and inhibition of apoptosis (36). Importantly, cancer-specific HSF1-bound genes ("HSF1 cancer signature") were found enriched in the biopsies of human breast, colon, and lung tumors and strongly correlated with poor patient outcomes underscoring the critical role of HSF1 in tumorigenesis (36). In agreement, another clinical study found HSF1 upregulation in 80% of breast cancers, which was also associated with high histologic grade and increased

mortality (37). Finally, the pivotal role of HSF1 in tumorigenesis is demonstrated in various animal cancer models. For example, genetic deficiency of HSF1 dramatically reduces mammary tumor formation in the Her2/Neu mouse model (38), tumorigenesis in the DMBA-induced skin carcinogenesis and in the mutp53 mouse models (31).

The connection between mutp53 and heat shock response has been known for nearly two decades (39). Thus, mutp53 and HSP90 (one of the most explored HSF1 transcription targets) were shown to physically interact, which was linked to cancer-specific mutp53 stabilization (28, 29, 39, 40). We and others subsequently demonstrated that pharmacological or RNAi-mediated inhibition of HSP90 leads to ubiquitination and proteasomal degradation of mutp53, mediated by the E3 ubiquitin ligases MDM2 and CHIP (28, 29, 40, 41). Moreover, it is likely that other E3 ligases might be involved as well. Thus, a recent report suggests that arsenic trioxide, a drug used to treat acute promyelocytic leukemia, cooperates with HSP90 inhibitors and promotes mutp53 degradation in tumor cells by the Pirh2 ubiquitin ligase (42). It would be interesting to see whether Pirh2 is inhibited by HSPs during malignant transformation similarly to MDM2 and CHIP (29, 40), leading to mutp53 aberrant stabilization, and fueling of its oncogenic properties.

An important evidence for mutp53-HSP90 oncogenic cooperation comes from the studies showing that HSP90 inhibition shows preferential cytotoxicity in mutp53 – rather than in wild-type p53 or p53null – cancer cells and destabilizes mutp53 (28, 29). *In vivo* studies also provide compelling evidence for mutp53-heat shock response oncogenic cooperation. Thus, HSF1 genetic deficiency prolonged median overall survival of mutp53 mice (R172H) in a dose-dependent manner, from 427 to 470 to >622 days in HSF1$^{+/+}$ vs. HSF1$^{+/-}$ vs. HSF1$^{-/-}$ animals, respectively (31). This strongly suggests that either (i) HSF1/HSPs directly maintain mutp53 levels/activity or (ii) the oncogenicity of mutp53 critically depends on HSF1 and/or HSF1-mediated transcriptional program, or both. Although mutp53 levels were not examined in this study (31), it is tempting to speculate that they were reduced in HSF1-deficient tumors as a result of insufficient transcriptional upregulation of HSP90, leading to restrained malignant transformation. In support of this idea, we previously showed that shRNA-mediated knockdown of HSF1 in mutp53 cancer cells induces rapid destabilization of mutp53 and reduces its half-life, along with reduction of HSP90 levels (29). Further studies are needed to confirm this idea *in vivo* and to test the other possible mechanisms of mutp53-HSF1 oncogenic cooperation. Besides that, it would be interesting to examine whether other HSF1 transcriptional chaperone targets besides HSP90, e.g., HSP70 and HSP27, also can stabilize mutp53 in cancer cells and via what mechanisms. Interestingly, a novel mechanism of mutp53 degradation via chaperone-mediated autophagy was recently reported. It has been shown that heat shock cognate protein 70 (HSC70), a constitutive cytosolic protein (*not* regulated by HSF1), can target mutp53 to lysosomal degradation, but only in non-proliferating tumor cells under the condition of proteasomal and macroautophagy inhibition (43). However, the importance of this mechanism in proliferating cancer cells remains to be elucidated.

Mutant p53 Induces HSF1 Transcriptional Activity via Her2 and EGFR

Although mechanisms regulating the heat shock response in tumor cells are not fully understood, it is well established that heat shock response strongly depends on the transcriptional activity of HSF1. Stress-activated transcriptionally competent form of HSF1 is a homo-trimer, which can be post-translationally modified resulting in HSF1 nuclear translocation and binding to HSP promoters (27, 30). In response to proteotoxic stress, HSF1 becomes phosphorylated at serine 326, which is essential for HSF1 transcriptional activity (27, 31).

A number of studies suggest that the Her2/EGFR2/Neu signaling pathway is an important activator of HSF1, at least in breast cancer. Thus, Her2 overexpression in MCF7 cells leads to increased HSF1 levels and its trimerization (44). Also, HSF1 is necessary and sufficient for Her2-induced transformation of normal breast epithelial cells MCF10A *in vitro* (45). Finally, HSF1 genetic knockout significantly reduces mammary tumorigenesis in the ErbB2 transgenic mouse model (38). Clinically, the presence of high levels of nuclear HSF1 in Her2-positive mammary tumors correlates with poor patient prognosis (37). Moreover, recent studies demonstrate existence of a linear signaling pathway between Her2 and HSF1 in Her2-positive breast cancer, both *in vitro* (13, 46, 47) and *in vivo* (4, 47). Thus, Schulz et al. showed that Her2 overexpression constitutively activates HSF1, resulting in stabilization of HSP90 clients, such as MIF, AKT, mutp53, and HSF1 itself (47). Moreover, pharmacological inhibition of Her2 strongly suppresses HSF1 activation *in vitro* and in the Her2 mouse transgenic model, which correlates with reduced mammary tumor progression (13, 47). Mechanistically, Her2 signals via the phosphoinositide-3 kinase (PI3K)–AKT axis to induce pSer326 phosphorylation of HSF1 and its transcriptional activity (47).

Intriguingly, the Lindquist group found that activation of HSF1 can be triggered not only by environmental and proteotoxic insults but also by genetic alterations. Thus, loss of the tumor suppressor gene neurofibromatosis type 1 (NF1) increases HSF1 levels and induces HSF1 phosphorylation at Ser326, which depends on dysregulated RAS/MAPK signaling, suggesting a key role of RAS/MAPK pathway in the transcriptional activation of HSF1 (35). Consistently, Stanhill et al. showed that RAS activation induces HSF1-mediated upregulation of HSP70 (48). These findings are strongly supported by clinical data. Thus, elevated expression of HSF1 and pSer326-HSF1 was found in surgical specimens of malignant peripheral nerve sheath tumors driven by loss of NF1 (35). Given the importance of HSF1 in cancer cell physiology (36), it will be interesting to see whether loss of other tumor suppressor genes, especially those leading to MAPK/PI3K pathway activation, could trigger transcriptional activity of HSF1 in different tumor types.

How exactly does mutp53 cooperate with HSF1 in cancer? On one hand, as mentioned above, HSF1 genetic ablation profoundly alleviates tumorigenesis driven by mutp53 *in vivo* (31), which could be due to insufficient levels of HSP90 to support mutp53 accumulation in tumors (29, 39, 40). On the other hand, our recent findings demonstrate that mutp53 is also an important

determinant of HSF1 activity. Thus, overexpression of various mutp53 alleles in cancer cell lines leads to upregulation of HSF1, with concurrent increase in heat shock response and prototypical HSP clients (13). Furthermore, we found that this mutp53-HSF1 positive feed-forward loop depends on growth factor receptor signaling, specifically EGFR and Her2 pathways, for HSF1 transcriptional activity (4, 13).

Since mutp53 is known to exert its GOF activities via other growth factor receptor signaling pathways besides Her2 and EGFR (see above), these pathways could also be involved in mutp53-HSF1 oncogenic cooperation. Preliminary indirect evidence of this possibility comes from the finding by the Linquist lab that HSF1 enables cellular transformation of mouse embryo fibroblasts via PDGFRβ signaling (31), which was shown to be positively regulated by mutp53 as well (15). However, it remains to be determined whether other growth factor receptor signaling pathways – besides EGFR and Her2 – can modulate HSF1 activity in a mutp53-dependent manner.

Recently, we found another, more direct mode of the mutp53-HSF1 circuit regulation in cancer cells. Thus, upon proteotoxic stress and nuclear translocation, phospho-activated pSer326-HSF1 interacts (directly or indirectly) with mutp53. Moreover, mutp53 facilitates HSF1 recruitment to specific DNA sites (HSEs) in target gene promoters and augments a broad pro-survival HSF1-induced transcriptional program, including expression of HSPs (13).

Finally, as mentioned above, HSF1 transcriptional activity can be stimulated by genetic alterations in cancer (35). Thus, the well-known ability of mutp53 to induce genomic instability (49) could in theory be another mechanism contributing to HSF1 activation in cancer, which remains to be tested.

Based on the described findings, we propose a novel mechanism of mutp53 GOF, via regulation of the heat shock response, as depicted in **Figure 1**. We propose that mutp53 through enhanced recycling (12) and/or stability of EGFR and Her2/ErbB2 – augments MAPK and PI3K signaling, leading to phospho-activation of HSF1. Concurrently, mutp53 directly interacts with activated HSF1 and facilitates its binding to specific promoter elements and stimulates transcription of HSPs. In turn, HSPs further stabilize their oncogenic clients, including EGFR, Her2, and mutp53 itself (via suppression of ubiquitin ligases MDM2, CHIP, etc.), reinforcing tumorigenesis (**Figure 1**). Importantly, this mechanism has been confirmed by *in vivo* studies. First, clinical data show a strong enrichment (83%) of the Her2-positive breast cancer in LFS women with p53 germline mutations, compared to 20–25% Her2 positivity in patients with sporadic breast cancer (50). This suggests that the presence of mutp53 germline mutations strongly predisposes Li–Fraumeni women specifically to the initiation of Her2-driven breast cancer. Second, we found a strong correlation between mutp53 and nuclear pSer326-HSF1 levels only in strongly Her2-positive (3+), but not in Her2-negative/ER/PR-positive human breast cancer specimens (13). Finally, we demonstrated that the Her2/ErbB2 signaling is much more amplified in mutp53 (R172H) compared to p53null tumor cells in the MMTV-ErbB2 mouse breast cancer model, leading to a more aggressive disease (4).

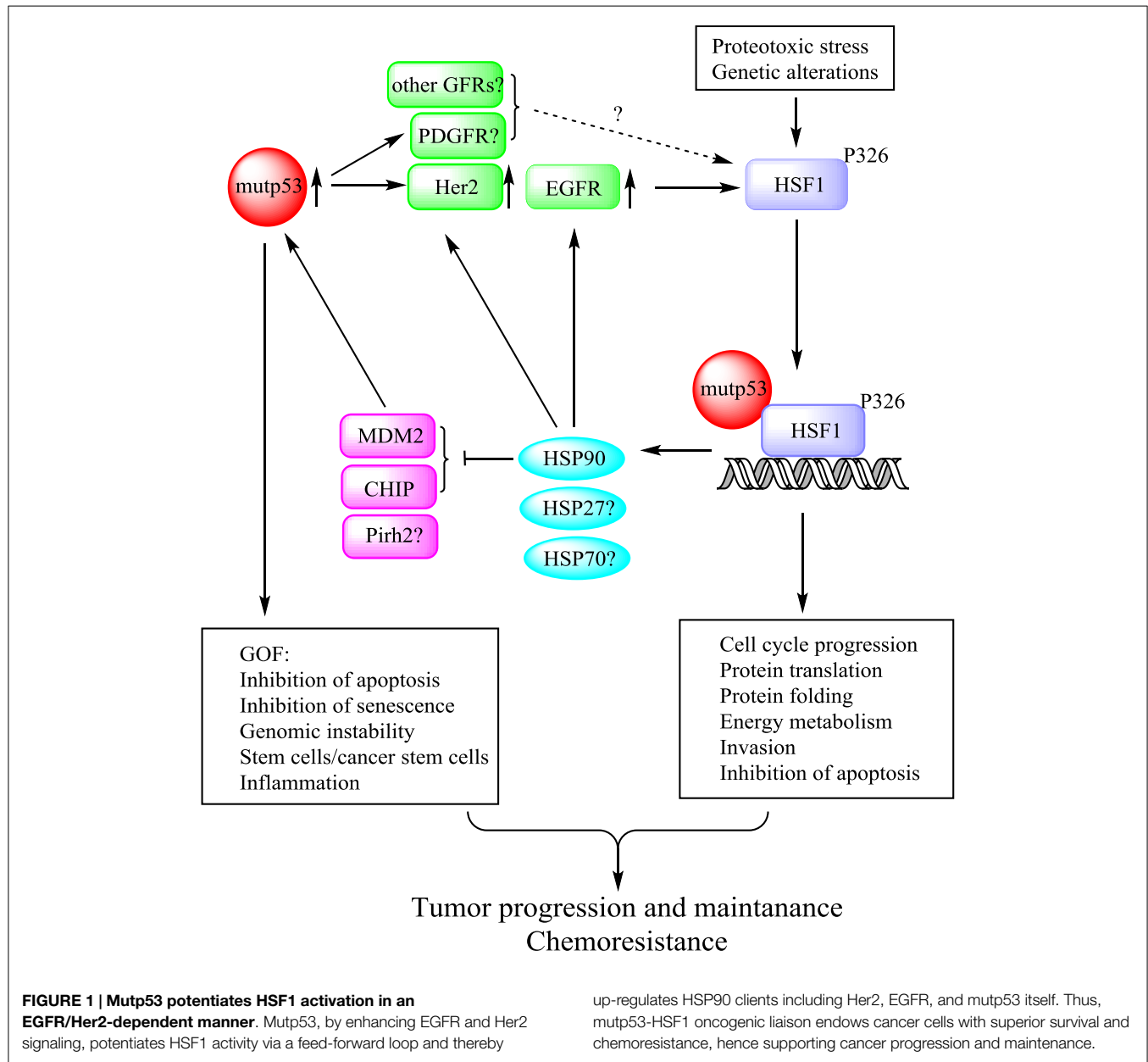Many physiological consequences can result from the described mutp53-HSF1 liaison in cancer cells. First, the resulting increased expression of HSPs endows cancer cells with a superior resistance to proteotoxic stress caused by harsh tumor environment (13). Second, it leads to enhanced stabilization of numerous tumor-promoting HSP oncogenic clients, including Her2, EGFR, and mutp53 themselves, which cell-autonomously further amplify this feed-forward circuit and oncogenesis. Third, HSPs have been shown to inhibit oncogene-induced senescence pathways in cancer cells (51). Thus, by enhancing HSP expression at early stages of tumorigenesis, mutp53 may facilitate disabling of oncogene-induced senescence and therefore empower tumor progression. Fourth, we showed that mutp53-HSF1-mediated amplification of Her2 pathway can promote expansion of mammary stem cells and induce cancer cell proliferation *in vivo* (4), which can enhance tumor initiation and progression. Finally, by augmenting the broad HSF1-dependent transcriptional program, mutp53 may promote global cancer-related changes, including cell-cycle progression, altered signaling pathways, metabolism, adhesion, protein translation, etc. (36).

In sum, it is evident that the functional oncogenic interaction between mutp53 and HSF1 can initiate a wide range of tumorigenic processes in the complex landscape of mutp53-harboring cancers.

Summary

While mutations in the p53 gene are prevailing in many types of cancer, specific therapeutic modalities tailored to mutp53-harboring cancers have not been developed in clinic. Three potential mutp53-targeted therapeutic strategies have been recently proposed: (i) restoration of wild-type p53 activity in mutp53 proteins (3), (ii) inhibition of mutp53-regulated downstream targets and pathways, e.g., proteins involved in integrin recycling (12), the mevalonate pathway (25), PDGFRβ signaling (15), etc. [reviewed in Ref. (3)], and (iii) mutp53 degradation (28, 29, 42). Overall, understanding the mechanisms underlying mutp53 oncogenic activity will no doubt have a profound translational impact. However, more in-depth studies are needed to establish whether these approaches will be clinically feasible and whether pharmacological targeting of relevant pathways will achieve preferential response in the patients with mutp53-harboring tumors.

The herein described novel oncogenic GOF role of mutp53 in the regulation of heat shock response – via enhanced receptor tyrosine kinase (Her2, EGFR) signaling and augmented HSF1 transcriptional activity – opens up novel therapeutic opportunities. We anticipate that the mutp53-HSF1 liaison, due to potentiating Her2 and/or EGFR pathways, sensitizes cancer cells to ErbB2 and EGFR targeted therapies. Thus, inhibition of Her2, EGFR, or their downstream effectors can intercept the sensitive mutp53-HSF1-Her2/EGFR circuitry and therefore be a potent approach for the treatment of Her2 (or EGFR) and mutp53 double-positive cancers. Convergence of the mutp53-HSF1 liaison on the Her2/EGFR pathways provides a strong rationale to test the targeted therapies that are currently on the market (e.g., Her2-targeted trastuzumab, pertuzumab, T-DM1, and lapatinib) specifically in mutp53-harboring cancers. The most promising cancer types expected to specifically respond to these therapies are Her2/mutp53 double-positive breast cancer [constituting 72% of all sporadic Her2-positive breast cancers (52)],

FIGURE 1 | Mutp53 potentiates HSF1 activation in an EGFR/Her2-dependent manner. Mutp53, by enhancing EGFR and Her2 signaling, potentiates HSF1 activity via a feed-forward loop and thereby up-regulates HSP90 clients including Her2, EGFR, and mutp53 itself. Thus, mutp53-HSF1 oncogenic liaison endows cancer cells with superior survival and chemoresistance, hence supporting cancer progression and maintenance.

pancreatic and non-small-cell lung cancer [both of which have high prevalence of mutp53 (1) and are commonly treated with EGFR inhibitor Erlotinib], and possibly esophageal cancer [43% mutp53-positive (1), 23% Her2-positive]. Although it remains to be determined whether the Her2-positive subtype of esophageal cancers is enriched in mutp53, similarly to breast cancer. Importantly, the data described here also predict that the patients with tumor types that commonly overexpress both, Her2/EGFR and mutp53, will need to be stratified according to their mutp53 status for the most efficient treatment outcomes.

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