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EXERCISE AND CANCER: FROM CLINICAL ASSOCIATION TO MECHANISTIC INSIGHTS

Topic Editors:
Yao Lin, Fujian University of Traditional Chinese Medicine, China
Geng Liu, University of Oxford, United Kingdom
Han-Xiang An, Xiamen University, China
Hang Fai Kwok, University of Macau, China

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EDITED AND REVIEWED BY
William C. Cho,
QEH, Hong Kong SAR, China

*CORRESPONDENCE
Yao Lin,
yaolin@fjtcm.edu.cn
Hang Fai Kwok,
hfkwok@um.edu.mo

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Editorial: Exercise and cancer: From clinical association to mechanistic insights

Yao Lin[1]* and Hang Fai Kwok[2,3,4]*

[1]Central Laboratory at the Second Affiliated Hospital of Fujian Traditional Chinese Medical University, Fujian-Macao Science and Technology Cooperation Base of Traditional Chinese Medicine-Oriented, Collaborative Innovation Center for Rehabilitation Technology, Fujian University of Traditional Chinese Medicine, Fuzhou, China, [2]Cancer Centre, Faculty of Health Sciences, University of Macau, Taipa, Macao SAR, China, [3]Department of Biomedical Sciences, Faculty of Health Sciences, University of Macau, Taipa, Macao SAR, China, [4]MoE Frontiers Science Center for Precision Oncology, University of Macau, Avenida de Universidade, Taipa, Macao SAR, China

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Editorial on the Research Topic
Exercise and cancer: From clinical association to mechanistic insights

Cancer is one of the leading causes of mortality worldwide. The association between physical exercise and many aspects of cancer, such as incidence and prognosis, has been well documented. In addition, many risk factors of cancer including obesity, aging, and inflammation are affected by physical exercise. Although it is widely recognized that physical exercise has a positive effect on cancer in terms of morbidity, prognosis, rehabilitation, and even therapy, there are issues in this field awaiting a deeper understanding. In this research topic, biological and biomedical scientists summarized or investigated the latest progress in multiple areas of this field.

Four studies investigated the effect of exercise in different cancer models. Suzuki et al. carried out an interesting research on the combined effects of exercise training and nutritional supplementation in cancer patients in the context of coronavirus (COVID-19). They proposed that combining dietary supplements and exercise training in cancer patients can boost immune responses against COVID-19 and probably improve vaccine responses. Li et al. evaluated the impact of swimming on murine colon cancer cell line CT-26 xenograft model. Swimming significantly attenuates tumor growth and muscle wasting, and suppresses inflammatory and apoptosis pathways. Kim et al. also used CT-26 cells to study the effect of high-intensity aerobic exercise on cancer (https://doi.org/10.3389/fmolb.2022.818470). Instead of using xenograft, they injected CT-26 cells via the tail vein to establish a cancer mouse model. They discovered that exercise improved positive results in comprehensive parameters such as food intake, weight gain and survival rate. Jin et al. carried out a meta-analysis of animal experiments to study the effects of exercise on breast cancer (https://doi.org/10.3389/fmolb.2022.843810). Based on their analyses, exercise could reduce tumor weight, the number of tumors per animal, and the

tumor incidence in breast cancer models of mice and rats. However, the standards of conducting and reporting animal works need to be improved.

In addition, another five studies investigated exercise and cancer from other angles. Zhu et al. studied the physical activity and cancer status among middle-aged and older Chinese. They reported that individuals who spent more than half an hour performing moderate or vigorous intensity activity every day were significantly less likely to report a cancer diagnosis than inactive individuals. Chen et al. constructed a modified model to predict malignancy in thyroid nodules with small size using ultrasound characters (https://doi.org/10.3389/fmolb.2021.752417), which may provide useful tools to evaluate the effect of exercise on early tumor development. Wu et al. investigated the association of the methylation and expression of the exercise-related toll like receptor-1 (TLR-1) gene with the prognosis and outcome of low-grade glioma (LGG). They found that TLR-1 can be a potential prognostic marker and may be involved in immune cell infiltration and immunotherapy in LGG. Ochi et al. analyzed the blood of patients from a 12-weeks trial to evaluate the effect of exercise on cancer-related fatigue (CRF). They suggested that blood polyunsaturated fatty acid (PUFA) balance may be associated with the effect of exercise on CRF. Jin et al. carried out a bibliometrics study on the molecular mechanisms of exercise on cancer. The authors discovered that altered metabolism, oxidative stress and apoptosis were current research hot spots in this field, and emerging research foci were generally around inflammation, epithelial mesenchymal transition (EMT) and adipokines.

Although covering a relatively broad range in the cancer field, it is a pity this research topic did not have any work on the bioactive material mediating the effect of exercise on cancer, which is an emerging promising direction in this field. For example, Bar-sagi et al. discovered that exercise-induced activation of the IL-15/IL-15Rα pathway promotes anti-tumor immunity in pancreatic cancer (Kurz et al., 2022). Likewise, Saxton et al. found that acute aerobic exercise-conditioned serum reduces colon cancer cell proliferation *in vitro* through interleukin-6 (Orange et al., 2022).

There is no doubt more such intermediate bioactive molecules such as proteins, miRNAs, metabolites and so on will be identified in the future. They are probably the key to unravel the complex functions of exercise on cancer. The deepening of our understanding of the mechanisms underlying the effect of exercise on cancer may 1 day help us harness the benefits of exercise without actually exercising.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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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Polyunsaturated Fatty Acids, Exercise, and Cancer-Related Fatigue in Breast Cancer Survivors

Yutaka Matsuoka[1]*, *Katsunori Tsuji*[1] and *Eisuke Ochi*[1,2]*

[1] Division of Health Care Research, Center for Public Health Sciences, National Cancer Center Japan, Tokyo, Japan, [2] Faculty of Bioscience and Applied Chemistry, Hosei University, Tokyo, Japan

Cancer-related fatigue (CRF) is one of the most frequently reported and disabling symptoms in cancer survivors. With its negative impact on the activities of daily living, work, social activities, and mood, CRF causes severe impairment of quality of life. A previous study showed that omega-6 polyunsaturated fatty acid (PUFA) supplementation unexpectedly reduced CRF compared with omega-3 PUFA supplementation and that omega-6 PUFA supplementation reduced pro-inflammatory serum markers in fatigued American breast cancer survivors. Meanwhile, a recent meta-analysis of individual patient data revealed significant benefits of exercise interventions on CRF. Recently, we completed our randomized controlled trial among early-stage Japanese breast cancer survivors, in which we examined the effect of baseline blood PUFA characteristics on change in CRF during the 12-week trial by exercise group and confirmed that increased Cancer Fatigue Scale (CFS) was associated with both docosahexaenoic acid (DHA) ($p = 0.06$) and omega-3 index ($p = 0.08$) at baseline in all participants ($n = 46$, omega-6/omega-3 ratio = 6.79, SD = 1.90). On the contrary, DHA at baseline was positively correlated with change in CRF ($r = 0.40$, $p = 0.06$) in the control group ($n = 24$, omega-6/omega-3 ratio = 7.0). Moreover, eicosapentaenoic acid (EPA) at baseline was positively correlated with leg strength ($r = 0.39$, $p = 0.10$) in the exercise group. In conclusion, blood PUFA balance might be associated with the effect of exercise on CRF. In addition, higher EPA in individuals who conducted exercise likely has a beneficial effect on muscle strength. Further investigation is needed to clarify the interaction between PUFAs and exercise for alleviating CRF.

Keywords: omega-3 fatty acids, ω-3, cancer-related fatigue, exercise, cancer survivorship

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Edited by:
Geng Liu,
University of Oxford, United Kingdom

Reviewed by:
Ying Hu,
Harbin Institute of Technology, China
Jian Zhao,
Shanghai University of Medicine and Health Sciences, China

***Correspondence:**
Eisuke Ochi
ochi@hosei.ac.jp
Yutaka Matsuoka
matsuoka-psy@umin.ac.jp

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Cancer-related fatigue (CRF) is one of the most frequently reported and disabling symptoms in cancer survivors. The primary candidates for causing fatigue are cancer treatment, particularly treatment with adjuvant chemotherapy, depression, pain, and sleep (Bower et al., 2000). With its negative impact on the activities of daily living, work, social activities, and mood, CRF causes severe impairment of quality of life. The phospholipid polyunsaturated fatty acids (PUFAs) are promising candidates for the reduction of CRF (Peppone et al., 2019) and are divided into two main types of PUFAs in the human body: the omega-6 PUFA series derived from *cis*-linoleic acid (LA, 18:2) and the omega-3 PUFA series derived from alpha-linolenic acid (ALA, 18:3) (Su, 2009). Peppone et al. (2019) demonstrated in their multicenter randomized controlled trial that omega-6 PUFA supplementation unexpectedly

TABLE 1 | Correlations between blood polyunsaturated fatty acid compositions and change in cancer-related fatigue over 12 weeks.

	All ($n = 46$)		Exercise ($n = 23$)		Control ($n = 23$)	
	r_s	p	r_s	p	r_s	p
Omega-6 PUFAs						
Linoleic acid	0.06	0.70	−0.28	0.20	0.28	0.20
Arachidonic acid	0.23	0.13	0.04	0.84	0.32	0.13
Total n-6 PUFAs	0.01	0.96	−0.36	0.09	0.26	0.22
Omega-3 PUFAs						
Eicosapentaenoic acid	0.18	0.24	0.33	0.12	0.18	0.42
Docosapentaenoic acid	0.19	0.22	0.20	0.36	0.24	0.27
Docosahexaenoic acid	0.28	0.06	0.20	0.35	0.40	0.06
Total n-3 PUFAs	0.23	0.13	0.29	0.17	0.27	0.21
Omega-3 index	0.26	0.08	0.28	0.20	0.37	0.08
Omega-6/omega-3 ratio	−0.28	0.06	−0.36	0.09	−0.28	0.19

Cancer-related fatigue was assessed using the Cancer Fatigue Scale.
The exercise group underwent home-based smartphone-supported high-intensity interval training using bodyweight three times a week for 12 weeks. The control group received treatment as usual.
r_s, Spearman's rank correlation coefficient.
PUFAs, Polyunsaturated fatty acids.

reduced CRF compared with omega-3 PUFA supplementation and that omega-6 PUFA supplementation reduced pro-inflammatory serum markers in fatigued American breast cancer survivors. Meanwhile, an earlier large-scale cross-sectional study found that a higher intake of omega-6 PUFAs relative to omega-3 PUFAs was associated with 1.8 times greater C-reactive protein and 2.6 times greater odds of CRF in 633 American breast cancer survivors (Alfano et al., 2012). Strikingly, the findings of these two studies are diametrically opposed, though we must keep in mind the differences in their designs. A weakness of the trial performed by Peppone et al. was that the above result was significant for only a single-item screening question but not for standard CRF measures. Furthermore, caution is needed in extrapolating the results of Americans, who consume a large amount of omega-6 PUFAs and a small amount of omega-3 PUFAs, to the population with high fish consumption.

A recent meta-analysis of individual patient data revealed significant benefits of exercise interventions on CRF (van Vulpen et al., 2020). Ochi revealed the effect of omega-3 PUFAs on improving muscle endurance, inflammatory reaction, and delayed onset muscle soreness (Ochi and Tsuchiya, 2018). We are working on clinical research to improve physical fitness and reduce CRF in breast cancer survivors through exercise, and we are also interested in analyzing whether PUFA balance in the body affects the efficacy of exercise on CRF and muscle strength.

Recently, we, in our randomized controlled trial among early-stage Japanese breast cancer survivors (Ochi et al., 2021), examined the effect of baseline blood PUFA characteristics on change in CRF assessed by the Cancer Fatigue Scale (CFS), which was designed to reflect the nature of fatigue experienced by cancer patients (Okuyama et al., 2000), during the 12 week trial by the exercise group (**Table 1**). Elevated CRF, defined as an increase in CFS between baseline and 12 weeks, was associated with both docosahexaenoic acid (DHA; Spearman's rank correlation $r_s = 0.28$, $p = 0.06$) and omega-3 index ($r_s = 0.26$, $p = 0.08$) at baseline in all participants ($n = 46$, omega-6/omega-3 ratio = 6.79, SD = 1.90). On the contrary, DHA at baseline was positively correlated with change in CRF ($r = 0.40$, $p = 0.06$) in the control group ($n = 24$, omega-6/omega-3 ratio = 7.0). Moreover, eicosapentaenoic acid (EPA) at baseline was positively correlated with leg strength ($r = 0.39$, $p = 0.10$) in the exercise group. In our phase II trial, the associations between PUFAs and CRF were not significant, but these trends were consistent with the findings of the trial performed by Peppone et al. We speculate that blood PUFA balance might be associated with the effect of exercise on CRF, and this effect might be clearer in cancer survivors. In addition, higher EPA in individuals who conducted exercise likely has a beneficial effect on muscle strength.

Considering the trial performed by Peppone et al. together with ours, omega-6 PUFAs might be beneficial for reducing CRF, while omega-3 PUFAs might have no benefit regardless of fish-eating habits. As no gold-standard treatment for CRF has been established, further investigation is needed to clarify the interaction between PUFAs and exercise for alleviating CRF. Self-management such as dietary modification and exercise must be both effective and easy to be an acceptable solution in cancer survivorship care.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the National Cancer

Center Japan on 28 February 2019 (No: 2018–274). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YM and EO conceived and designed the study. YM, KT, and EO drafted the main text and table. All authors reviewed the manuscript, contributed to the acquisition, analysis, and interpretation of data, and approved the completed version of the manuscript.

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ORIGINAL RESEARCH
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Methylation and Expression of the Exercise-Related TLR1 Gene Is Associated With Low Grade Glioma Prognosis and Outcome

Lichun Lu[1†], *Yifang Hu*[2†], *Chen Wang*[1†], *Feng Jiang*[3*] and *Chuyan Wu*[4*]

[1]Department of Neurology, The Affiliated Suzhou Science & Technology Town Hospital of Nanjing Medical University, Suzhou, China, [2]Department of Geriatrics, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China, [3]Department of Neonatology, Obstetrics and Gynecology Hospital of Fudan University, Shanghai, China, [4]Department of Rehabilitation Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

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Edited by:
Hang Fai Kwok,
University of Macau, China

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Qingshui Wang,
Fujian Normal University, China
Vikas Singh,
Rockefeller University, United States

***Correspondence:**
Chuyan Wu
chuyan_w@hotmail.com
Feng Jiang
dxyjiang@163.com

[†]These authors have contributed equally to this work

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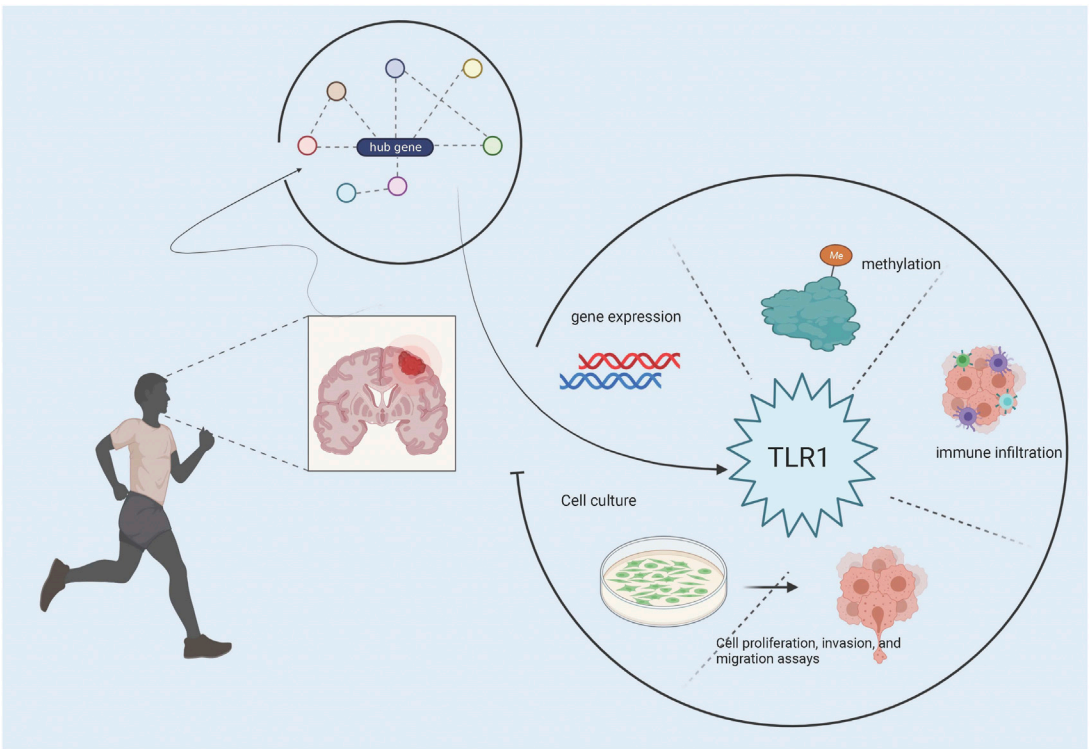
Background: Exercise improves function, reduces disability, maintains independence, and improves quality of life for low-grade glioma (LGG) patients. Exercise can also improve the effectiveness of cancer treatment. The goal of this research was to find potential exercise related genes that may be used to predict exercise levels and may be used as a biomarker for cancer outcomes.

Methods: The GSE111551 database was thoroughly examined in this research, and the resulting conclusion of exercise-related genes was reached. The protein interaction network (PPI) was used to examine the differentially expressed genes (DEGs). Then the exercise-related gene TLR1 was chosen. The expression, methylation degree, prognosis, and immune relevance of TLR1 were investigated using bioinformatics. In addition, we verified the role of TLR1 in Glioma cell lines.

Results: LGG patients with reduced TLR1 expression and hypermethylation had a better overall survival (OS) and progression free survival (PFS), using the TCGA database. Low TLR1 expression and hypermethylation of TLR1 were found to be independent biomarkers for OS using Cox regression. Furthermore, the CGGA database was used to confirm the prognostic function of TLR1 in this cancer. Finally, most methylation sites of TLR1 were strongly correlated with immune infiltration and immune checkpoint. Then, reducing TLR1 expression substantially slowed the cell cycle and decreased LGG cell proliferation, emigration, and infiltration *in vitro*.

Conclusions: Exercise-related gene TLR1 has the potential to be a useful prognostic biomarker, and it is thought to be involved in immune cell infiltration and immunotherapy in LGG.

Keywords: exercise-related genes, methylation, prognosis, immune cell infiltration, low grade glioma



GRAPHICAL ABSTRACT | Exercise-related TLR1 gene plays a certain role in the prognosis judgment and immunotherapy of LGG.

INTRODUCTION

Tumors derived from the neuroepithelium are called gliomas, accounting for 29% of the central nervous system cancers, which is the most common primary intracranial tumor (Du et al., 2021). The most prevalent identified malignant cancer in the brain is low-grade glioma, which has a large degree of inherent heterogeneity in terms of tumor biological conduct (Ostrom et al., 2013). Despite extensive treatment for LGG, which includes neurosurgical resection, chemotherapy, and radiotherapy, therapeutic resistance and tumor recurrence appear to be unavoidable (Cancer Genome Atlas Research Network et al., 2015). Any LGG patients are slow to improve, whereas others develop high-grade glioblastoma, which has a poor prognosis (Tan et al., 2020).

Exercise has a strong theoretical basis for being an effective intervention for managing symptoms of brain cancer and treating side effects (Schmidt et al., 2015; Nelson, 2016). Currently, animal experiments and clinical experiments have confirmed the encouraging relationship between appropriate physical exercise and improving the survival results of patients with malignant tumors of the nervous systemanimal (Carson et al., 2007; Wu et al., 2010; Cormie et al., 2015; Tantillo et al., 2020). As a consequence, the voluntary physical exercise program can be used in the therapeutic environment as an auxiliary and non-invasive therapy for glioma, enhancing patient's quality of life. In other cancer patients and chronic patients, clinical studies also have confirmed the efficacy of appropriate exercise in combating physical impairment, cognitive impairment, and psychological effects such as depression and anxiety (Cormie et al., 2015). However, more research is needed to determine the best exercise treatment plan for these patient's specific needs in order to improve protection and efficacy. When recommended and monitored properly, the possible rehabilitation impact of selected exercise intervention in neurooncology is impressive, particularly because exercise is a reasonably inexpensive and simply to access medication with little adverse side effects.

Toll-like receptors (TLRs) are a kind of non-specific immunity (innate immunity) protein molecule that also serves as a link between non-specific and specific immunity. The protein encoded by TLR1 gene is a member of the Toll-like receptor (TLR) family, which is widely expressed at higher levels than other TLR genes. TLR1 was identified as a TLR member that recognizes triacyl lipopetides; in TLR1 knockout mice, macrophages produced less inflammatory cytokine in response to triacyl lipopetides and lipoproteins from mycobacteria (Yu et al., 2021). It was verified in an animal experiment that TLR1 is expressed in mouse brain neurons, astrocytes, and microglia and may be implicated in the development of epilepsy (Wang et al., 2015). The link between TLR1 and exercise has been studied in a limited number of studies. TLR1 expression was considerably lower after post-acute exercise and after 2 h recovery compared to samples obtained at rest, according to relevant studies (Lancaster et al., 2005; Gleeson et al., 2006). However, studies on the link between long-term exercise and TLR1 expression are currently lacking.

To find genes associated with exercise, we used the GSE111551 dataset from the Gene Expression Omnibus (GEO) database (Hao et al., 2021). We discovered genes that are differentially expressed after exercise. The gene expression level, methylation level, and clinicopathological features of TLR1 were investigated using the Cancer Genome Atlas (TCGA) database. Our findings indicate that TLR1, which is linked to exercise, can play a role in the production and incidence of LGG. It maybe plays a certain role in the prognosis judgment and immunotherapy therapy of gliomas (**Graphic Abstract** and **Figure 1**).

MATERIALS AND METHODS

Processing of Data and Network of Protein-Protein Interactions Analysis

The GEO database was used to obtain GSE111551. The transcriptional histograms of 13 healthy male subject's before and after exercise (running, 3 times/wk, 60min, 18w) were found in the database. LGG patient's clinical information and transcriptional profiles were then downloaded from the TCGA (Gao et al., 2013) and CGGA database (Hu et al., 2018) (http://www.cgga.org.cn/). The protein-protein interaction (PPI) network was utilized to find key genes and gene modules associated with exercise. The Search Tool for the Retrieval of Interacting Genes (STRING) database was used to build the PPI network (http://www.stringdb.org/). Then, using the online resource Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia. cancer-pku. cn/index.html), we evaluated the differences in TLR1 mRNA expression in normal and LGG tissues (Tang et al., 2017).

Methylation Site Analysis and Correlation Comparison With Expression

We downloaded the methylation profiles of patients with LGG from TCGA database via the Cbioportal website (https://www.cbioportal.org/). We analyzed the CpG sites of TLR1 gene. Then, using Pearson correlation analysis, the TLR1 CpG sites whose methylation was most significantly associated with TLR1 mRNA expression were identified.

Survival Analysis of Gene Expression and Methylation Sites Using TCGA-Low-Grade Glioma Dataset

The Kaplan-Meier survival analysis was used to demonstrate the survival link between the high-risk and low-risk groups. In the experiment, the gene expression and prognosis of different groups were examined using heatmap and scatter dot plot. We evaluated the ROC curve's predictive abilities in order to determine the prediction's accuracy. Correlation analysis was used to establish relationships between the risk score and the patient's clinicopathological variables. To validate the independent prediction model, we have utilized univariate and multivariate Cox regression analyses.

Validation of Prognostic Significance in CGGA and Creation of a Projected Nomogram

We used univariate and multivariate Cox regression analysis to verify the prediction effect in CGGA database. In order to provide a valid clinical standard measure for LGG patients in terms of 1-, 3-, and 5-years survival, a nomogram based on risk values as well as other clinicopathological characteristics was developed. The calibration curves were then utilized for assessing the concordance between patients anticipated and observed.

Relationship Between Gene Expression, Methylation Level and Immune Infiltration

CIBERSORT(Newman et al., 2015; Charoentong et al., 2017), ESTIMATE (Yoshihara et al., 2013), MCPcounter (Shi et al., 2020), TIMER algorithms (Li et al., 2017), QUANTISEQ (Finotello et al., 2019), EPIC (Racle et al., 2017), and XCELL (Aran et al., 2017) were evaluated to measure cellular function or immune effects between low and high risk groups according to TLR1 expression. A Heatmap was used to reveal variations in immune reaction under various algorithms. Furthermore, single-sample gene set enrichment analysis (ssGSEA) (Yi et al., 2020) was utilized to compare and identify the tumor-infiltrating immune cell subsets. Previous literature also yielded a possible immunological check-point.

Biological Process Analysis

The TCGA dataset had two types of glioma patients: those with belevated TLR1 expression and those with low expression. With a false discovery rate of less than 0.05, the genes that varied in expression between the two groups were selected. Gene terms with a p value less than 0.01 and $|\log FC| \geq 1$ were deemed important. The role of TLR1 in glioma was then investigated using GO analysis.

Cell Culture and Transfection

Glioma cell lines (NHA, A172, U87, U251, T98, LN229) were acquired from the cell bank of the Chinese Academy of Sciences (Shanghai). RPMI 1640 (Gibco, Gaithersburg, MD, United States) was cultured with 10% fetal bovine serum (HyClone, Logan, United States) and 1% penicillin/streptomycin (Gibco) in an incubator at 37°C and 5% CO_2. Transfections were performed applying OPTI-MEM (Invitrogen) and Lipofectamine 3000 by the manufacturer's instructions. The siTLR1-1 (5′- AACACAACTAACTACAGATTA CA -3′), siTLR1-2 (5′-ACUGAUAUCAAGAUACUGGAT-3′) and siNC were bought from Tsingke (Nanjing, China) and introduced into cells at a concentration of 50 nM. The transfected cells were harvested at 24 h after transfection.

Cell Proliferation, Invasion, and Migration Assays

The Cell Counting Kit-8 (CCK-8) and colony formation assays were applied to explore the ability of proliferation of cancer cells in different groups. In CCK-8 experiment, a total of 2,500 cancer cells

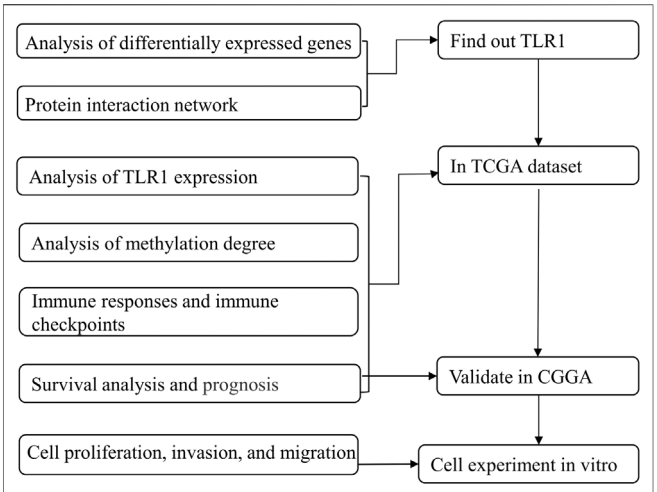


FIGURE 1 | Flow progress diagram.

were added into each well of 96-well plate. 10μL of CCK-8 solution (Dojindo Laboratiories, Kumamoto, Japan) was added into 96-well, then the absorbance of each well was analyzed at 450 nm after an incubation at 37°C for 2 h. For colony formation experiment, 1,000 cells of different groups were added into each well of a six-well plate. The culture medium was changed every 72 h. Crystal violet and 4% paraformaldehyde were applied to stain and fix the cells when the appearance of colonies could be recognized. The wound healing and transwell assays were applied to explore the ability of cellular migration and invasion.

Western Blotting Analysis

After being washed using PBS, the cells were lysed with RIPA (radioimmunoprecipitation assay) solution containing a protease inhibitor. The acquired proteins were then quantified using a bicinchoninic acid protein assay (BCA) kit, after which 20 mg of total protein was separated on 8% or 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) under electric field. The separated proteins were blotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore, United States), which was then blocked with 5% bovine serum albumin (BSA) solution for 1 h and incubated with primary antibodies against GAPDH (1:1,000, Proteintech, United States), RBM15 (1:1,000, Proteintech, United States), METTL14 (1:1,000, Proteintech, United States), PDCD1 (1:2,000, Proteintech Group, United States), CTLA4 (1:1,000, novus biotechnologies, United States), HNRNRC (1:1,000, abcam, United States), and BTLA (1:1,000, abcam, United States) overnight at 4°C. On the second day, the membrane was incubated with secondary antibodies (1:2,500) at room temperature for 1 h after three times of washes with TBST (Tris-buffered saline-Tween). After being washed for another three times, bands of conjugate proteins were visualized via a ChemiDoc™ MP Imaging system (Bio-Rad Laboratories) with GADPH as the internal control.

Statistical Analysis

The value of the risk parameter was calculated using Kaplan-Meier survival curves. To see whether the risk factors were independent of clinical variables including age, gender, grade, and mutation, researchers used multivariate cox analysis and stratified data analysis. $p < 0.05$ was found statistically important. For all statistical studies, R software (v3.6.1) was utilized.

RESULTS

Identification of Exercise-Related Gene TLR1 of PPI Network

Twenty-six transcriptome data subsets were collected from the GSE111551 database. The database was used to examine the baseline and exercise groups' mRNA expression levels. There were 958 differentially expressed genes (DEGs) between the baseline and after exercise groups (**Figure 2A**). TLR1 was found to be down-regulated among them. Then we further made the difference of TLR1 between the two groups. Wilcox test study revealed a large differential between the two classes, with TLR1 expression decreasing dramatically in the exercise community (**Figure 2B**). Then we selected 2,628 survival differentially expressed genes from TCGA-LGG database and 131 differentially expressed genes from the intersection of the two genes (**Figure 2C**). Using the String database, a PPI network of differentially expressed genes was developed. 131 nodes are included in this network and may play a crucial role in aerobic exercise (**Figure 2D**). The first 10 genes were chosen by Cytohubba plugin (Cao et al., 2018) (**Supplementary Figure S1**) and sequentially ordered as follow: TLR1, SIRT1, CCNA2, HIST1H2AC, EED, SCD, ELAVL1, ACADVL, NIP7, UCP2. We selected the gene TLR1 at the core of the network.

TLR1 Expression and Methylation in Low-Grade Glioma

With GEPIA, we studied the RNA-sequencing results of 518 LGG tissues from TCGA and 207 normal samples from the GTEx project and discovered that TLR1 mRNA was strongly expressed in LGG tissues but not in normal tissues (**Figure 3A**). We found a negative association ($r = 0.17$, $p < 0.0001$) between TLR1 expression and TLR1 DNA methylation, as seen in (**Figure 3B**). **Figure 3C** clearly shows the distribution of eight TLR1 CpG locations. Then, using Pearson correlation analysis, the TLR1 CpG sites where methylation was highest closely associated with TLR1 mRNA expression were identified (**Figures 3D–K**).

The Therapeutic and Prognostic Importance of TLR1 Expression According to TCGA Site

According to a Kaplan-Meier survival study, LGG patients with elevated TLR1 expression had a lower overall survival (OS) and disease-free survival (DFS) period (**Figures 4A,B**). Furthermore, patients with elevated TLR1 expression had shorter progression free survival (PFS) than those with poor TLR1 expression (**Figure 4C**).

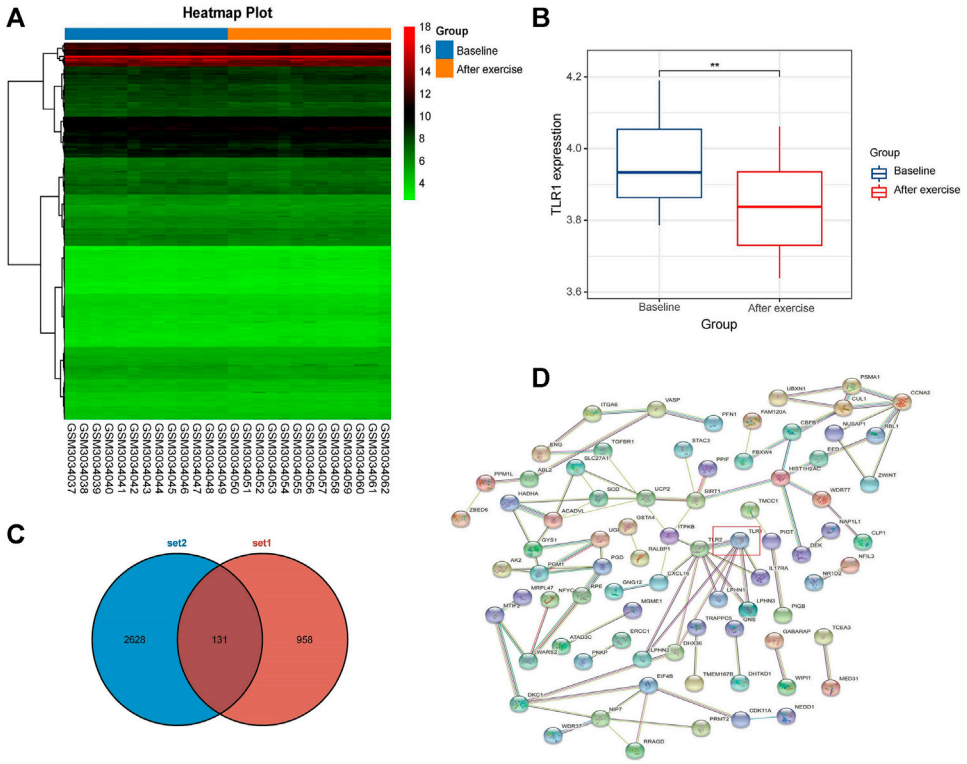


FIGURE 2 | (A) DEGs hierarchical cluster analysis between baseline and post training groups in GSE111551 database. 958 DEGs related to exercise were identified. Red, up-regulated genes, blue, down-regulated genes **(B)** The expression of TLR1 in exercise group **(C)** Venn map of two gene sets **(D)** 131 exercise-related DEGs were included in PPI network. The nodes indicated proteins. The edges represented protein's interaction.

Taking cg09316306 as an example, elevated amounts of methylation at the selected CpG sites were also correlated with a stronger OS and PFS in LGG patients, according to Kaplan-Meier plots (**Figures 4D–F**). The relationship between other CpG sites and survival can be seen in **Supplementary Figure S2**. The LGG patients in the TCGA were then classified into low and high subgroups based on the median TLR1 expression or methylation value. The chi-square test was used to investigate the specific association of TLR1 expression and methylation with a set of clinical characteristics. TLR1 expression was found to be highly associated with age (p = 0.0263) and grade (p < 0.0001), as seen in **Table 1**. Similarly, age (p = 0.0061), grade (p < 0.0001) and TLR1 expression (p = 0.0166) had an impact on the degree of TLR1 methylation.

TLR1's Prognostic Significance Validated in Low-Grade Glioma From CGGA

To confirm the prognostic importance of TLR1, we downloaded RNA-seq details and clinical data of 1,008 patients through CGGA. To validate TLR1's prognostic importance, we used Kaplan-Meier research, which revealed that high TLR1 expression predicted low OS. We used univariate and multivariate Cox regression analysis to further validate TLR1's independent prognostic significance (**Figures 5A,B**). TLR1, as seen in the forest diagram, maybe an independent prognostic biomarker (p = 0.019, hazard ratio = 1.232 (1.035–1.467)). In CGGA, PRS type (p = 0.022, hazard ratio = 3.616 (1.203–10.872), grade (p < 0.001, hazard ratio = 2.255 (1.637–3.106), 1p19q codeletion (p < 0.001, hazard ratio = 0.371 (0.218–0.630), and IDH mutation (p = 0.006, hazard ratio = 0.641 (0.468–0.878) could all be separate prognostic variables. According to a Kaplan-Meier survival study, LGG patients with elevated TRL1 expression had shorter overall survival (OS) (**Figure 5C**).

We created a nomogram to develop a quantitative tool for LGG prognosis (**Figure 5D**). Specific variables were assigned points using the point scale in the nomogram based on the multivariate Cox study. We quantify the cumulative points for each patient by summing the points of all variables and normalizing them to distribution of 0–100 by drawing a horizontal line to decide the value of each variable. By drawing a longitudinal line between the complete point axis and each prognosis axis, we may quantify the average survival rates for LGG patients at 1, 3, and 5 years, which can aid appropriate clinicians in developing clinical decision making for LGG patients.

Examining TLR1's Interactions With Immune Cell Infiltration

Figure 6A shows a heatmap of immunological responses depending on the algorithms. According to ssGSEA of TCGA-LGG data, correlation analysis of immune cell subtypes and associated activities showed that T cell functions such as cytolytic, check-point,

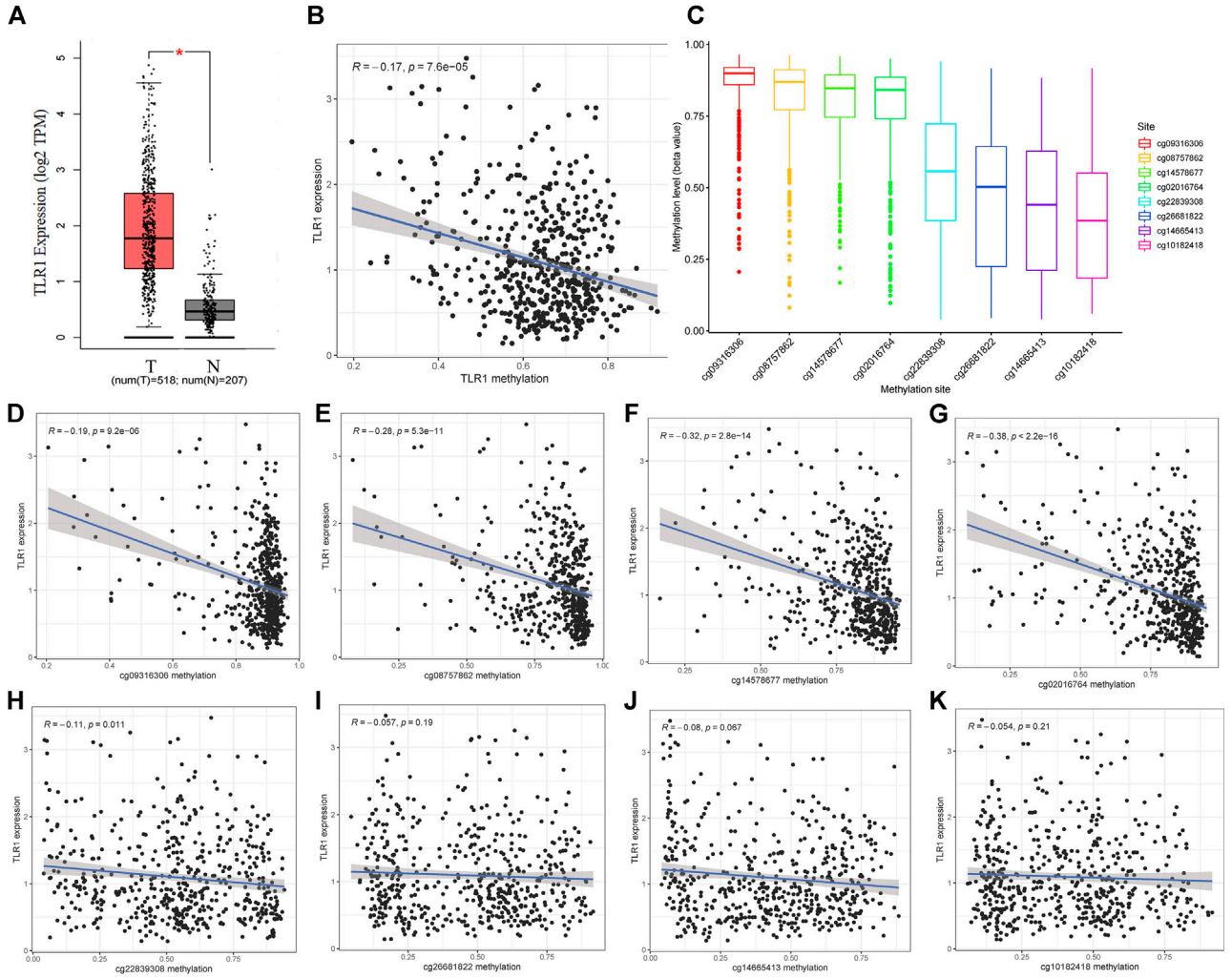


FIGURE 3 | The expression and methylation of TLR1 in LGG tissues and normal tissues revealed by bioinformatic analysis. **(A)** TLR1 mRNA is strongly expressed in LGG tissues in TCGA dataset. **(B)** The expression of TLR1 was negatively regulated by DNA methylation. **(C)** The distribution of 8 TLR1 DNA promoter CpG sites. **(D–K)** Correlation analysis ofTLR1 CpG sites and TLR1 mRNA expression.

TABLE 1 | Correlation between TLR1 mRNA expression/methylation and clinicopathologic features in TCGA database.

Covariates	Type	Total	ARID5A expression		P value	ARID5A methylation		P value
			High	Low		High	Low	
Age	<40	260 (49.34%)	143 (54.37%)	117 (44.32%)	0.0263	146 (55.51%)	114 (43.18%)	0.0061
	≥40	267 (50.66%)	120 (45.63%)	147 (55.68%)		117 (44.49%)	150 (56.82%)	
Grade	G2	260 (49.34%)	102 (38.78%)	158 (59.85%)	<0.0001	158 (60.08%)	102 (38.64%)	<0.0001
	G3	267 (50.66%)	161 (61.22%)	106 (40.15%)		105 (39.92%)	162 (61.36%)	
Gender	female	239 (45.35%)	109 (41.44%)	130 (49.24%)	0.0872	111 (42.21%)	128 (48.48%)	0.1737
	male	288 (54.65%)	154 (58.56%)	134 (50.76%)		152 (57.79%)	136 (51.52%)	
expression	High	263 (49.91%)	263 (100%)	0 (0%)	0	117 (44.49%)	146 (55.3%)	0.0166
	Low	264 (50.09%)	0 (0%)	264 (100%)		146 (55.51%)	118 (44.7%)	
methylation	High	263 (49.91%)	117 (44.49%)	146 (55.3%)	0.0166	263 (100%)	0 (0%)	0
	Low	264 (50.09%)	146 (55.51%)	118 (44.7%)		0 (0%)	264 (100%)	

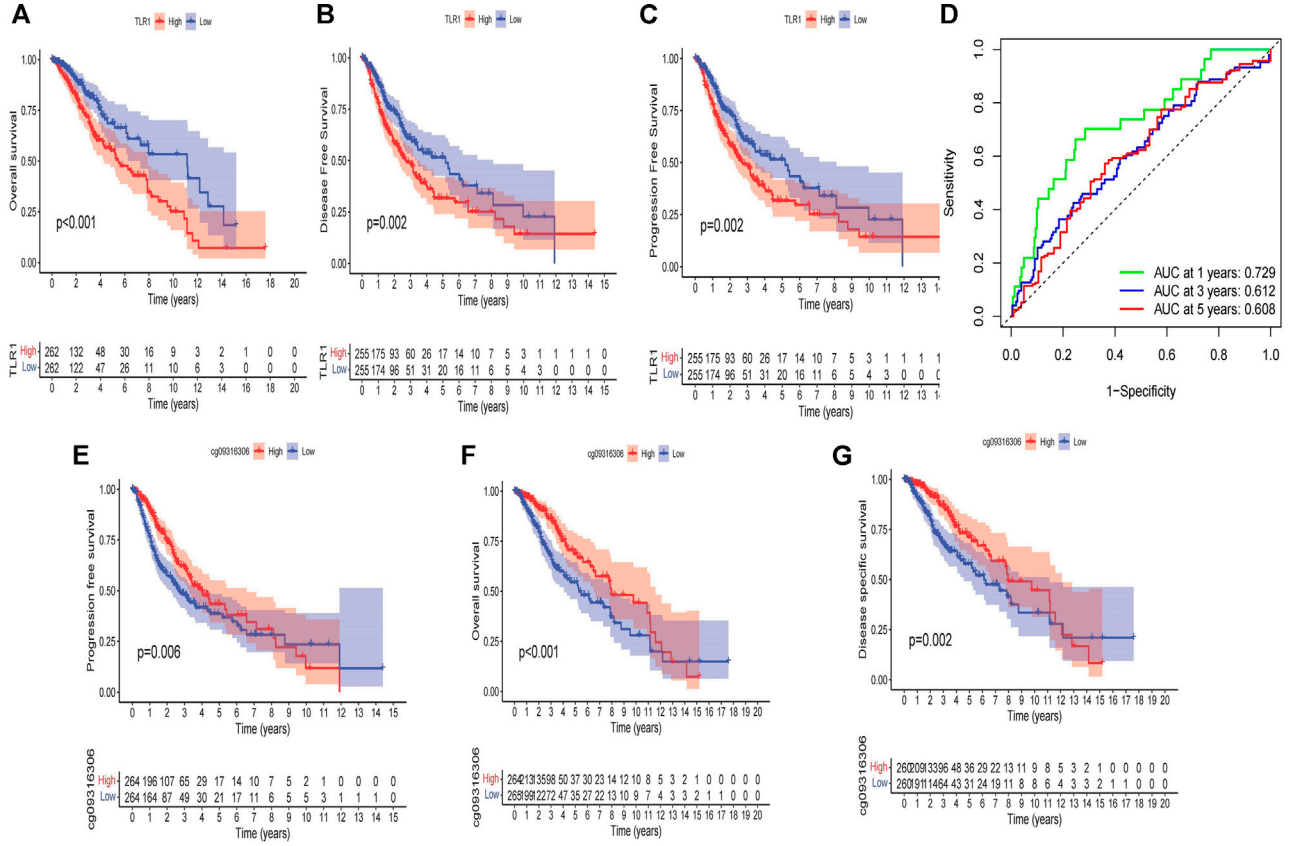


FIGURE 4 | Kaplan-Meier curves. **(A)** over survival in all patients, grouped by low and high TLR1 expression. **(B)** disease-free survival in all patients, grouped by low and high TLR1 expression. **(C)** progression free survival in all patients, grouped by low and high TLR1 expression. **(D)** AUC values for predicting LGG survival rates at 1, 3, and 5 years. **(E)** progression free survival in all patients, grouped by low and high methylation level of cg09316306 site. **(F)** over survival in all patients, grouped by low and high methylation level of cg09316306 site. **(G)** disease-specific survival in all patients, grouped by low and high methylation level of cg09316306 site.

MHC class1, co-inhibition, and co-stimulation were substantially different between the two groups **Figure 6B**. Because checkpoint inhibitor-based immunotherapies are so important, we looked at the differences in immune checkpoint expression between the two groups. Between the two groups of patients, we discovered a significant variation in the expression of PDCD1, CTLA4, and BTLA (**Figure 6C**). There were significant variations in the expression of 5 methyltransferases such as HNRNPC, METTL14, and RMB15 when the m^6A expression-related mRNA was compared between the two groups (**Figure 6D**).

Interaction Between TLR1 Methylation Site and Immune Cell Infiltration

The methylation site cg09316306 was taken as an example. **Figure 7A** shows a heatmap of immunological responses depending on the algorithms. According to ssGSEA, correlation analysis of immune cell subtypes and associated activities showed that T cell functions such as cytolytic, check-point, MHC class1, co-inhibition, and co-stimulation were all substantially different between the two groups (**Figure 7B**). We then looked at the differences in immune checkpoint expression between the two groups. Between the two groups of patients, we discovered a significant variation in the expression of PDCD1, CTLA4, BTLA, and VTCN1 (**Figure 7C**). There were significant variations in the expression of 9 methyltransferases such as HNRNPC, METTL14, and RMB15 when the m^6A expression-related mRNA was compared between the high and low methylation levels groups (**Figure 7D**). Similarly, we also analyzed the relationship between the remaining 7 CpG sites and immune invasion. The results showed that 3 methylation CpG sites had no significant correlation with immune checkpoint (**Supplementary Figure S3**).

Analysis of TLR1-Related Biological Processes

GO enrichment analysis revealed that TLR1 is involved in pathways of oxidative stress, chemical stress, reactive oxygen species metabolic process, cellular response to oxidative stress, response to ketone, response to corticosteroid, response to a steroid hormone, response to the metal ion, unsaturated fatty acid metabolic process and fatty acid metabolic process among others (**Figure 8**). It is worth noting that in these numerous biological processes, Oxidative stress can cause DNA

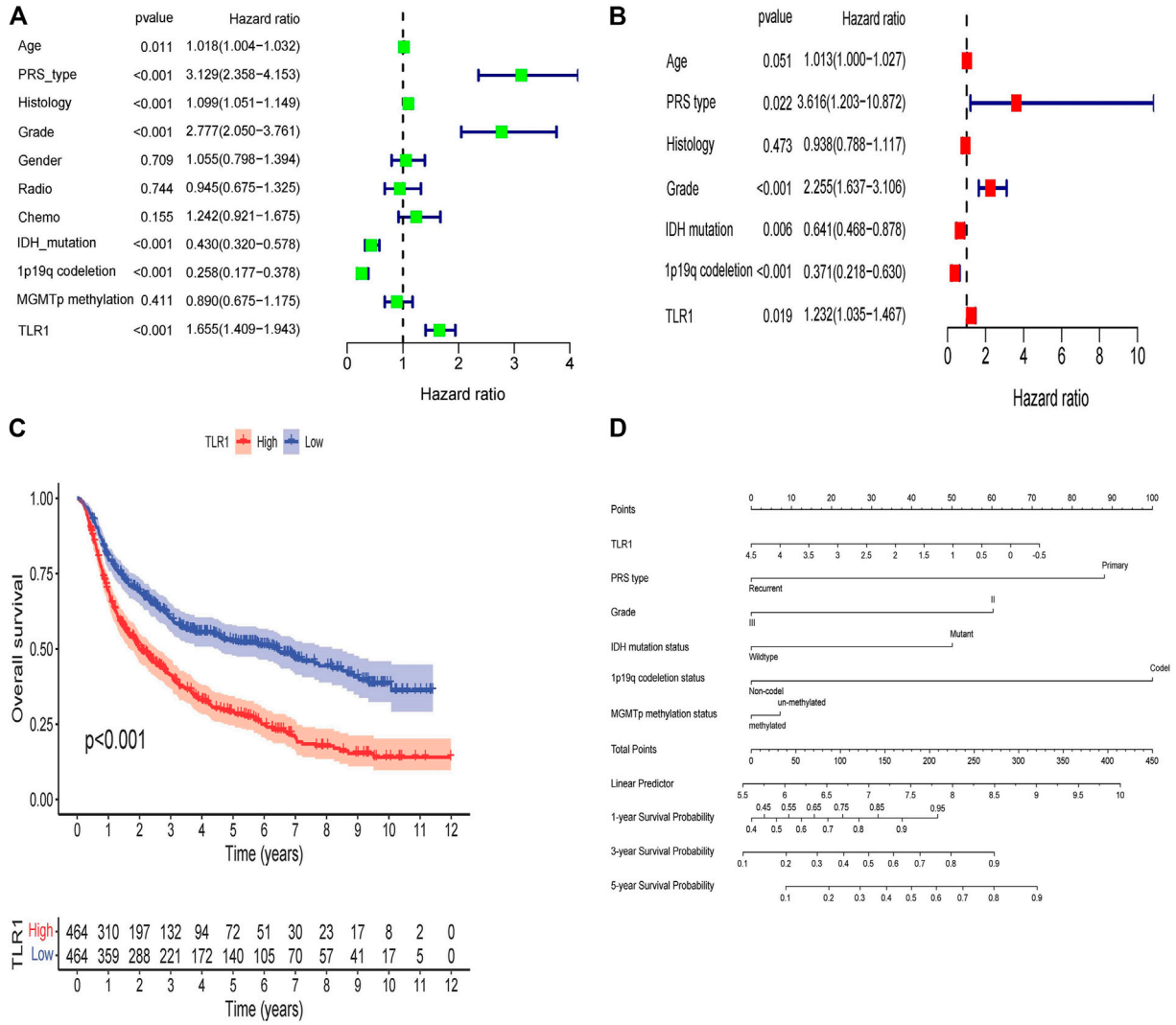


FIGURE 5 | TLR1's prognostic significance validated in LGG from CGGA. **(A)** The univariate and **(B)** multivariate Cox regression in LGG from CGGA. **(C)** TLR1 expression and overall survival in LGG patients in CGGA cohort. **(D)** Nomogram for predicting 1-, 3-, and 5-years OS of LGG patients in the CGGA cohort.

base changes, strand breaks, increased expression of proto-oncogenes, and inactivation of tumor suppressor genes. It is linked to the onset and progression of numerous cancers.

Knockdown of TLR1 Suppresses the Malignant Phenotype of Glioma *in vitro*

To explore the role of TLR1 in glioma, the expression of TLR1 in the six glioma cell lines (NHA, A172, U87, U251, T98, LN229) was analyzed respectively. The expression of TLR1 in the U251 cell line was higher than in other cell lines (**Figure 9A**). Then, the U251 cell line was selected for functional analysis. Using qRT-PCR, we have detected the efficiency of TLR1-siRNA (**Figure 9B**). The results of colony formation and CCK8 experiments demonstrated that lower expression of TLR1 significantly inhibited the ability of proliferation and colony formation of U251 cells (**Figures 9C,D**). The results of the transwell experiment indicated that the U251 cells exhibited significantly decreased invasion ability upon TLR1 knockdown (**Figure 9E**). The results of the wound healing experiment indicated that knockdown of TLR1 significantly inhibited the migration of U251 cells (**Figure 9F**). We measured the expression of PDCD1, CTLA4, BTLA and methyltransferases HNRNPC, METTL14 and RMB 15 in U251 cells with and without siTLR1-1, respectively. We found that the expression of six proteins in siTLR1-1 decreased (**Supplementary Figure S4**). These findings showed that TLR1 may be a glioma promoter. To uncover the underlying processes, further study is required.

DISCUSSION

Medium-intensity and rhythmic activity, which is marked by low intensity, speed, and long length, is referred to as aerobic exercise (Dahamsheh et al., 2019). The GEO data set selected in this study

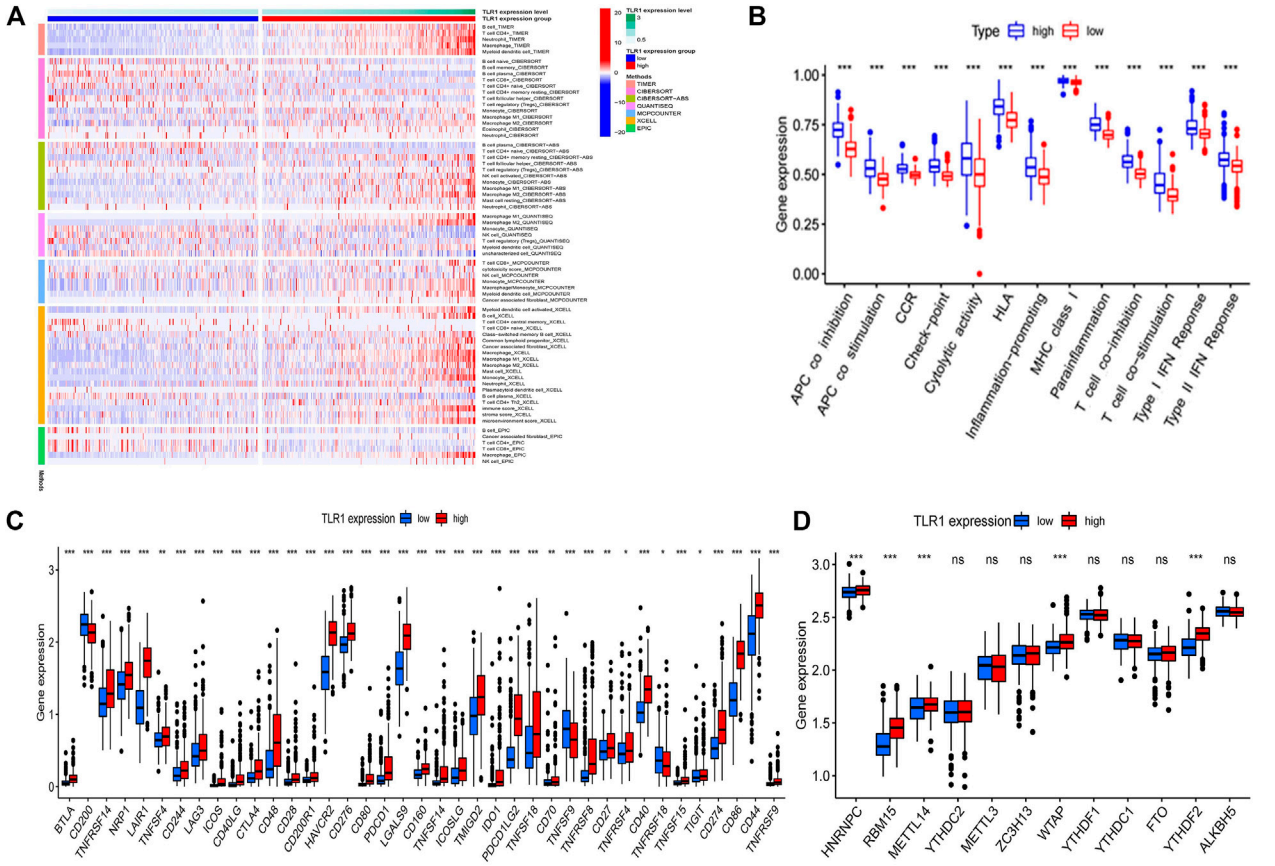


FIGURE 6 | The Correlation of TLR1 expression with immune infiltration level in LGG. **(A)** the heatmap of immune responses based on different algorithms. **(B)** ssGSEA for the association between immune cell subpopulations and related functions. **(C)** immune checkpoints expression among the high and low groups. **(D)** The expression of m6A-related genes between high and low TLR1 risk group.

adopts the way of running. This type of exercise uses oxygen to fully burn sugar in the body while still consuming body fat, improving cardiopulmonary function, preventing osteoporosis, and regulating psychological and mental state (Santos et al., 2017). Epidemiological data show that exercise can prevent the occurrence of some cancers and reduce the risk of disease recurrence (Schmidt et al., 2015), which promotes extensive research on exercise intervention for cancer patients.

Epigenetics is the study of how non-gene sequence changes affect gene expression levels (Zhang et al., 2016). Methylation is the most stable epigenetic mechanism, and epigenetic modification also plays an important role in exercise adaptation. A large number of studies have confirmed the interaction between exercise and epigenetics (Liang et al., 2021). Recently, more and more attention has been paid to the application of exercise in tumor diseases. We found the difference gene TLR1 between baseline group and post training group in the data set, and TLR1 was differentially expressed in low-grade glioma and normal population in TCGA data set. In LGG tissues, TLR1 mRNA expression was negatively associated with the methylation of TLR1. We discovered that TLR1 expression and methylation were linked to several important characteristics, such as histological and molecular forms. The importance of TLR1 hypermethylation and low expression in LGG patient's positive prognosis was supported by a Cox regression model.

A significant number of studies have shown that irregular DNA methylation is essential in the onset and development of LGG (Mathur et al., 2020). In this study, we confirmed that TLR1 methylation status affects the expression of TLR1mRNA. TLR1 methylation was negatively correlated with TLR1 mRNA expression in LGG tissues. TLR1's low expression in LGG tissues may be explained by this negative association. Then, we found The methylation of five CpG sites in the DNA promoter was closely related to TLR1 mRNA expression. We also looked into the function of TLR1 DNA methylation and CpG sites locus in prognosis. We found that hypermethylation of TLR1 at this locus was corrected with good OS and improved PFS in LGG, and we further verified it by multivariate regression. As a result of the methylation of CpG sites, TLR1 is negatively controlled, and the methylation status of TLR1 can be an important predictor of OS and PFS.

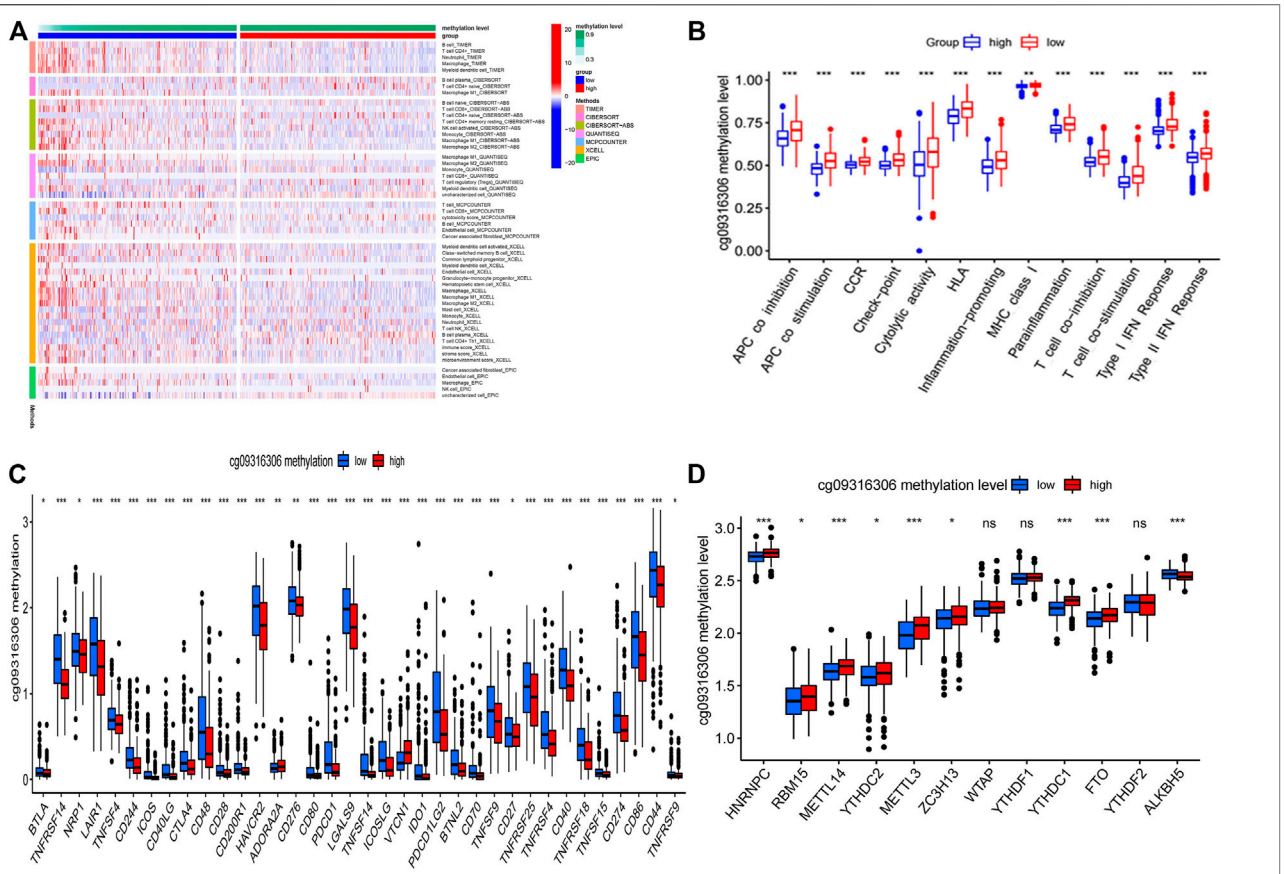


FIGURE 7 | The Correlation of TLR1 methylation site cg09316306 and immune cell infiltration. **(A)** the heatmap of immune responses based on different algorithms. **(B)** ssGSEA for the association between immune cell subpopulations and related functions. **(C)** immune checkpoints expression among the high and low groups. cg09316306 site methylation risk groups. **(D)** The expression of m6A-related genes between high and low cg09316306 site methylation risk group.

Several genes linked to glioma pathogenesis have been discovered, but the prognostic and clinical importance of the TLR1 gene in glioma remains uncertain. In the report of the TLR1 gene, elevated expression of TLR1 is corrected with a good prognosis of pancreatic cancer (Lanki et al., 2019), but it has never been studied in LGG. In this analysis, we used the TCGA database to investigate the connection between low TLR1 expression and survival rates, and we double-checked the findings using the CGGA database. Encouragingly, in LGG patients, low TLR1 expression is highly associated with improved OS than elevated TLR1 expression. Multivariate Cox regression further confirmed this conclusion, suggesting that decreased TLR1 expression is a powerful prognostic factor for OS in LGG patients. TLR1 is a successful biomarker for assessing the prognosis of LGG patients, according to our findings.

One of the most frequent causes of cancer development is chronic local inflammation, which may be caused by a change in the microenvironment (Shacter and Weitzman, 2002). The

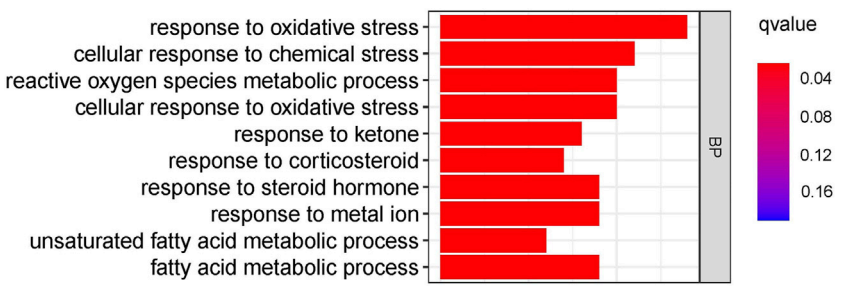


FIGURE 8 | GO enrichment analysis of TLR1-related biological pathways in glioma.

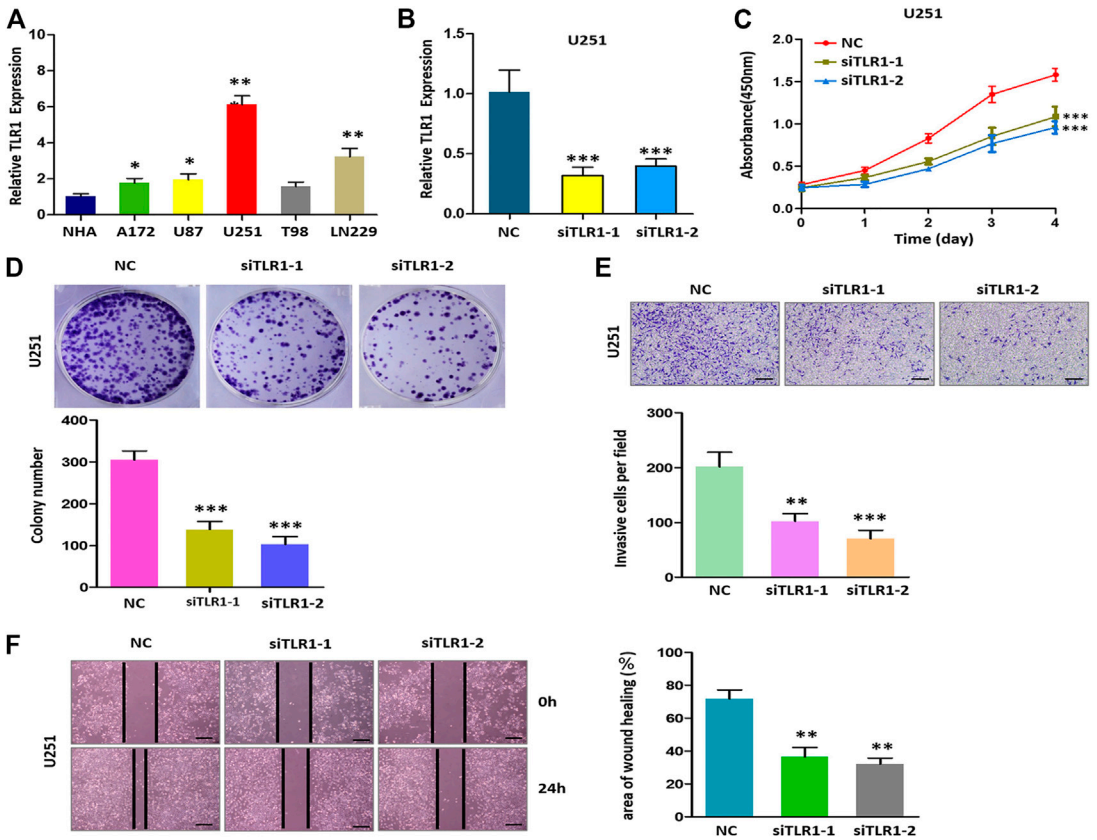


FIGURE 9 | Decreased the expression of TLR1 inhibits proliferation, invasion, and migration of glioma cells *in vitro*. **(A)** Relative expression of TLR1 in 6 cell lines. **(B)** qRT-PCR to detect the relative silencing levels of TLR1 in the U251 cell line. **(C)** The CCK-8 assay was applied to detect the efficiency of TLR1 knockdown on the proliferation of the U251 cell line. **(D)** Images of the colony formation assay after knockdown of TLR1 in U251 cell line. **(E)** Images of the transwell assay results after knockdown of TLR1 in U251 cell line. **(F)** Representational images of the wound healing assay.

function of local inflammation is complex, because some features are conducive to tumor progression, while others can prevent tumor progression (Schulz et al., 2019). The relationship between tumors and inflammation has always been a concern. In recent years, more and more researchers use experimental data to show that tumor-associated inflammation can promote tumor growth and progress by promoting angiogenesis and metastasis, subverting anti-tumor immune response, and changing the sensitivity of tumor cells to chemotherapy drugs (Weenink et al., 2019). Persistent and uncontrollable inflammatory microenvironments can also trigger gene mutation and lead to tumorigenesis (Chauhan and Trivedi, 2020). TLRs, or toll-like receptors, are important mediators of local inflammation. They identify foreign molecules and activate the NF-B pathway, causing inflammatory cytokines and interferon-gamma to be released (Li et al., 2010; O'Neill et al., 2013). The finding that TLR1 expression correlates with immune cell markers suggests that TLR1 can regulate tumor immunity in LGG. In addition, we found that 5 of the 8 methylation sites were closely related to the immune checkpoint. At the same time, it opens a new way for the immunotherapy of LGG. Furthermore, reducing TLR1 expression substantially slowed the cell cycle and decreased LGG cell proliferation, emigration, and infiltration *in vitro*.

Our review has certain inherent limits. First of all, because there are relatively few studies on exercise in cancer patients, we screened the exercise genes from normal people. Second, we were unable to establish the predictive role of TLR1 methylation in LGG owing to the limitations of the CGGA database. Third, the TCGA database is the only one that contains PFS content, and the connection between TLR1 expression and PFS cannot be checked in other databases. Finally, while enrichment analysis enabled us to investigate the biological process of TLR1 in gliomas, the comprehensive mechanism of linking TLR1 expression and TLR1 methylation with LGG development needs additional biomedical research. The recent findings, on the other hand, are encouraging and call for further research into discovering potential prognostic biomarkers of LGG.

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.

AUTHOR CONTRIBUTIONS

LL and FJ designed this study. CW and YH analyzed the data and wrote the manuscript. CW collected the data. FJ analyzed the data. CW revised the manuscript. All authors approved the final version for submission.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2021.747933/full#supplementary-material

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Modified Models for Predicting Malignancy Using Ultrasound Characters Have High Accuracy in Thyroid Nodules With Small Size

Shan Jiang[1,†], *Qingji Xie*[1†], *Nan Li*[1]*, *Haizhen Chen*[2]* *and Xi Chen*[2]*

[1]Department of Vascular Thyroid Surgery, Affiliated Union Hospital, Fujian Medical University, Fuzhou, China, [2]Department of Surgery, Ruijin Hospital Affiliated of Shanghai Jiaotong University School of Medicine, Shanghai, China

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Edited by:
Yao Lin,
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***Correspondence:**
Nan Li
linan@fjmu.edu.cn
Haizhen Chen
chz11011@rjh.com.cn
Xi Chen
chenxi_thyroid_js@163.com

[†]These authors have contributed equally to this work

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To assess the malignancy risk of thyroid nodules, ten ultrasound characteristics are suggested as key diagnostic markers. The European Thyroid Association Guidelines (EU-TIRADS) and 2015 American Thyroid Association Management Guidelines (2015ATA) are mainly used for ultrasound malignancy risk stratification, but both are less accurate and do not appropriatetly classify high risk patients in clinical examination. Previous studies focus on papillary thyroid carcinoma (PTC), but follicular thyroid carcinoma (FTC) and medullary thyroid carcinoma (MTC) remained to be characterized. Thus, this study aimed to determine the diagnostic accuracy and establish models using all ultrasound features including the nodule size for predicting the malignancy of thyroid nodules (PTC, FTC, and MTC) in China. We applied logistic regression to the data of 1,500 patients who received medical treatment in Shanghai and Fujian. Ultrasound features including taller-than-wide shape and invasion of the thyroid capsule showed high odds ratio (OR 19.329 and 4.672) for PTC in this dataset. Invasion of the thyroid also showed the highest odds ratio (OR = 8.10) for MTC. For FTC, the halo sign has the highest odds ratio (OR = 13.40). Four ultrasound features revealed distinct OR in PTC nodule groups with different sizes. In this study, we constructed a logistic model with accuracy up to 80%. In addition, this model revealed more accuracy than TIRADS in 4b and 4c category nodules. Hence, this model could well predict malignancy in small nodules and classify high-risk patients.

Keywords: diagnostic, ultrasound characteristics, malignancy of thyroid nodules, nodule size, logistic model

INTRODUCTION

Thyroid cancer, including papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and medullary thyroid carcinoma (MTC), is one of the least painful carcinoma, which develops into a solid malignant tumor. In recent years, the incidence and detection of thyroid carcinoma has been on the rise worldwide. Over the past 20 years, thyroid carcinoma has shown higher incidence rates, surpassing those of breast carcinoma and lung carcinoma (Vigneri et al., 2015; Seib and Sosa, 2019). The malignant rate of the thyroid nodule is more than 10% (Brito et al., 2014; Al Nofal et al., 2016; Singh Ospina et al., 2016). At present, clinical guidelines suggest that all patients should have ultrasound examination and combine with clinical factors

TABLE 1 | Association between ten ultrasound characteristics and PTC thyroid malignancy in the bivariate analysis (n = 1,385).

Features	Positive		Negative		OR	95%CI		χ^2-mo	p Values
	Malignant	Benign	Malignant	Benign					
ECHO	958	324	42	61	4.294	2.842	7.961	53.074	3.213E-13
IRE	356	93	644	292	1.736	1.329	6.004	16.099	6.012E-05
TTW	797	65	203	320	19.329	14.205	24.312	464.049	6.32E-103
EF	584	161	416	224	1.953	1.539	6.368	30.086	4.133E-08
ITC	237	24	763	361	4.672	3.015	8.187	54.314	1.709E-13
CDFI	185	79	815	306	0.879	0.655	5.381	0.610	0.435
MCAL	147	69	853	316	0.789	0.577	5.323	1.954	0.162
Round	32	113	968	272	0.080	0.053	54.926	200.028	2.059E-45
AUR	16	6	984	379	1.027	0.399	3.246	0.034	0.854
UPL	248	97	752	288	0.979	0.747	5.491	0.007	0.934

The characteristics include the following: 1) hypo-echogenicity (ECHO); 2) irregular or micro-lobulated margins (IRE); 3) taller-than-wide ratio >1 (TTW); 4) echogenic focus (EF); 5) invasion of the thyroid capsule (ITC); 6) blood flow by color Doppler flow imaging (CDFI); 7) microcalcification (MCAL); 8) round; 9) halo sign (AUR); and 10) up-location (UPL).

to determine further validation tests, such as fine-needle aspiration biopsy (FNAB). As ultrasound assessment has wide availability, is not complex, and does not involve exposure to ionizing radiation, it has become a key diagnostic step to assess the risk of carcinoma in patients (Singh Ospina et al., 2016).

Research studies conducted in the United States have reported an association between the risk of malignancy and the following features: hypo-echogenicity (ECHO), taller-than-wide (TTW) shape, irregular edge (IRE), echogenic focus (EF), and invasion of the thyroid capsule (ITC) (Wolinski et al., 2014; Chambara and Ying, 2019). Nevertheless, none of these characteristics can be used as a single reliable factor to identify malignancies of the thyroid (Brito et al., 2014; Al Nofal et al., 2016; Singh Ospina et al., 2016). Brito et al. identified TTW (11.1; 95%CI: 6.6–18.9) and internal calcifications (6.8; 95%CI: 4.5–10.2) as the ultrasound features with the highest diagnostic odds ratio (OR). Campanelle et al. found that the top diagnostic OR belongs to TTW, absent halo sign, EF, and IRE. Remonti et al. revealed that TTW, EF, and absence of elasticity have the highest OR. Recently, Wettasinghe et al. suggested that EF, IRE, and ECHO are typical for diagnostic OR. Not only diagnostic OR, the specificity and sensitivity of these ultrasound parameters are variable in different studies. However, PTC accounted for more than 90% of all thyroid malignant tumors, and these data almost indicate risk factors for PTC. Other ultrasound features such as the halo sign (AUR) and location were minimally used as risk factors in clinical studies. Actually, other rare types of thyroid cancer can also have regional lymph node metastasis at the same time of blood metastasis, such as FTC and MTC. It was suggested that the evaluation of thyroid nodules based on rare types of cancer is also an important process of diagnosis and treatment of thyroid cancer. These topics still remained to be studied.

Thus, the diagnostic accuracy of these ultrasound features is not high. No significant evidence currently exists for any single characteristic. Clinically, it usually defines the risk according to the number of ultrasound features, which has great uncertainty (Oliveira et al., 2018). The European Thyroid Association Guidelines for ultrasound malignancy risk stratification (EU-TIRADS) suggested that the numbers of high-risk features including ECHO, TTW, IRE, EF, and ITC from 1 to > −4 could indicate the risk form 5 to 80%. The weight factor of each ultrasound feature and the combination pattern are not considered in US-TIRADS, which could play a critical role in the prediction of PTC. The 2015 American Thyroid Association Management Guidelines (2015ATA) suggested that nodules with high suspicion of malignancy (70–90%) has ECHO with one or more features, including IRE, MCAL, and TTW. The intermediate suspicion of malignancy in 2015ATA is 10–20%, where nodules only have ECHO. According to US-TIRADS and 2015ATA, recommended FNABs in 4b type of nodules only have one or more ultrasound feature than 4a. Also, recommended surgical operations in 4c type of nodules only have one or more ultrasound feature that 4b. However, which features among the five factors were not considered to validate the risk is not known. An alternative approach to accurately predict malignancy of thyroid nodules is building a mode to count the probability based on all five ultrasound features.

Moreover, the ultrasound assessment is still not solid and convincing to predict malignancy of thyroid nodules. FNAB was considered as the most conclusive method in clinical examination, whereas patients are not fully accepted to FNAB before carcinoma is finally determined. Even FNAB has limitations in clinical implementation. The nodule size, location, texture, and other factors will restrict the operation process of FANB. Importantly, when the nodule is less than 5 mm, FANB is more difficult due to the limitation of the puncture tool. Finally, these factors will reduce the accuracy of puncture. Thus, improvement of ultrasound assessment using typical ultrasound characteristics together with a nodule size is valuable in the current stage.

Thus, the present study aimed to establish models to finely explain the probability of thyroid nodules malignancy (PTC, FTC, and MTC) using currently revealed ultrasound features with high risk and nodule size. The analysis of more characteristics and prediction of the probability of malignant thyroid nodules to avoid confirmatory experiments will have an important impact on the guidelines and clinical recommendations.

Patients and Methods

Patients and Data Collection

The study was reviewed and approved by the Ethics Committee of Fujian Medical University and the Ethics Committee of Ruijin

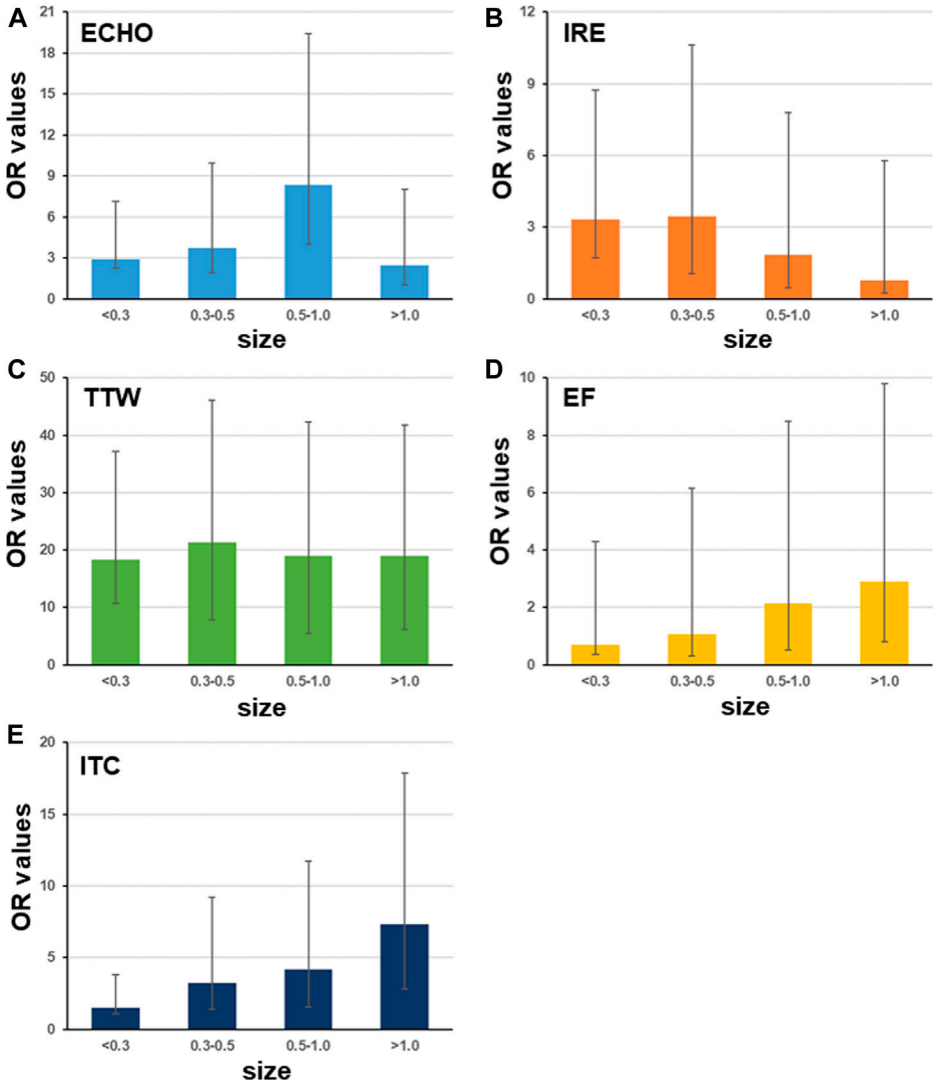

FIGURE 1 | Odds ratio of ultrasound characteristics differs with nodule size. The patients are classified into groups with different nodule sizes including <0.3 cm, 0.3–0.5 cm, 0.5–1 cm, and >1 cm (sample size is 33, 187, 499, and 281, respectively). Among five ultrasound characteristics, only four have OR values with significance < 0.05. Bars indicate the 95%CI.

Hospital Affiliated to the Shanghai Jiao Tong University Medical School. From January 2015 to August 2019, 1,505 consecutive patients (with clinical diagnosis) at Ruijin Hospital and Fujian Union Hospital were included in the study. Among them, 385 had benign tumors, 1,000 had PTC, 80 had FTC, and 40 had MTC. The malignant thyroid carcinoma was confirmed as PTC, FTC, or MTC by pathologic diagnosis after thyroidectomy. The benign cases were confirmed by FNAB or pathologic diagnosis after thyroidectomy. We randomly selected 500 PTC and 193 benign cases for developing the prediction model, and 500 PTC and 192 benign cases for validation. All FTC or MTC cases were used for developing the prediction model.

Ultrasound scanning was performed by several radiologists from two hospitals, who have many years of experience in thyroid ultrasound scanning. Ten ultrasound characteristics of the thyroid nodules in all patients were assessed by the radiologist, including 1) hypo-echogenicity (ECHO); 2) irregular or micro-lobulated margins (IRE); 3) taller-than-wide ratio >1 (TTW); 4) echogenic focus (EF); 5) invasion of the thyroid capsule (ITC); 6) blood flow by color Doppler flow imaging (CDFI); 7) microcalcification (MCAL); 8) round; 9) halo sign (AUR); and 10) up-location (UPL). These ten ultrasound indicators are based on internationally valid judgments of whether nodules are malignant tumors according to 2015ATA and EU-TIRADS. According to the number of 1) to 5) ultrasound features, the PTC patients were classified into 4A (one feature), 4B (two), 4C (three or four), and V (five). Their risk for PTC is 10, 50, 85, and 100%, respectively, which is the least upper bound of EU-TRADS. However, their accuracy needs further verification, which we intend to explore in further studies.

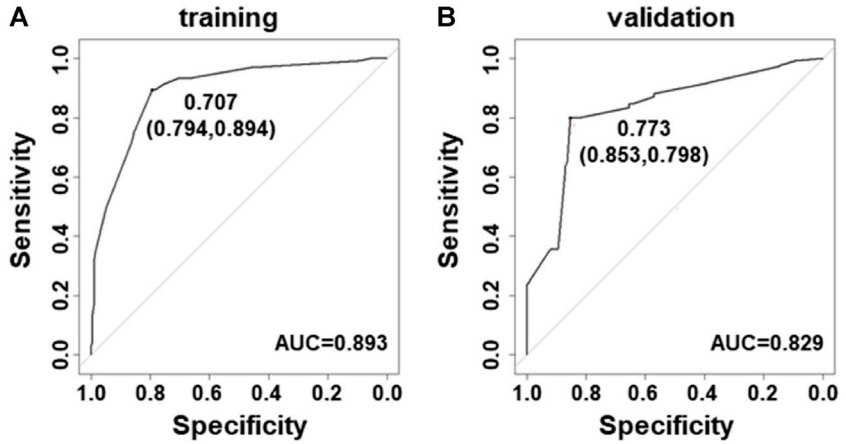

FIGURE 2 | Receiver–operating characteristic curves for the prediction model in PTC. (A) Curve of the model derived from modeling data (AUC = 0.893). (B) Curve of the model from validated data (AUC = 0.829).

The following clinical features were recorded: birth date, sex, height, weight, T-nodule size (in cm), and the presence of antibodies to thyroid peroxidase (TPOAb), as may occur in Hashimoto's disease. This disease manifests as an autoimmune attack on the thyroid. Clinical features were matched with five ultrasound characteristics, and the odds ratio and 95% confidence intervals were calculated for patients with a malignant single cancer.

Statistics and Models

The dependent variable was malignancy, and the independent variables were the ultrasound features. The level of significance, at which the validity of independent variables was evaluated, was set at $p \leq 0.05$. The odds ratio and 95% confidence interval were calculated for both training and test datasets.

Based on these characteristics, an equation indicating the probability of a malignant tumor was derived and applied to the data of the validation group. In PTC's case, the test derivation prediction model (derivation set) was applied to the randomly selected 50% of patients, and the data of the remaining 50% provided the validation set. In FTC and MTC cases, all data were used for the prediction model.

The proposed models were assessed using a receiver–operating characteristic (ROC) curve, which is a plot of sensitivity against specificity. Sensitivity was defined as the proportion of positives identified correctly. Specificity was defined as the proportion of negatives identified correctly. The area under the ROC curve was calculated, and this provided the proportion of true results, including both the true positives and true negatives.

RESULTS

Clinical Characteristics

This study included patients all of whom underwent ultrasound scanning. PTC in patients were confirmed by pathologic diagnosis after operation. The majority comprised female patients (79.6%). The age range of the patients was 15–74 years, with a mean age of 46.3 years and a median age of 47 years (standard deviation, 11.6 years).

Association Between Ultrasound Characteristics of Nodules and Thyroid Cancers

A univariate analysis of ten potential ultrasound predictors of PTC in nodules is summarized in **Table 1**. Six characteristics

TABLE 2 | Association between ten ultrasound characteristics and FTC thyroid malignancy in the bivariate analysis (n = 465).

Features	Positive		Negative		OR	95%CI		χ^2-mo	p Values
	Malignant	Benign	Malignant	Benign					
ECHO	54	324	26	61	0.391	0.227	5.413	11.011	0.0009056
IRE	25	93	55	292	1.427	0.842	4.511	1.406	0.236
TTW	11	65	69	320	0.785	0.394	3.701	0.274	0.601
EF	36	161	44	224	1.138	0.701	4.556	0.160	0.689
ITC	7	24	73	361	1.442	0.599	3.276	0.330	0.566
CDFI	44	79	36	306	4.734	2.857	7.685	38.725	4.879E-10
MCAL	23	69	57	316	1.848	1.066	4.722	4.235	0.040
	22	113	58	272	0.913	0.533	4.265	0.039	0.844
AUR	14	6	66	379	13.399	4.971	13.482	37.114	1.114E-09
UPL	15	97	65	288	0.685	0.373	4.082	1.173	0.279

TABLE 3 | Association between ten ultrasound characteristics and MTC thyroid malignancy in the bivariate analysis (n = 425).

Features	Positive		Negative		OR	95%CI		χ^2-mo	p Values
	Malignant	Benign	Malignant	Benign					
ECHO	36	324	4	61	1.6944	0.58201	3.5822	0.557	0.455
IRE	18	93	22	292	2.5689	1.32085	4.911	7.114	0.008
TTW	9	65	31	320	1.4293	0.64959	3.5483	0.452	0.501
EF	21	161	19	224	1.5378	0.80054	4.1923	1.281	0.258
ITC	14	24	26	361	8.0994	3.7502	9.1357	33.380	7.581E-09
CDFI	21	79	19	306	4.2811	2.19495	6.4464	18.858	1.408E-05
MCAL	10	69	30	316	1.5266	0.71278	3.7	0.777	0.378
	19	113	21	272	2.1778	1.12763	4.63	4.759	0.029
AUR	3	6	37	379	5.1216	1.23001	4.1934	3.638	0.056
UPL	17	97	23	288	2.1945	1.12544	4.581	4.682	0.030

showed statistically significant relationships ($p < 0.05$) with thyroid malignancy including ECHO (OR = 4.294), IRE (OR = 1.736), TTW (OR = 19.329), EF (OR = 1.953), ITC (OR = 4.672), and Round (0.080). TTW and ITC showed much higher OR levels than other characteristics. Neither odds ratio of CDFI, MCAL, AUR, or UPL has statistical significance ($p > 0.05$) in our patients.

In FTC patients, four characteristics showed statistically significant relationships ($p < 0.05$) with thyroid malignancy including ECHO (OR = 0.391), CDFI (OR = 4.734), MCAL (OR = 1.848), and AUR (OR = 13.399) (**Table 2**).

In MTC patients, six characteristics showed statistically significant relationships ($p < 0.05$) with thyroid malignancy including IRE (OR = 2.569), ITC (OR = 8.099), CDFI (OR = 4.281), ROUND (OR = 2.178), AUR (OR = 5.122), and UPL (OR = 2.195) (**Table 3**).

Relationship Between Nodule Size and Ultrasound Characteristics in PTC

Small nodule size could lead to troubles in ultrasound image judgment and FNAB. Hence, it could be an effective factor for diagnosis and operation in clinical examination. The OR values of five ultrasound characteristics in patients with different nodule sizes were counted. The patients were classified into four groups according to their nodule size being < 0.3, 0.3–0.5, 0.5–1, and > 1 cm, respectively. Results displayed that ECHO had a dynamic OR values in all four groups with a maximum in the 0.5–1 cm group and a minimum in the > 1 cm group (**Figure 1A**). IRE had a decreasing pattern with increasing nodule size (**Figure 1B**). Four groups had similar OR values for TTW (**Figure 1C**). EF and ITC had an increased tendency with maximum OR values in the > 1 cm group (**Figures 1D,E**).

Prediction Model and ROC Curves for PTC

The clinical predictive model of PTC in the thyroid nodule expressed the relationship between the probability of malignancy and the identified ultrasound characteristics, as follows:

1) Probability of PTC = $e^x / (1 + e^x)$, where e is the base of natural logarithms;

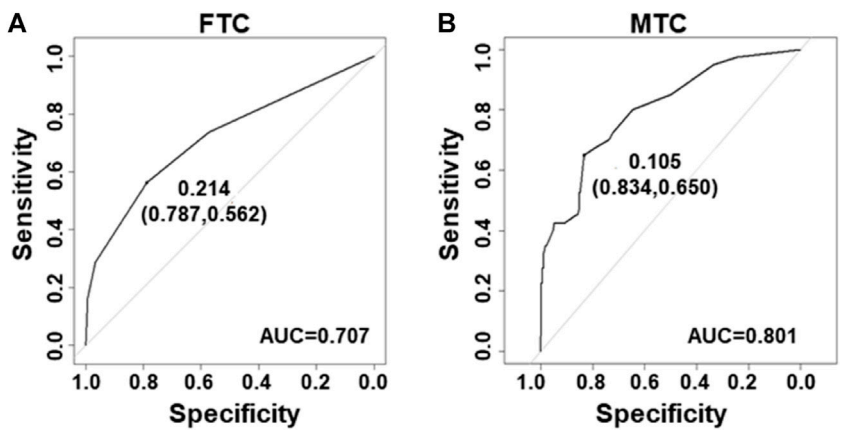

FIGURE 3 | Receiver–operating characteristic curves for the prediction model in FTC and MTC. **(A)** Curve of the model derived from FTC data (AUC = 0.707). **(B)** Curve of the model from MTC data (AUC = 0.801).

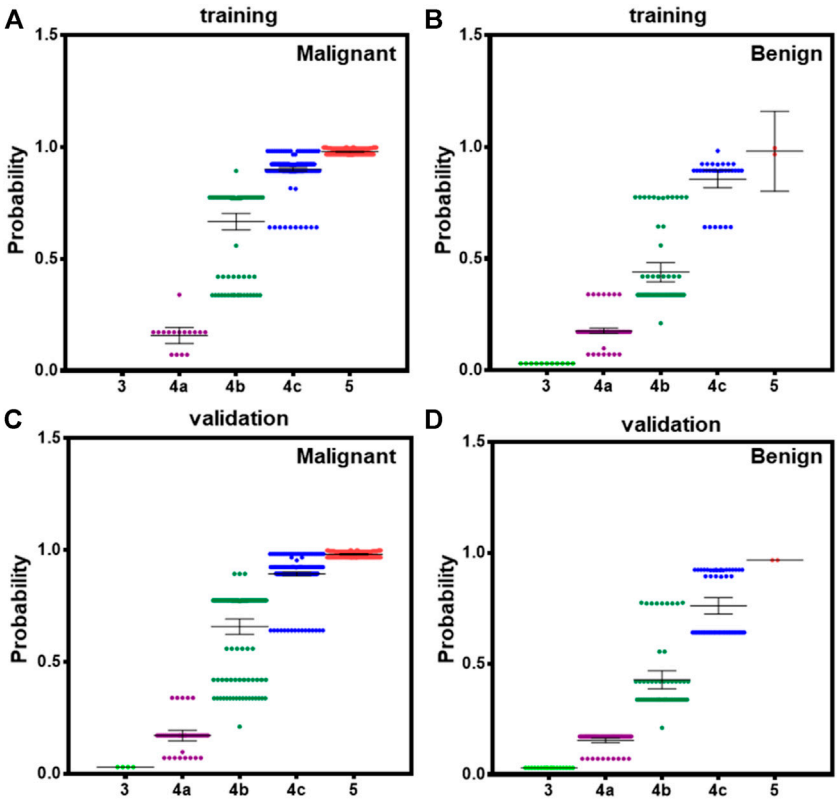

FIGURE 4 | The averaged probability of malignancy predicted by models in different category malignant nodules according to TI-RADS. **(A)** Malignant cases derived from modeling data, **(B)** benign cases form modeling data, **(C)** malignant cases derived from validation data, and **(D)** benign cases form validation data. Bars indicate the 95%CI.

TABLE 4 | Results of the logistic regression analysis to determine the association between ultrasound characteristics and PTC malignancy.

Coefficients	Estimate	Std.	z	Pr(>|z|)
(Intercept)	–3.474	0.517	–6.716	1.860E-11
ECHO	1.899	0.489	3.883	1.030E-04
IRE	1.252	0.276	4.534	5.790E-06
TTW	2.810	0.240	11.731	2.000E-16
EF	0.901	0.236	3.824	1.320E-04
ITC	2.791	0.436	6.397	1.580E-10

2) $x = -3.474 + 1.899 \times ECHO + 1.252 \times IRE + 2.810 \times TTW + 0.901 \times EF + 2.791 \times ITC$, where the presence of symptoms was scored as 1 if the patient had related symptoms and 0 if not. The statistical results are shown in **Tables 3**, **4**.

Next, the ROC curves were developed for modeling and validation datasets. The benign and malignancy could be distinguished by the prediction models. The area under the ROC curves (AUC) was 0.893 in datasets for model construction (**Figure 2A**), and 0.829 in the validation dataset (**Figure 2B**).

Prediction Model and ROC Curves for FTC and MTC

The clinical predictive model of FTC and MTC in the thyroid nodule expressed the relationship between the probability of malignancy and the identified ultrasound characteristics, as follows:

3) Probability of FTC = $e^x/(1+e^x)$, where e is the base of natural logarithms;
4) $x = -2.461 + 0.787 \times IRE + 1.433 \times CDFI + 2.812 \times AUR$, where the presence of symptoms was scored as 1 if the patient had related symptoms and 0 if not.
5) Probability of MTC = $e^x/(1+e^x)$, where e is the base of natural logarithms;
6) $x = -3.634 + 0.860 \times IRE + 1.763 \times ITC + 1.553 \times CDFI + 0.695 \times UPL$. The statistical results are shown in **Tables 5**, **6**.

Next, the ROC curves were developed for the FTC and MTC datasets. The area under the ROC curves (AUC) was 0.707 in datasets for FTC (**Figure 3A**) and 0.801 for MTC (**Figure 3B**).

TABLE 5 | Results of the logistic regression analysis to determine the association between ultrasound characteristics and FTC malignancy.

Coefficients	Estimate	Std.	z	Pr(>|z|)
(Intercept)	–2.461	0.218	–11.274	2.000E-16
IRE	0.787	0.296	2.656	7.900E-03
CDFI	1.433	0.279	5.145	2.670E-07
AUR	2.182	0.542	4.023	5.740E-05

TABLE 6 | Results of the logistic regression analysis to determine the association between ultrasound characteristics and mTC malignancy.

Coefficients	Estimate	Std.	Error	Pr(>|z|)
(Intercept)	–3.634	0.352	–10.327	2.00E-16
IRE	0.860	0.394	2.184	2.890E-02
ITC	1.763	0.438	4.029	5.600E-05
CDFI	1.553	0.376	4.136	3.530E-05
UPL	0.696	0.375	1.856	6.350E-02

Models Revealed Different Probability in 4b/c Types Thyroid Nodules Between PTC and Benign Cases

According to 2015ATA, nodules with ECHO plus one or more of ultrasound risk features are high suspicion malignancy (70–90%). Both BI-RADS and TI-RADS classified thyroid nodules into 0–6˚ according the number of positive ultrasound features. However, the combination of ultrasound features was not considered in the assessment of the probability. We calculated the probability in all PTC and benign nodules according to our model, and showed the average in 3–5 TI-RADS types. In the training dataset, malignant 4b nodules have significantly higher probability than benign 4b nodules, and malignant 4c nodules have a small difference with benign 4c nodules (**Figures 4A,B**). In validation datasets, both 4b and 4c type malignant nodules have significantly higher probability than benign nodules (**Figures 4C,D**). These results suggest that our module could better indicate the risk of PTC than both BI-RADS and TI-RADS.

DISCUSSION

In this study, five typical ultrasound characteristics were analyzed as predictor of thyroid malignancy. It was congruent with the findings of most previous studies (Moon et al., 2008; Wettasinghe et al., 2019; Alam et al., 2014). ECHO nodules showed a sensitivity of 87.1% in another study conducted on 500 patients (Papini et al., 2002). Previous studies suggest that marked hypo-echogenicity is commonly associated with benign tumors and rarely observed in cases of malignancy (Nachiappan et al., 2014; Kim et al., 2002). However, using only ECHO as malignancy characteristics showed only low specificity. It is different with a previous study in which Remonti et al., (2015) reported that the absence of elasticity, with a sensitivity of 87.9% and a specificity of 86.2%, achieved the best diagnostic accuracy in a study of 52 patients (Remonti et al., 2015). In patients with 0.5–1.0 cm nodule size, ECHO has much bigger OR values than in other nodule sizes (**Figure 1A**). Next, the incidence of IRE is proportional to the risk of malignancy (Papini et al., 2002; Remonti et al., 2015). In our results, nodules with a small size (<0.5 cm) has higher OD of IRE than big nodules (**Figure 1B**). It suggested that IRE is more valuable for prediction of PTC in small nodules. Another study indicated that a taller-than-wide shape is very specific for distinguishing malignant from benign thyroid nodules in both unilateral and bilateral cancer (Moon et al., 2008). In this study, we found that OR values of TTW did not vary in nodules with different sizes (**Figure 1C**). It suggested that the nodule size has a limit effect on OR values of TTW. Previous studies suggested that EF and ITC are reliable indicators of diagnostic accuracy (Wettasinghe et al., 2019) (Nachiappan et al., 2014). Our results showed that both EF and ITC have biggest OR values in nodules with size >1 cm. In general, while using ECHO, IRE, EF, and ITC to assess risk of PTC, one should also consider the nodule size; then none of these five features would be a risk factor for FTC and MTC according to OD values (**Table 4**). Instead of them, IRE, CDFI, and AUR have significant high OD values for FTC. In MTC's case, IRE, ITC, CDFI, and UPL are significant risk factors. Here, CDFI, AUR, and UPL are not risk factors for PTC.

Next, integration of five ultrasound characteristics to predict thyroid malignancy is more reliable than only one. We assessed individual ultrasound characteristics separately to counteract the overlap that occurs when more than one ultrasound characteristic is considered (Frates et al., 2006). A multivariate model is more appropriate. We developed logistic models for prediction of PTC. The model could increase the accuracy to 89 and 83% in modeling and validation datasets, respectively (**Figure 2**), and this might be due to the fact that the model considered the weight factor of ultrasound features and the combination pattern. In general, our model could more easily give a reliable prediction than using only one ultrasound characteristic. Also, logistic models for prediction of FTC and MTC were built with an accuracy of 70 and 80%, respectively (**Figure 3**).

In addition, definition of high-risk category (4b and 4c, 10–85%) following 2015ATA did not consider the detail number of ultrasound features, whereas our result clearly revealed that malignancy and benign nodules with two or three of ultrasound features have significantly different risk probability based on our logistical model (**Figure 4**). This could facilitate the clinical performance to convince patients for FNAB and operation.

In conclusion, it is difficult to predict the malignancy of thyroid nodules using ultrasound features. Also applying ultrasound features to assess PTC is restricted by the nodule size. Here, our modified logistic model using ECHO, TTW, IRE, EF, and ITC could give more than 80% accuracy for predicting the PTC.

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

The study was reviewed and approved by the Ethics Committee of Fujian Medical. University and the Ethics Committee of Ruijin Hospital Affiliated to The Shanghai Jiao Tong University Medical School.

AUTHOR CONTRIBUTIONS

XC and SJ designed the experiments. SJ, QX, HC, and NL collected the data. XC, SJ, and QX did most analysis. SJ and XC wrote themanuscript.

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REVIEW
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Molecular Mechanisms of Exercise on Cancer: A Bibliometrics Study and Visualization Analysis *via* CiteSpace

Dongling Zhong[1†], *Yuxi Li*[2†], *Yijie Huang*[3†], *Xiaojuan Hong*[2*], *Juan Li*[1*] *and Rongjiang Jin*[1*]

[1]School of Health Preservation and Rehabilitation, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]School of Acupuncture Moxibustion and Tuina, The Third Affiliated Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China

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Yao Lin,
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Weilin Liu,
Fujian University of Traditional Chinese Medicine, China

***Correspondence:**
Xiaojuan Hong
365358649@qq.com
Rongjiang Jin
cdzyydxjrj@126.com
Juan Li
785939016@qq.com

[†]These authors have contributed equally to this work and share first authorship

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Objective: To analyze the research hot spots and frontiers of molecular mechanisms of exercise on cancer *via* CiteSpace.

Method: Related publications in the Web of Science Core Collection Science Citation Index Expanded were retrieved from inception to November 27th, 2021. Then we used CiteSpace to generate network maps and identify top authors, institutions, countries, keywords, co-cited authors, journals, references and research trends.

Results: A total of 1,130 related publications were retrieved. The most productive author and journal were Lee W Jones and PLOS ONE. Hanahan D and Warburg O were the most cited authors. Fudan University and Shanghai Jiao Tong University were the leading institutions, while China was the leading country. Top-cited authors and references generally focused on the epidemiology and hallmarks of cancer. Top five keywords with both high frequency and high betweenness centrality were breast cancer, aerobic glycolysis, oxidative stress, gene expression, skeletal muscle. Keyword "warburg effect" ranked first with the highest citation burst, while "inflammation", "hepatocellular carcinoma", "epithelial mesenchymal transition", and "adipose tissue" were emerging research foci.

Conclusion: This study analyzed the research hot spots and frontiers of molecular mechanisms of exercise on cancer *via* CiteSpace. Based on the results, altered metabolism (aerobic glycolysis, insulin resistance, myokines), oxidative stress, gene expression and apoptosis were hot-research mechanisms of exercise on cancer. Emerging research foci of mechanisms were generally around inflammation, epithelial mesenchymal transition and adipokines. In addition, future studies could carry in-depth research of interactions between different mechanisms and try to elucidate the recommended doses and intensities of exercise for cancer, especially in breast, colorectal, prostate cancer and hepatocellular carcinoma.

Keywords: exercise, cancer, molecular mechanisms, bibliometrics, visualization analysis, citespace

INTRODUCTION

Cancer is one of the leading causes of disability and mortality worldwide. According to latest estimates of the International Agency for Research on Cancer (IARC) (https://www.iarc.who.int/), there were about 19.3 million new cases of cancer, 10 million cancer deaths worldwide in 2020. In 2040, there will be 28.4 million new cancer cases.

Exercise plays an essential role in the management of cancer, especially in cancer prevention, cancer progression control, cancer-related outcomes improvement (Galvao et al., 2010; Speck et al., 2010; Magne et al., 2011). According to a study involving 430,000 people, leisure-time physical activity was associated with lower risks of several cancer types (Moore et al., 2016). In addition, exercise could reduce cancer risk factors such as obesity (Swift et al., 2018), inflammation (Metsios et al., 2020) and improve health-related outcomes in cancer survivors (Campbell et al., 2019). On the contrary, physical inactivity could increase the risk of colon cancer (Wolin et al., 2009) and breast cancer (Kyu et al., 2016). Therefore, multiple organizations including the American Cancer Society (ACS) (Rock et al., 2012), the American College of Sports Medicine (ACSM) (Schmitz et al., 2010), Exercise and Sports Science Australia (ESSA) (Hayes et al., 2019) have published exercise guidelines for cancer survivors.

Due to the rapid development of technologies, the possible molecular mechanisms of exercise are being illuminated, which may be possibly related to changes in the serum markers level, inflammation markers, oxidative stress and so on (Hojman et al., 2018). However, research hot spots and frontiers of this field remain unclear.

CiteSpace is a Java-based application to analyze and visualize the hot spots and research frontiers in the scientific literature of a discipline or knowledge domain in a certain period with metrology, co-occurrence analysis and cluster analysis (Chen, 2004; Chen, 2006). In this study, we intended to analyze the hot spots and research frontiers of molecular mechanisms of exercise on cancer via CiteSpace, which may help us understand the curative and preventive effects of exercise for cancer better.

METHODS

Search Strategy

We retrieved articles in the Web of Science Core Collection Science Citation Index Expanded (SCI-Expanded) from inception to November 27th, 2021, using the following terms: exercise, neoplasms and molecular mechanism. **Table 1** shows the detailed search strategy.

Inclusion and Exclusion Criteria

Peer-reviewed published original articles or reviews about the molecular mechanisms of exercise on cancer were included.

Exclusion criteria were: 1) conference abstracts or corrigendum documents; 2) unpublished articles; 3) repeated publications; 4) unrelated articles.

Bibliometrics and Visualization Analysis

We exported retrieved articles in plain text format with full records and references, named "download_XXX.txt" and then imported into CiteSpace 5.8.R3 for further analysis. When mapping visualization knowledge figures, we followed the main procedural steps of CiteSpace, including time slicing, thresholding, modeling, pruning, merging, and mapping (Chen, 2004). Central concepts of CiteSpace includes burst detection, betweenness centrality, and heterogeneous networks, which can help to timely visualize the research status, hot spots, and frontiers (Chen, 2006). Nodes in different maps represent authors, institutions, countries or keywords. Size of nodes indicates the frequency of occurrence or citation, and color of nodes indicated the occurrence or citation years. Besides, nodes with purple trims suggests high betweenness centrality, which are often identified as hot spots or turning points in a field.

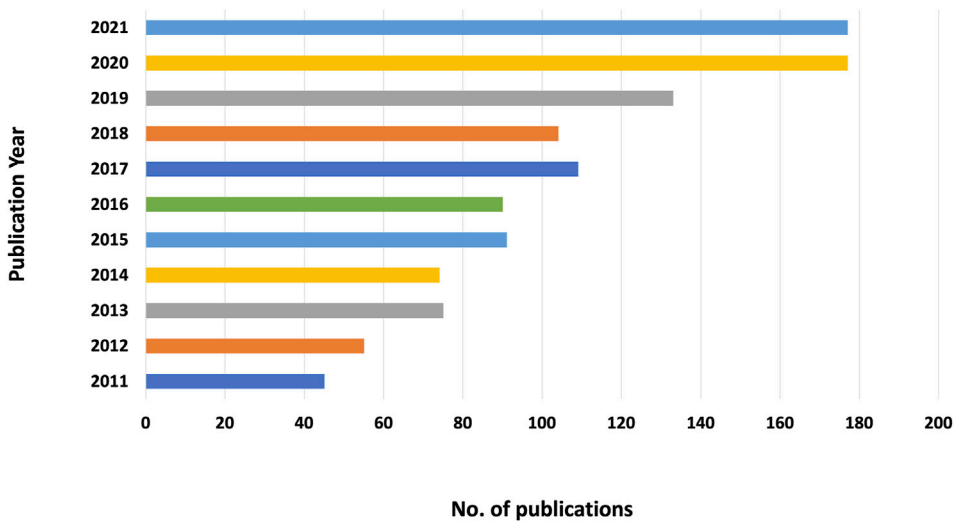

FIGURE 1 | Annual trend of publications.

TABLE 1 | Search strategy.

Set	Search query
#1	TS=(cancer* or tumor* or tumour* or neoplas* or malignan* or carcinoma* or adenocarcinoma* or choricarcinoma* or leukemia* or leukaemia* or metastat* or sarcoma* or teratoma* or melanoma* or lymphoma* or myeloma*)
#2	{TS=[physical* near/5 (fit* or activit* or movement*)]} OR [TS=(exercis* or aerobic* or walk* or endurance* or training or tai ji or yoga or tai-chi or tai-ji or tai chi or taiji* or pilates)]
#3	(#2) AND (#1)
#4	[TS=(molecular mechanism*)] AND (#3)

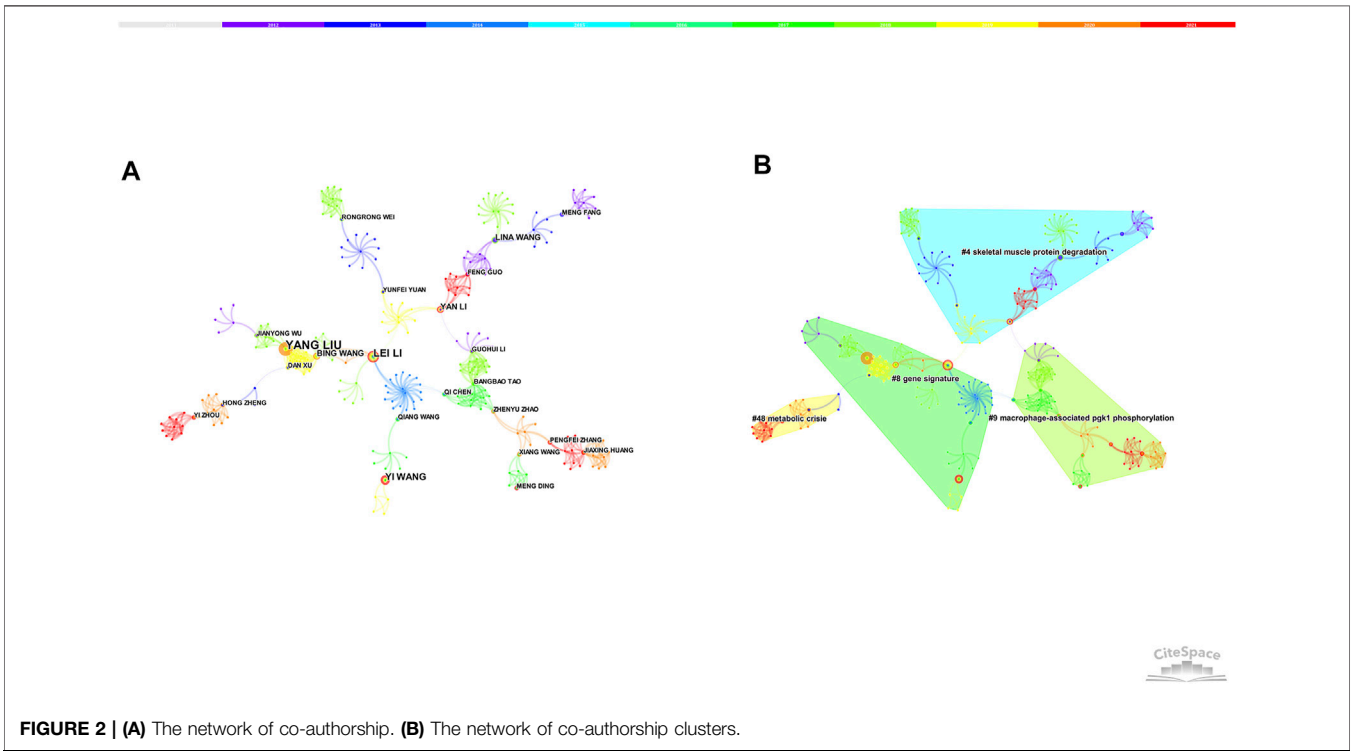

FIGURE 2 | (A) The network of co-authorship. **(B)** The network of co-authorship clusters.

RESULTS

Distribution of Articles by Publication Years

After removing 61 unqualified records, 1,130 articles were obtained. **Figure 1** shows that the number of publications has generally increased with some fluctuations, ranging from 45 to 177 publications. From 2019 to 2020, the number of related publications increased the most (33 publications), indicating that more and more researchers are beginning to pay attention to this field.

Co-Authorship, Co-Institution, and Co-Country

We analyzed publications with time slicing of 1 year and the top 50 levels of most-cited or occurred items from each slice. Tree ring history was selected as node display pattern. Lines between nodes represent cooperation, and the color of lines indicates the first cooperation year.

7,588 nodes and 23,925 links composed of the merged co-authorship network, and we chose to visualize the largest connected component only (**Figure 2A**). The co-authorship network shows prolific authors and the collaboration among them. The most productive author was Lee W Jones with a total of 8 articles, followed by Jing Li (6 articles) and Yang Liu (6 articles). Although many authors have published relevant articles, there was little collaboration among them. Besides, the centrality of authors was relatively low, suggesting that more high-quality and large-scale collaborations are needed in the future.

In general, the silhouette value is used to evaluate the clusters. If the silhouette value is over 0.7, the cluster is efficient and convincing. Four clusters (the silhouette value of 4 clusters exceeded over 0.9) were produced by log-likelihood ratio, mainly around skeletal muscle protein degradation, gene signature, metabolic crisis, macrophage-associated PGK-1 phosphorylation (**Figure 2B**).

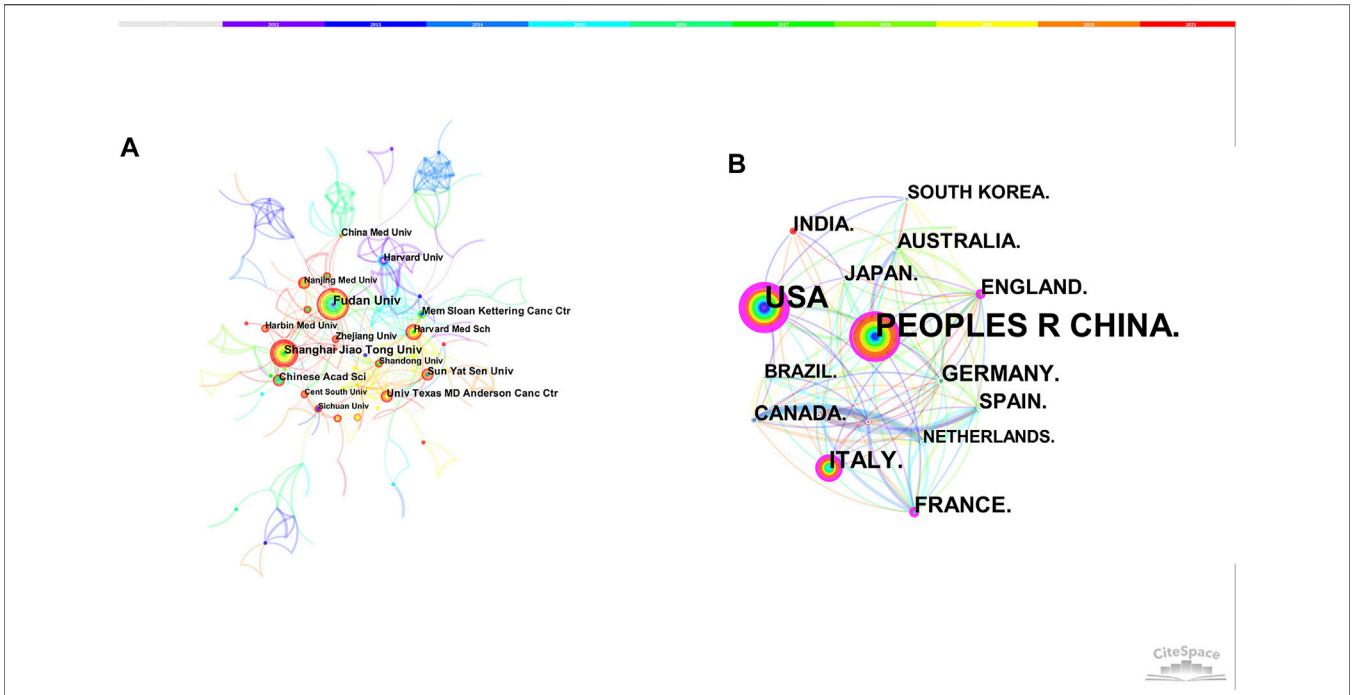

FIGURE 3 | (A) The network of co-institution. **(B)** The network of co-country.

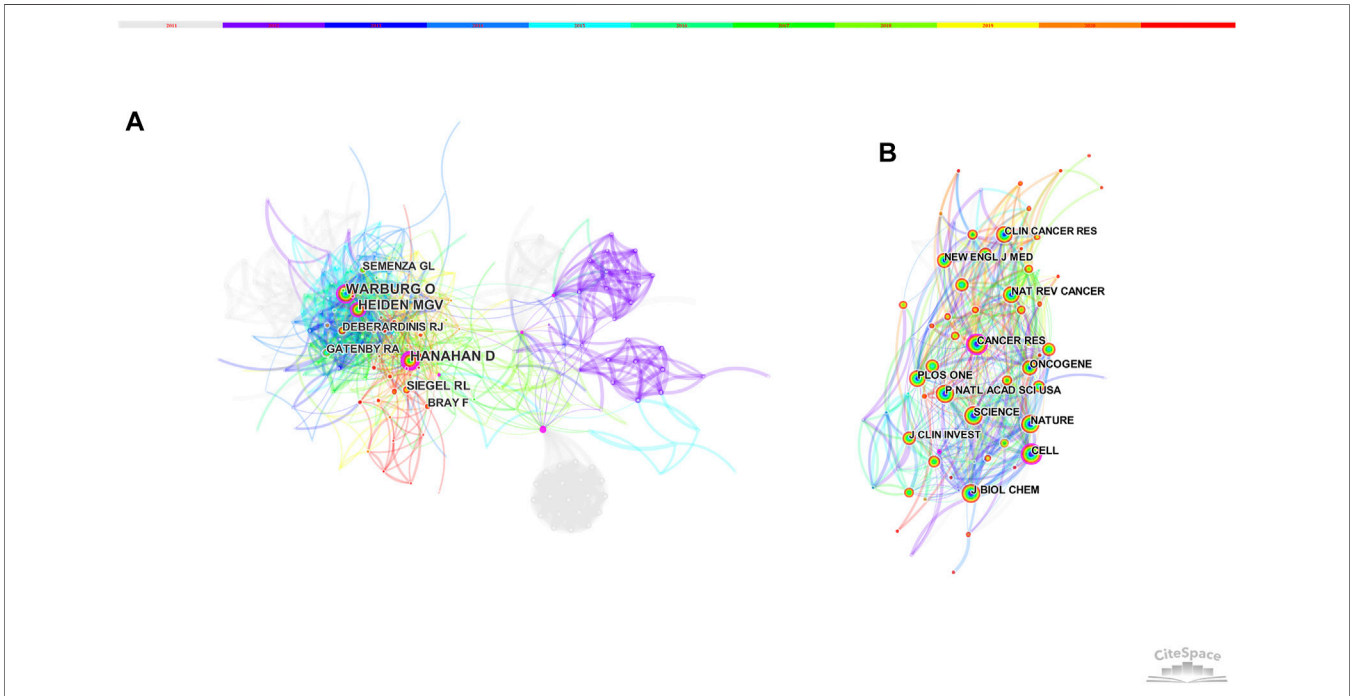

FIGURE 4 | (A) The network of author co-citation. **(B)** The network of journal co-citation.

The merged co-institution network map is shown in **Figure 3A**, with 1,568 nodes and 5,227 links. The top five institutions were Fudan University (36 articles), Shanghai Jiao Tong University (36 articles), Sun Yat-sen University (22 articles), Chinese Academy of Sciences (21 articles) and Memorial Sloan Kettering Cancer Center (19 articles).

The merged co-country network consists of 86 nodes and 433 links. People's Republic of China was the leading country (427

TABLE 2 | The top five cited journal.

Rank	Frequency	Cited journal	IF	Centrality	Cited journal	IF
1	638	PLOS ONE	3.2	0.15	Cancer research	12.701
2	634	Nature	49.962	0.14	Cell	41.584
3	629	Cancer research	12.701	0.1	The biochemical journal	3.857
4	627	Proceedings of the national academy of sciences of the United States of America	9.58	0.09	The journal of clinical investigation	14.808
5	587	Cell	41.584	0.08	International journal of cancer	7.396

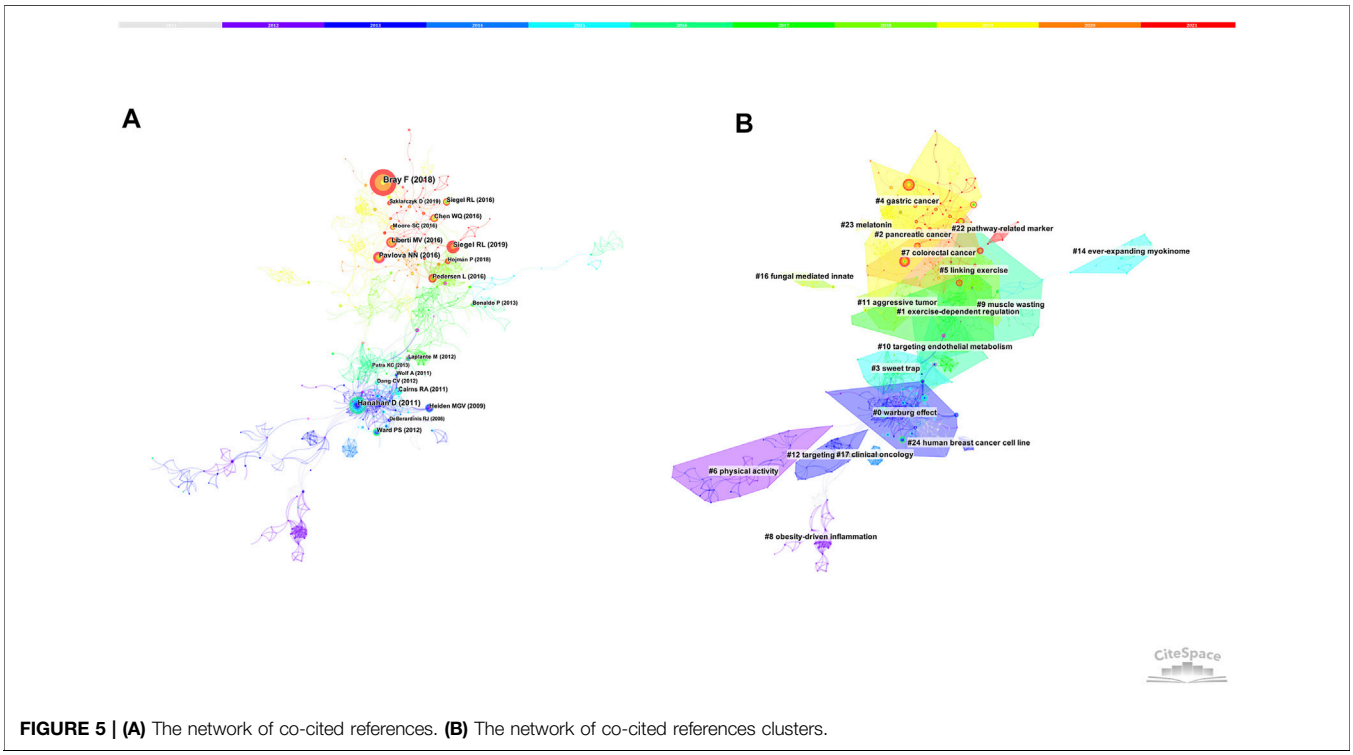

FIGURE 5 | (A) The network of co-cited references. **(B)** The network of co-cited references clusters.

articles), followed by United States (316 articles), Italy (87 articles), Germany (57 articles) and France (51 articles). Except for Germany, the betweenness centrality of other countries were both over 0.1, illustrating the important contribution of these countries in this area (**Figure 3B**).

Author and Journal Co-Citation

Figure 4 displays the network of author and journal co-citation. The most-cited authors were Hanahan D (130 citations) and Warburg O (130 citations), followed by Heiden MGV (119 citations), Siegel RL (74 citations), Semenza GL (62 citations) and Deberardinis RJ (61 citations). Besides, Hanahan D, Warburg O and Heiden MGV were also high-centrality authors (**Figure 4A**).

The top-ranked journal by citation counts was PLOS ONE with 638 citations, followed by Nature (634 citations), Cancer Research (629 citations), Proceedings of the National Academy of Sciences of the United States of America (627 citations) and Cell (587 citations) (**Figure 4B**). Besides, Cancer Research and Cell got both high frequency and high betweenness centrality, indicating its critical role in this field (**Table 2**).

References Co-Citation

Figure 5A and **Table 3** show the top co-cited references with high frequency and high betweenness centrality. The first co-cited reference was published by Bray F et al., which provided a status report on the global burden of cancer (Bray et al., 2018), The reference published by Hanahan D introduced the hallmarks of cancer, which may affect the development of anti-cancer therapies (Hanahan and Weinberg, 2011). Siegel RL provided the cancer statistics of United States in 2019 (Siegel et al., 2019). Pavlova NN made a summary of the emerging 6 hallmarks of cancer metabolism (Pavlova and Thompson, 2016). Liberti MV summarized the explanations and controversies for the function of Warburg Effect (Liberti and Locasale, 2016).

Other high-cited publications were as follows: Pedersen L et al. revealed that exercise-induced muscle-derived interleukin-6 (IL-6) was involved in natural killer (NK) cell redistribution, thus reduced the incidence and growth of cancer (Pedersen et al., 2016). Hojman P et al. concluded that the tumor growth-inhibitory effect of exercise was probably mediated by several different mechanisms (the cellular immune system and exercise-induced myokines) (Hojman et al.,

TABLE 3 | The top five cited references.

Rank	First author	Country	Frequency	Centrality	Year	Cited references	Journal	IF
1	Bray F	France	53	0	2018	Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries	CA: a cancer journal for clinicians	508.702
2	Hanahan D	Switzerland	35	0.06	2011	Hallmarks of cancer: the next generation	Cell	41.584
3	Siegel RL	United States	26	0.01	2019	Cancer statistics, 2019	CA: a cancer journal for clinicians	508.702
4	Pavlova NN	United States	24	0.11	2016	The emerging hallmarks of cancer metabolism	Cell Metabolism	27.287
5	Liberti MV	United States	21	0.02	2016	The warburg effect: How does it benefit cancer cells?	Trends in biochemical sciences	13.807

2018). Moore SC et al. found that leisure-time physical activity was associated with lower risks of many cancers (Moore et al., 2016).

Figure 5B shows the largest 19 clusters of co-cited references. **Figure 6** displays the top 41 references with strongest citation bursts, which indicating the emerging trends or increasing interests in the field. Generally, the most co-cited references usually got the strongest citation bursts.

Keywords Co-Citation and Clusters

Keywords are the high-level summary. High-frequency and high-centrality keywords often reflect hot research topics in this field. We analyzed publications with time slicing of 1 year and the top 30 levels of most-cited or occurred items from each slice. 149 nodes and 1,138 links composed of the merged co-occurring keywords network. **Table 4** presents the top 10 keywords with high frequency and centrality. 6 clusters were produced by log-likelihood ratio, including metabolic syndrome, aerobic glycolysis, metabolic reprogramming, pharmacological suppression, molecular switch and atherosclerosis (**Figure 7**).

Keywords With Citation Bursts

Figure 8 presents the top 33 keywords with citation bursts. The blue line indicates the time interval, while the red line indicates the time period when a keyword had a burst (Chen et al., 2014). Keywords "warburg effect" with the strongest citation bursts appeared in 2014, indicating the importance of glucose metabolism in cancer cells. The most recent keywords with citation bursts occurred in 2019 were "inflammation", "hepatocellular carcinoma" and "adipose tissue". In addition, "epithelial mesenchymal transition" also continued to 2021.

DISCUSSION

Summary of Findings

A total of 1,130 related articles were retrieved eventually. In the past several years, increasing researchers have begun to study the molecular mechanisms of exercise on cancer, forming a group of prolific authors represented by Lee W Jones, Jing Li and Yang Liu. China was the leading country in this field and Chinese universities have published many relevant studies. Top-cited authors and references generally focused on the epidemiology and hallmarks of cancer. Top five keywords with both high frequency and high betweenness centrality were breast cancer, aerobic glycolysis, oxidative stress, gene expression, skeletal muscle. Keyword "warburg effect" ranked first with the highest citation burst, while "inflammation", "hepatocellular carcinoma", "epithelial mesenchymal transition", and "adipose tissue" were emerging research foci.

Research Hotspots on Molecular Mechanisms of Exercise on Cancer

Based on the results of CiteSpace, we summarized the hot-research molecular mechanisms of exercise on cancer (**Figure 9**).

Metabolism

Aerobic Glycolytic/Warburg Effect

Altered glycolysis/TCA cycle has been proved to be one of hallmarks of cancer (Liberti and Locasale, 2016; Pavlova and Thompson, 2016). Highly up-regulated glycolytic phenotype could induce local acidosis, which is conducive to tumor development and invasion. Warburg effect is characterized by accelerated aerobic glycolytic metabolism even if there is sufficient oxygen supply, and is common in most malignant tumors (Cairns et al., 2011). In healthy athletes, increased systemic lactate levels during repeated high-intensity anaerobic exercise could inhibit glycolysis and net lactate production (Hollidge-Horvat et al., 1999). Evidence indicated that high-intensity anaerobic exercise was shown to inhibit the Warburg-type highly glycolytic (Hofmann, 2018). Animal studies also demonstrated that high-intensity anaerobic exercise training may have stronger effects on tumor growth compared with moderate-intensity aerobic exercise (Bacurau et al., 2007; de Lima et al., 2008; Paceli et al., 2012). However, the correlation between the host and cancer metabolism is related to the duration, time, intensity, and movement mode of exercise (Koelwyn et al., 2017).

Insulin/Insulin-Like Growth Factors

Insulin and the insulin-like growth factors (IGFs) family play an essential role in regulating cell growth and apoptosis, as well as proliferation and differentiation of cancer cells (Hankinson et al., 1998; Christopoulos et al., 2015). Besides, insulin-like growth factor binding protein 3 (IGFBP-3) could inhibit cell proliferation and promote cell apoptosis by regulating local IGF-1 concentration and the anti-apoptosis effect of IGFBP-3 can be inhibited in breast cancer cells (Kim et al., 2004). Irwin et al. revealed that the decrease in IGF-I and IGFBP-3 caused by exercise may explain the link between higher

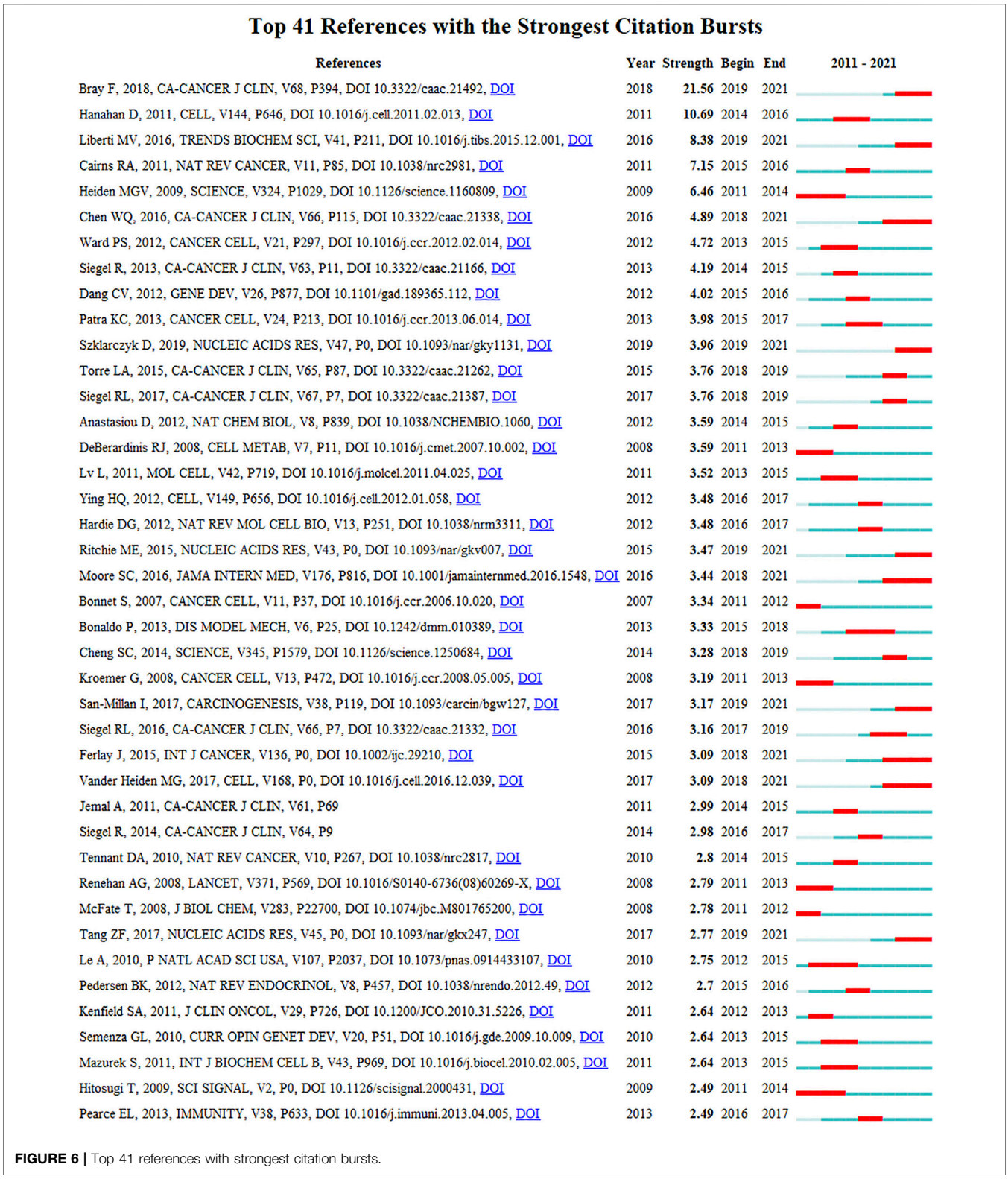

FIGURE 6 | Top 41 references with strongest citation bursts.

levels of physical activity and the survival rate of breast cancer patients (Irwin et al., 2009). Another study indicated that changes in insulin levels and/or changes in body fat or fat deposition by exercise may mediate breast cancer prognosis in part (Ligibel et al., 2008).

Myokine/Apoptosis

During exercise, paracrine factors coordinate tissue remodeling in response to skeletal muscle contraction. Factors secreted from muscle cells may influence cancer cell growth. Lack of physical activity

TABLE 4 | Top 10 keywords in terms of frequency and centrality.

Rank	Frequency	Keywords	Centrality	Keywords
1	128	Breast cancer	0.18	Oxidative stress
2	113	Aerobic glycolysis	0.14	Breast cancer
3	83	Oxidative stress	0.14	Aerobic glycolysis
4	80	Gene expression	0.14	Gene expression
5	69	Skeletal muscle	0.12	Skeletal muscle
6	69	Metabolism	0.07	Colorectal cancer
7	45	Colorectal cancer	0.06	Apoptosis
8	40	Apoptosis	0.06	Prostate cancer
9	32	Hepatocellular carcinoma	0.05	Insulin resistance
10	30	Insulin resistance	0.04	Metabolism

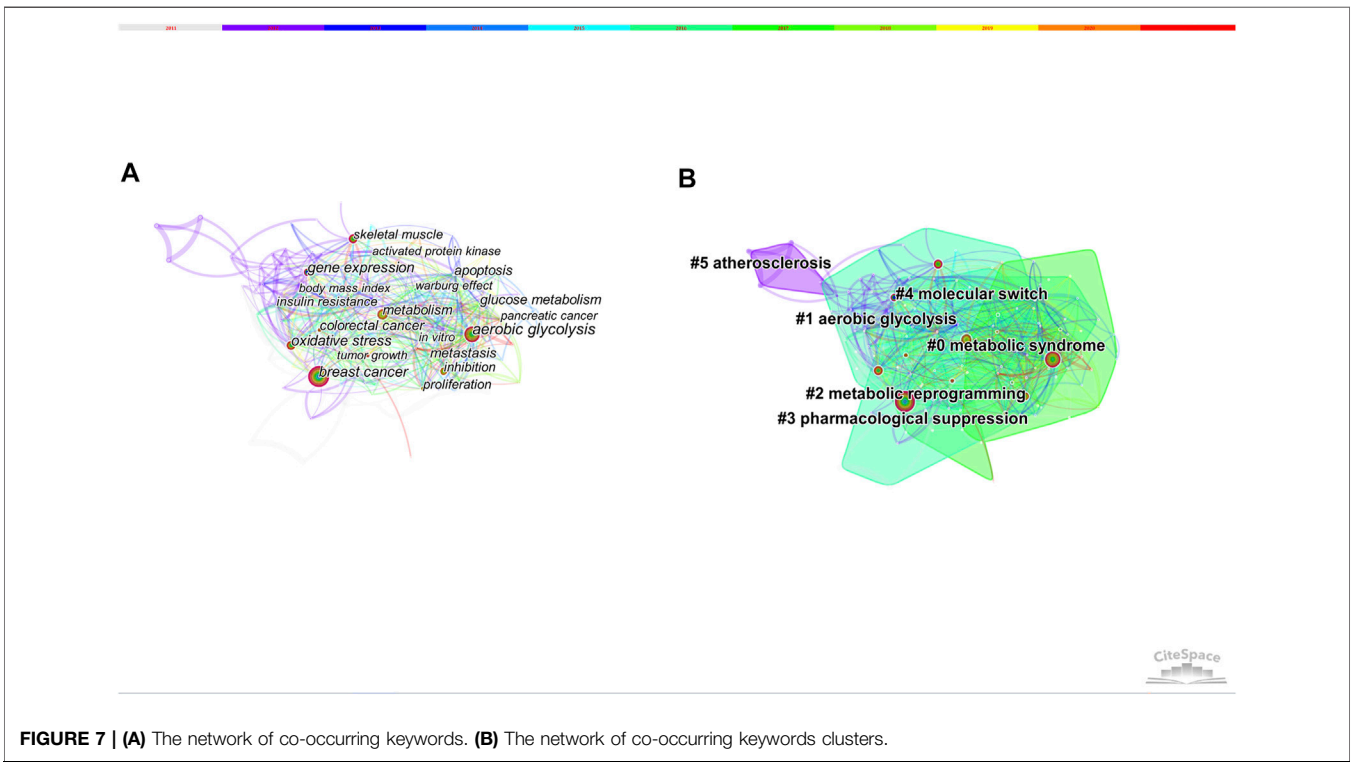

FIGURE 7 | (A) The network of co-occurring keywords. **(B)** The network of co-occurring keywords clusters.

probably leads to changes in myokine response. Evidence showed that myokines, such as the IL-6 superfamily, may mediate some of the inhibitory effects of exercise on mammary cancer cell proliferation (Hojman et al., 2011). Moreover, exercise can induce apoptosis of tumor cells in skeletal muscle. DNA microarrays were used to compare the transcriptome of muscle tissue in young and old mice (sedentary and exercised), and the results showed that exercise was able to stimulate the secretion of secreted protein acidic and cysteine-rich (SPARC) from muscle tissues and SPARC could inhibit colon tumorigenesis by increasing apoptosis (Aoi et al., 2013). Animal studies also suggested that moderate-intensity training may inhibit cancer cell proliferation and induce apoptosis (Westerlind et al., 2002).

Oxidative Stress

High levels of oxidative stress is another hallmark of cancer (Cairns et al., 2011), which is caused by the imbalance between the production and elimination of reactive oxygen species (ROS). Increased ROS levels are generally detrimental to cells, and could promote tumor formation via inducing DNA damage, pro-inflammatory cytokines (Naik and Dixit, 2011) and activating the nuclear factor-κB (NF-κB) pathway (Gloire et al., 2006). Studies showed that exercise was able to improve antioxidation and counteract the negative consequences of oxidative stress by modulating systemic oxidative status (SOS) and DNA repair capability (Tomasello et al., 2017; Simioni et al., 2018). However, controversy exists since strenuous exercise may enhance oxidative stress.

Cancer Types

According to the high frequency keywords, breast cancer, colorectal cancer and hepatocellular carcinoma are main research cancer types of mechanisms of exercise. Possible underlying mechanisms of

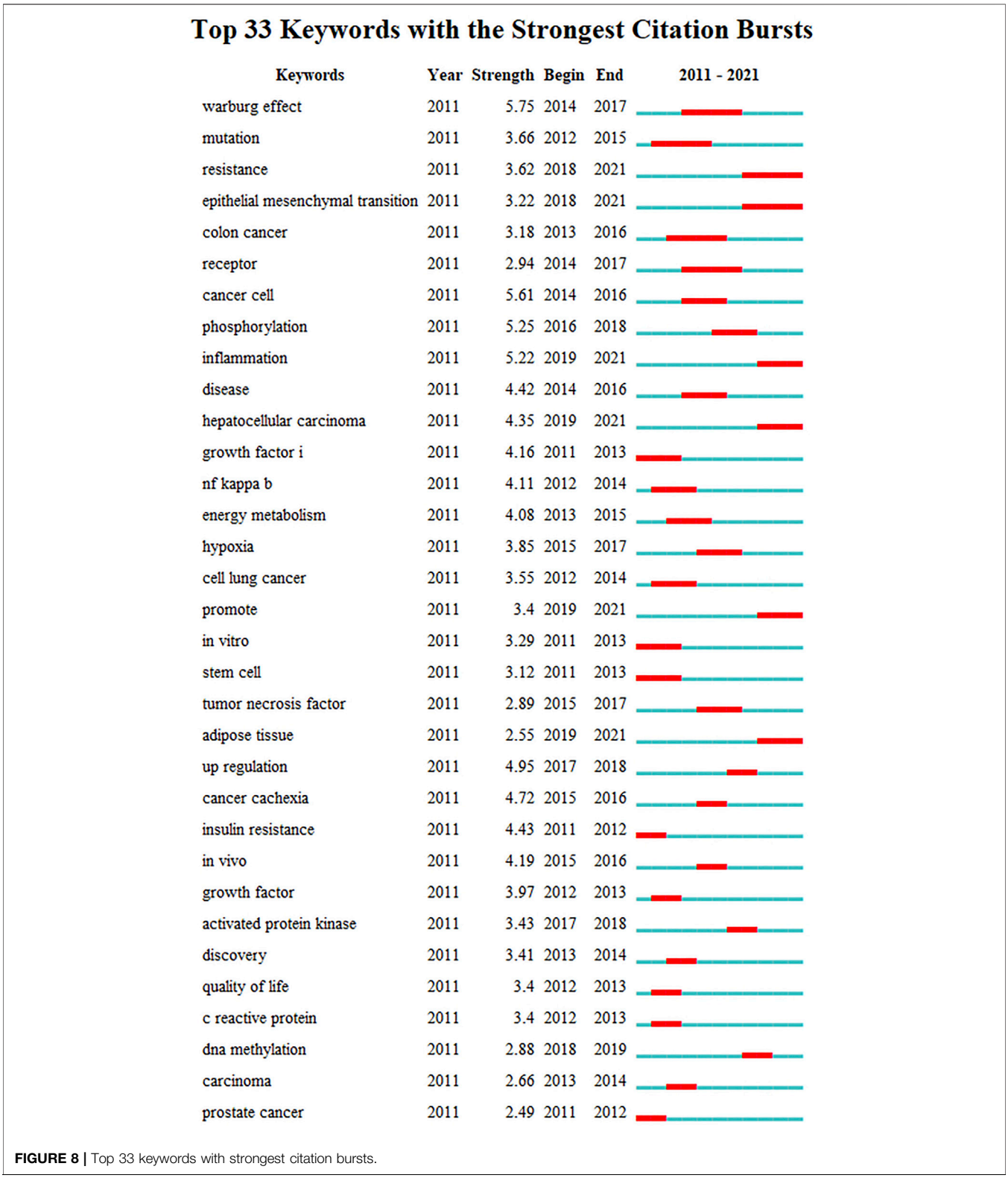

FIGURE 8 | Top 33 keywords with strongest citation bursts.

breast cancer includes a reduction of sex hormones, metabolic hormones, adipokines and oxidative stress, and an improvement of the immune function (de Boer et al., 2017). Hayes et al. also outlined the mechanisms of exercise for colorectal and prostate cancer through harnessing the immune system (Hayes et al., 2016; Song and Chan, 2018). Besides, hepatocellular carcinoma was one of the most recent keywords with citation bursts, which has attracted the attention of researchers. Exercise can attenuate the progression of

FIGURE 9 | Summary of the hot-research mechanisms.

hepatocellular carcinoma related to changes in key signaling pathways, cellular proliferation, tumor vascularization, and necrosis (Saran et al., 2018).

Emerging Areas on Molecular Mechanisms of Exercise on Cancer

From the citation bursts analysis, the most recent keywords with citation bursts occurred in 2019 was inflammation. Chronic inflammation is bound up with the development and progression of cancer (Gleeson et al., 2011; Friedenreich et al., 2012). Numerous molecules such as tumor necrosis factor (TNF)-α, interleukin-1 (IL-1) and IL-6 are common biomarkers of inflammation associated with cancer, and they could be regulated by the transcription factor NF-κB (Aggarwal et al., 2009). Khosravi et al. has proved the anti-inflammatory effects of exercise, especially in prostate and breast cancer survivors (Khosravi et al., 2019). Besides, recent studies suggested that obesity related-excess adipose tissue could promote the neoplasia and progression of tumor through adipose tissue inflammation (circulating cytokines such as TNF-α and IL-6) (Campbell et al., 2009; Iyengar et al., 2013). Accompanied by weight loss, exercise could attenuate adipose tissue inflammation in aged 20–40 years, overweight men (Auerbach et al., 2013; Ahmadizad et al., 2014) and obese postmenopausal breast cancer survivors (Dieli-Conwright et al., 2018).

The NF-κB family is composed of transcription factors and plays a complex and key role in the regulation of immune responses and inflammation (Tilborghs et al., 2017). NF-κB could be activated by almost all infectious agents links with cancer, e.g. human papillomavirus (James et al., 2006), HIV(DeLuca et al., 1996) and Helicobacter pylori (Keates et al., 1997). It was proved that exercise training could prevent tumor-induced TWEAK/NF-κB signaling pathway in skeletal muscle and had a beneficial effect on fiber cross-sectional area and metabolism. This exercise-induced muscle remodeling was related to less malignant mammary lesions in tumor-bearing animals (Padrao et al., 2017).

These findings provide new insights into the potential anti-cancer role of exercise. Since exercise has different parameters, future studies could carry in-depth research of interactions between different mechanisms and try to elucidate the recommended doses and intensities of exercise for cancer, especially in breast, colorectal, prostate cancer and hepatocellular carcinoma.

Compared with other reviews, our study based on Citespace provided a visualized research hotspots and frontiers. However, there are still some limitations in this study. Due to the limitation of Citespace software, we only searched SCI-Expanded and analyzed studies published in English. Therefore, the data may not be comprehensive enough. Articles published in other languages and databases need further research.

CONCLUSION

This study analyzed the research hot spots and frontiers of molecular mechanisms of exercise on cancer via CiteSpace. Based on the results, altered metabolism (aerobic glycolysis, insulin resistance, myokines), oxidative stress, gene expression and apoptosis were hot-research mechanisms of exercise on cancer. Emerging research foci of mechanisms were generally around inflammation, epithelial mesenchymal transition and adipokines. In addition, future studies could carry in-depth research of interactions between different mechanisms and try to elucidate the recommended doses and intensities of exercise for cancer, especially in breast, colorectal, prostate cancer and hepatocellular carcinoma.

AUTHOR CONTRIBUTIONS

RJ and JL designed the study. DZ and YL drafted the manuscript. XH revised the manuscript. YH analyzed the data. All authors contributed to the article and approved the submitted version.

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Swimming Attenuates Muscle Wasting and Mediates Multiple Signaling Pathways and Metabolites in CT-26 Bearing Mice

Jiapeng Li[1,2†], Qiurong Xie[3,4†], Liya Liu[3,4], Ying Cheng[3,4], Yuying Han[3,4], Xiaoping Chen[3,4], Jia Lin[5], Zuanfang Li[3,4], Huixin Liu[3,4], Xiuli Zhang[3,4], Haichun Chen[2,6], Jun Peng[3,4] and Aling Shen[3,4]**

[1]The Department of Physical Education, Fujian University of Traditional Chinese Medicine, Fuzhou, China, [2]School of Physical Education and Sport Science, Fujian Normal University, Fuzhou, China, [3]Academy of Integrative Medicine, Fuzhou, China, [4]Fujian Key Laboratory of Integrative Medicine in Geriatrics, Fuzhou, China, [5]Rehabilitation Industry Institute, Fujian University of Traditional Chinese Medicine, Fuzhou, China, [6]Provincial University Key Laboratory of Sport and Health Science, School of Physical Education and Sport Sciences, Fujian Normal University, Fuzhou, China

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***Correspondence:**
Jun Peng
pjunlab@hotmail.com
Aling Shen
saling86@hotmail.com

[†]These authors have contributed equally to this work and share first authorship

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Objectives: To investigate the effects of swimming on cancer induced muscle wasting and explore its underlying mechanism in CT-26 bearing mice.

Methods: BALB/c mice (n = 16) injected with CT-26 cells were divided into two groups, including Tumor group (n = 8) and Swimming group (n = 8). Another 8 un-injected mice were set as Control group. Mice in Swimming group were subjected to physical training for swimming twice per day for 30 min intervals and 6 days per week for a total of 4 weeks. The tumor volume was monitored every 3 days and tumor weight was measured at the end of experiment. The changes of muscle function, pathological and cell apoptosis of quadriceps muscles were further assessed, and its underlying mechanisms were further explored using multiple biological technologies.

Results: Swimming obviously alleviated tumor volume and weight in CT-26 bearing mice. Moreover, swimming attenuated the decrease of muscle tension, autonomic activities, and increase of muscle atrophy, pathological ultrastructure, as well as cell apoptosis of quadriceps muscles in CT-26 bearing mice. Furthermore, swimming significantly down-regulated the protein expression of NF-κB, p-NF-κB, TNF-α, IL-1β, IL-6 and Bax, while up-regulated the expression of Bcl-2. Further differential expressed metabolites (DEMs) analysis identified a total of 76 (in anion mode) and 330 (in cationic mode) DEMs in quadriceps muscles of CT-26 bearing mice after swimming, including taurochenodeoxycholic acid, taurocholic acid, ascorbic acid and eicosapentaenoic acid.

Conclusion: Swimming attenuates tumor growth and muscle wasting, and by suppressing the activation of NF-κB signaling pathway mediated inflammation, reducing the level of Bax medicated cell apoptosis, as well as modulating multiple metabolites might be the importantly underlying mechanisms.

Keywords: swimming, colorectal cancer, muscle wasting, NF-κB, metabolite

INTRODUCTION

Cancer has become a leading cause of death worldwide, including in China, with an increasing burden of cancer incidence and mortality observed over the past half century (Wei et al., 2020). Recently statistics indicated that there are 18.1 million new cases and 9.6 million cancer deaths worldwide in 2018, of which, nearly 24% (4.3 million) of these cancer cases and 30% (2.9 million) of deaths have occurred in China (Sung et al., 2021). As the population ages, the number of cases of cancer will continue to rise significantly (Chen et al., 2016). Many cancers trigger rapid muscle wasting, a condition also known as cachexia, that in turn leads to resistance to treatment, low quality of life and death (Baracos et al., 2018). Though couples of therapeutic strategies have been adopted in an attempt to alleviate cancer cachexia and improve the quality of life of cancer patients (Kumar et al., 2010). Due to the complex processes of cancer induced cachexia, most of these therapeutic approaches based on a single target, have failed so far. Therefore, efforts to further explore the strategies on attenuating cancer induced cachexia may ultimately improve treatment response and quality of life of cancer patients.

As one of the primary causes of morbidity and mortality associated with cancer, cachexia is most commonly observed in individuals with advanced cancer, presenting in 80% of these patients (Fukawa et al., 2016). One of prominent clinical features of cancer-induced cachexia is muscle wasting, which is a complex phenomenon and characterized by significant decrease in muscle fiber cross-sectional area, myonuclear number, protein content and muscle strength (Musolino et al., 2016). These structural and functional changes in muscles deteriorate the quality of life and even lead to human disease and death (Dutt et al., 2018).

The pathophysiologic mechanisms underlying muscle wasting in cachexia are complex, including systemic inflammation (Aversa et al., 2017). Multiple cytokines, including TNF-α, interleukin-1 (IL-1), IL-6 and IFN-γ, had been reported to play an essential role in the induction of cancer-related muscle wasting (Argiles and Lopez-Soriano, 1999). Increasing studies revealed that the elevated levels of proinflammatory cytokine TNF-α in patients play an important role in the evolvement of muscle wasting (Jekell et al., 2004) *via* inducing the breakdown of mature myotubes (Guttridge et al., 2000). With the stimulation of TNF-α, the activation of transcription factor NF-κB is obviously increased, which in turn promotes muscle wasting by inhibiting the synthesis of MyoD and muscle regeneration, as well as promoting atrophy (Bowen et al., 2015). The activation of NF-κB further promotes the synthesis of cytokines, which can contribute to muscle wasting as described above (Thoma and Lightfoot, 2018).

These studies suggested that inhibiting cancer induced inflammation by reducing the levels of pro-inflammation cytokines and activation of NF-κB pathway represent a strategy for treatment of cancer induced muscle wasting. Recently both epidemiological studies and clinical trials revealed that the prognosis of physically active cancer patients is improved due to the exercise performed after cancer diagnosis as opposed to exercise habits before the disease (Irwin et al., 2008). Since exercise strongly related to skeletal muscle, exercise training has long been proposed to counteract muscle wasting in cachexia (Zhang et al., 2019). Proper physical exercise increases muscle mass and volume, enhances immune system function and improves metabolism and body composition (Re Cecconi et al., 2019).

Due to the difficulty for cancer patients to perform high-intensity exercises, low-intensity exercises, like swimming might be more acceptable. However, as a major kind of aquatic exercise, the benefits of swimming and its underlying mechanisms on cancer induced muscle wasting remains largely unknown. In the current study, we evaluated the benefits of swimming on tumor growth, muscle wasting, and its underlying mechanisms.

MATERIALS AND METHODS

Reagent and Antibodies

Fetal bovine serum (cat. no. 10099141), RPMI 1640 (cat. no. 1049101), Trypsin-EDTA (cat. no. 25200072), penicillin-streptomycin (cat. no. sv30010) and the BCA protein assay kit (cat. no. 23225) were purchased from Thermo Fisher Scientific (Sunnyvale, CA, USA). Antibodies against interleukin-6 (IL-6) (cat no. ab208113), NF-κB (cat. no. Abm40053), p-NF-κB (cat. no. Abm50373) and tubulin (cat. no.11H10) were purchased from Abcam (Cambridge, United Kingdom), while interleukin-1β (IL-1β) (cat. no. 12242S), Bax (cat. no.14796), Bcl-2 (cat. no. 2876) antibodies and Horseradish peroxidase (HRP)-conjugated secondary antibody (cat. no.7074) were bought from Cell Signaling Technology (Danvers, MA, USA). TNF-α (cat. no. 41504) antibody was obtained from SAB (MD, USA). Hematoxylin solution (cat. no. G1140) and eosin solution (cat. no. G1100) were purchased from Solarbio (Beijing, China). RIPA lysis buffer (cat. no. P0013, Beyotime, Shanghai, China), protease inhibitor (cat. no. 539131-10VLCN, MCE, NJ, USA) and phosphatase inhibitors (cat. no. 4906845001, Roche, Basel, Switzerland) were used for protein extraction. Matrigel (cat. no. 354234) was purchased from BD Biosciences (San Jose, CA, USA).

Cell Culture

The CT-26 murine colon carcinoma cell line was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium (cat. no. 8121369) supplemented with 10% of FBS (cat. no. 2254375CP) and 1% of penicillin (100 U/ml) and streptomycin (100 μg/ml) in a 37°C, 5% of CO_2, humidify of incubator. Cells were sub-cultured at 80–90% of confluence.

Animal Experiments

Male BALB/c mice (5–6 weeks; 20.8 ± 1.4 g) were purchased from Shanghai SLAC Laboratory Animal Co. (Shanghai, China) and acclimatized for 1 week before used. Mice were housed under a specific pathogen-free (SPF) condition with a 24–28°C of temperature, 60 ± 5% of humidity, 12-h dark/light cycle. Food and water were given ad libitum throughout the experiment. Animal care and experiments were performed in strictly accordance with the "Guide for the Care and Use of

Laboratory Animals" and the "Principles for the Utilization and Care of Vertebrate Animals", and approved by the Committee of Fujian University of Traditional Chinese Medicine (No. FJTCM IACUC 2019042).

Construction of mouse Xenograft.—After the acclimation period, CT-26 cells (1×10^6 cells/ml) in a total volume of 100 µl of PBS containing 50% matrigel were injected subcutaneously into the right flank area of the mice ($n = 16$). After seeding of 3 days, the tumor-bearing mice were randomly divided into Tumor group ($n = 8$) and Swimming group ($n = 8$) according to tumor volume. Another 8 un-injected mice were set as Control group.

Swimming training—In the current study, CT-26 bearing mice in the swimming group were subjected to physical training in water (30 ± 2°C) twice per day for 30 min intervals. This training was conducted 6 days per week for a total of 4 weeks. The mice participated in voluntarily swim for an estimated 10 min at initially, then floated on the water, and swam intermittently, each time extended for 10 min until 30 min. In order to make the mice swim continuously for longer duration, we used a stick to pull the water to drive them.

Tumor Volume and Weight

Measurement of tumor volume and tumor weight— During the experiment, the electronic vernier caliper was used to measure the major (L) and minor (W) diameter of tumors. The tumor volume was calculated every 3 days on the basis of the following formula: tumor volume = $L \times W^2/2$. At the end of experiment, the mice were anaesthetized with isoflurane and tumor tissues were removed and weighed, followed by fixed with 4% of formaldehyde until used.

Grip Force Assessment and Autonomic Activity Test

Skeletal muscular tension of mice was quantified by the grip-strength test. The grip-tension device (cat. no. DS2-20N) was obtained from Xinruan Corp. (Shanghai, China) and was comprised of a mesh tension test board connected to an isometric force transducer. Basically, the grip-tension meter was positioned horizontally, and the mice were held by the tail and lowered towards the device. The mice were allowed to grasp the mesh tension test board and pulled backwards in the horizontal plane. The force applied to the bar just before it lost grip was recorded as the peak tension and carried out five times for each mouse. The strength test was performed before the first training, after the 2nd and 4th week of training.

Autonomic activity of mice was measured by the multifunctional mice independent activities recorder (cat. no. ZZ6, Taimeng Corp, Chengdu, China). The mice were placed in a dark compartment and covered with a light-shielding lid. The mice activities recorder with 36 infrared sensing points was used to count the number of mice that stood continuously within 30 min.

HE Staining

The cross-sectional tissues of quadriceps femoris from each mouse were fixed with 4% paraformaldehyde (PH 7.4) for 24 h, processed, embedded in paraffin, and cut into 4-µm-thick sections. The sections were dewaxed and dehydrated. For histologic assessments, sections were stained with hematoxylin solution for 60 s, differentiated with 1% hydrochloric acid ethanol for 3 s, and stained with eosin-phloxine solution for 20 s. The tissues on each slide were added to a coverslip and imaged at magnification of 200 using a Leica DM400B microscope (Leica, Wetzlar, Germany).

Transmission Electron Microscopy Analysis

Tissue pieces of quadriceps muscle (about 1 mm^3) from each mouse were immersion-fixed for 2 h at 4°C in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.2 M phosphate buffer (pH 7.4), washed, and then postfixed in 1% osmium tetroxide. After rinsing in the phosphate buffer, the tissue pieces were dehydrated in ascending grades of ethanol ending with propylene oxide and embedded in epoxy resin. Semithin sections (1 µm) were prepared using ultra microtome (Leica, Germany) and stained with 1% toluidine blue. Ultrathin sections (90 nm) were cut, mounted on copper grids and stained with 2% uranyl acetate for 10 min, followed by lead citrate staining for 10 min and examined in a transmission electron microscope (Hitachi H-7650, Hitachi High-Technologies Corporation. Japan).

TUNEL Staining of Muscle Cells

TUNEL staining was performed to detect muscle cell apoptosis, according to the instructions. After dewaxing and gradient alcohol, the sections were incubated in proteinase K working solution at 37°C in a humidified atmosphere for 15 min. TUNEL reaction mixture (50 µl) was added and incubated for 60 min at 37°C. After rinsed with PBS for 3 times, 50 µl of converter-peroxidase was added to the sections and incubated at 37°C for an additional 30 min, and then rinsed with PBS for three times, incubated with the 100 µl diaminobenzidine substrate. Counterstained with hematoxylin and analyzed by light microscopy (Leica DM400B, Germany) at ×400 magnification. The cells with brown nucleus were defined as apoptotic cells. The percentage of apoptotic cells was calculated as the ratio of the number of TUNEL-positive cells to the total number of cells.

Immunohistochemical Analysis

The sections from each group were subjected to antigen retrieval and the endogenous peroxidase activity, and blocked with 3% hydrogen peroxide. After blocking non-specific proteins at room temperature for 10 min, the sections were incubated with primary antibodies against Bax, Bcl-2, TNF-α, IL-1β, IL-6, NF-κB or p-NF-κB (all in dilution, 1:200) at 4°C overnight. After washed with PBS, the slides were incubated with HRP-conjugated secondary antibody and then washed with PBS. The sections were then incubated with DAB as the chromogen, followed by counterstaining with diluted hematoxylin. After staining, five randomly selected images from each sample were taken at a magnification of ×400, and the average percentage of positive stained cells in each field was counted using Image-Pro Plus (Media Cybernetics, Rockville, MD, USA).

Analysis of Western-Blotting

The muscle tissues were homogenized and total protein were extracted using RIPA lysis buffer, and centrifuged for 10 min (12,000 rpm, 4°C), the supernatant with total protein was aspirated into a new clean tube. The BCA Protein Assay Kit was used to determine protein concentrations according to the manufacturer's protocol. Equal amounts of total protein from each sample were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamidegel electrophoresis (PAGE), transferred to polyvinylidene fluoride (PVDF) membrane (cat. no. ISEQ00010, Millipore, MA, USA). Non-specific protein interactions were blocked by incubation with 5% non-fat milk in Tris-buffered saline with Tween 20 (50 mM Tris-HCl, 150 mM NaCl and 0.05% Tween 20; pH 7.6) at room temperature for 2 h. Membranes were incubated with primary antibodies against Bcl-2, Bax or p-NF-κB (all in 1:1,000 dilution) overnight at 4°C followed by incubation with an HRP-conjugated secondary antibody (1:5,000 dilution) at room temperature for 2 h. Proteins were detected using a chemiluminescence detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and were visualized using enhanced chemiluminescence reagent (Beyotime, Shanghai, China). Tubulin was used as internal control.

Metabolomics Study

Quadricep muscles of mice from both Tumor group ($n = 5$) and the Swimming groups ($n = 5$) were collected and stored at −80°C. For metabolic profiling, archived quadriceps muscle samples were ground and mixed with 1:10 (w/v) ice cold extraction solution (a mixture of water, methanol, acetonitrile acentone with the volume ratio of 1:3:3:3 respectively). The mixtures were shaken at room temperature for 10 min, and then placed in the refrigerator at -20°C for 30 min, followed by centrifugation of the mixtures for 15 min (14,000 g, 4°C). The supernatant fractions were collected for LC-MS analysis. A pooled quality control (QC) sample was prepared by mixing equal amounts of each quadricep muscle sample. LC-MS analysis was performed on Atlantis PREMIER BEH C18 AX VanGuard FIT Column using the UHPLC 3000 System coupled to a Q Exactive System (Thermo Fisher Scientific), at both positive and negative ion modes. The metabolites were identified by MZCloud and ChemSpider. The MetaboAnalst (http://www.metaboanalyst) was used to data analysis and data visualization, including PCA (Principal Component Analysis), Cluster heat map, Metabolite Collection and Enrichment Analysis (MCEA), and Metabolite Pathway Analysis (METPA).

Statistical Analysis

Statistical analyses were performed using the SPSS statistical program (SPSS/PC+, version 22.0, Chicago, IL, USA). The results were presented as the mean value ±standard deviation (SD). Three group data used One-way analysis of variance (ANOVA) to compare statistical significance when the data met the normal distribution. Two group data used Student's T-TEST. Differences associated with $p < 0.05$ were considered statistically significant.

RESULTS

Swimming Attenuates Tumor Growth of CT-26 Cells *In Vivo*

To assess the benefits of swimming on tumor growth, CT-26 cells were transplanted into BALB/c mice and followed by quantitative daily swimming. Monitor of tumor volume indicated that swimming obviously alleviated tumor volume, when compared to Tumor group (**Figure 1A**; $^{*}p < 0.05$ *vs*. Tumor group). Consistently, determination of tumor weight confirmed that swimming significantly attenuated tumor weight (**Figure 1B**; $^{*}p < 0.05$, *vs*. Tumor group).

Swimming Alleviates the Decrease of Muscle Function and Autonomic Activity of CT-26 Bearing Mice

Tumor growth was accompanied by a significant decrease in muscle tension in the Tumor group at the 2nd and 4th weeks (**Figure 2A**, $^{\#}p < 0.05$ vs. Controlgroup), which was attenuated in the Swimming group (**Figure 2A**, $^{*}p < 0.05$ vs. Tumor group). Similarly, compared to the Tumor group, swimming significantly limited the decrease in autonomic activity at the 2nd and 4th weeks (**Figure 2B**, $^{\#}p < 0.05$ vs. Control group; $^{*}p < 0.05$ vs. Tumor group).

Swimming Attenuates Muscle Wasting and Pathological Ultrastructure of CT-26 Bearing Mice

Observation of pathological changes of quadriceps muscles by HE staining revealed a significant decrease of muscle fibers and cross sectional areas in the Tumor group suggesting a sign of muscle wasting, which were attenuated in Swimming group (**Figure 3A**). Observation of microscopic structural of muscle by transmission electron microscopy indicated a neatly arranged myofibrils of quadriceps with complete structure, distributedion of mitochondria near Z-line, and alternated light and dark bands of myofibrils with clearly I band and A band respectively (**Figure 3B**). However, we observed damaged or irregularly arranged myofibrils of quadriceps, with less or without clearly Z line and M line, as well as vacuolation and shrinkage of mitochondria in myofibrils of quadriceps in Tumor group, which were attenuated in Swimming group (**Figure 3B**).

Swimming Reduced Cell Apoptosis, Decreased the Expression of Bax and Promoted the Expression of Bcl-2 in Quadriceps Muscle of CT-26 Bearing Mice

TUNEL analysis of quadriceps muscle tissues from each group revealed that the percentage of apoptotic quadriceps muscle cells was significantly increased in Tumor group, while were attenuated in Swimming group (**Figure 4A**, $^{\#}p < 0.05$ *vs*. Control group; $^{*}p < 0.05$ *vs*. Tumor group). Moreover,

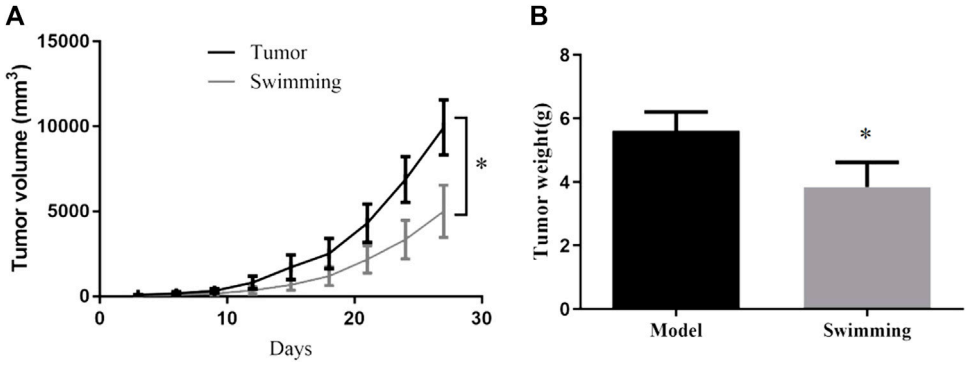

FIGURE 1 | Effects of swimming on tumor volume and tumor weight of CT-26 bearing mice. **(A)** Tumor volume was monitored during the exercise period for 28 days. **(B)** Tumor weight was determined by electronic scale at the end of the experiment. Data are present as mean ± SD. *$p < 0.05$, *vs* Tumor.

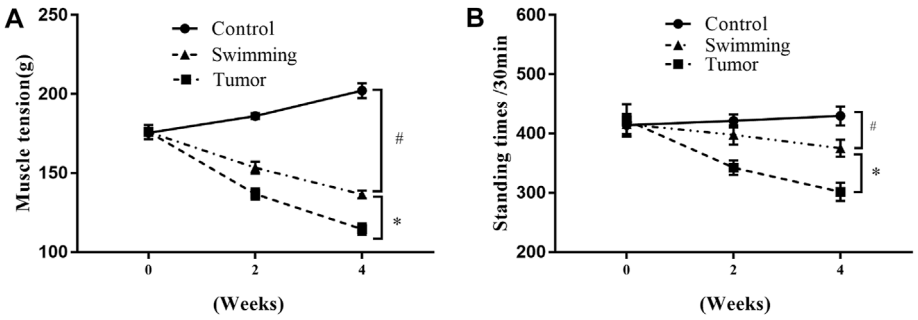

FIGURE 2 | Effects of swimming on muscle function in transplanted tumor mice. **(A)** Skeletal muscular tension of mice was quantified by the grip-strength test. The strength test was performed before training, after the 2nd and 4th weeks of training. **(B)** Autonomic activity of mice was measured by the multifunctional mice independent activities recorder. The autonomous activity of the mice was recorded. Data are shown as mean ± SD. #$p < 0.05$, *vs*. Control; *$p < 0.05$, *vs*. Tumor.

further determination of Bax and Bcl-2 protein expression by IHC revealed that the expression level of pro-apoptotic protein Bax was decreased while that of anti-apoptotic Bcl-2 was obviously elevated in quadriceps muscle tissues of mice in Swimming group, which compared with Tumor group (**Figure 4B**, $^{\#}p < 0.05$ *vs* Control group; $^{*}p < 0.05$ *vs*. Tumor group).

Swimming Decreased the Levels of Inflammatory Cytokines in Quadriceps Muscle Tissues of CT-26 Bearing Mice

To further explore the effects of swimming on attenuation muscle wasting and apoptosis, we further assessed the effects of swimming on level of inflammatory cytokines in quadriceps muscle tissues. As showed in **Figure 5A**, determination of inflammatory cytokines in muscle tissues from each group demonstrated that the level of IL-6 (**Figure 5A**, $^{\#}p < 0.05$ vs Control group) and TNF-α (**Figure 5B**, $^{\#}p < 0.05$ *vs*. Control group), as well as IL-1β (**Figure 5C**, $^{\#}p < 0.05$ *vs*. Control group) were obviously increased in the muscle of Tumor group, while were attenuated in that of Swimming group (**Figures 5A–C**; $^{*}p <$ 0.05 *vs*. Tumor group).

Swimming Alleviated the Activation of NF-κB Signaling Pathway in Quadriceps Muscle Tissues of CT-26 Bearing Mice

To further explore the underlying mechanism of swimming on reducing the elevated levels of inflammatory cytokines, we further determined the activation of the NF-κB signaling pathway. As shown in **Figures 6A–C**. IHC analysis reveal that the levels of both NF-κB (**Figure 6A**) and p-NF-κB (**Figure 6B**) increased in quadriceps muscle tissues of mice in Tumor group (all $^{\#}p < 0.05$ *vs*. Control group), while all were attenuated in Swimming group (all $^{*}p < 0.05$ *vs*. Tumor group). Consistently, Western-blotting analysis further confirmed that the protein expression of p-NF-κB significantly increased in the muscle tissues of Tumor group, which were decreased in that of the Swimming group (**Figure 6C**).

Swimming Improves Quadriceps Muscle Metabolism of CT-26 Bearing Mice

Based on the benefits of swimming on preventing tumor growth and muscle wasting, we further determined the metabolomic profiling of quadriceps muscle obtained from mice of both Tumor and Swimming groups. PCA score plots clearly showed

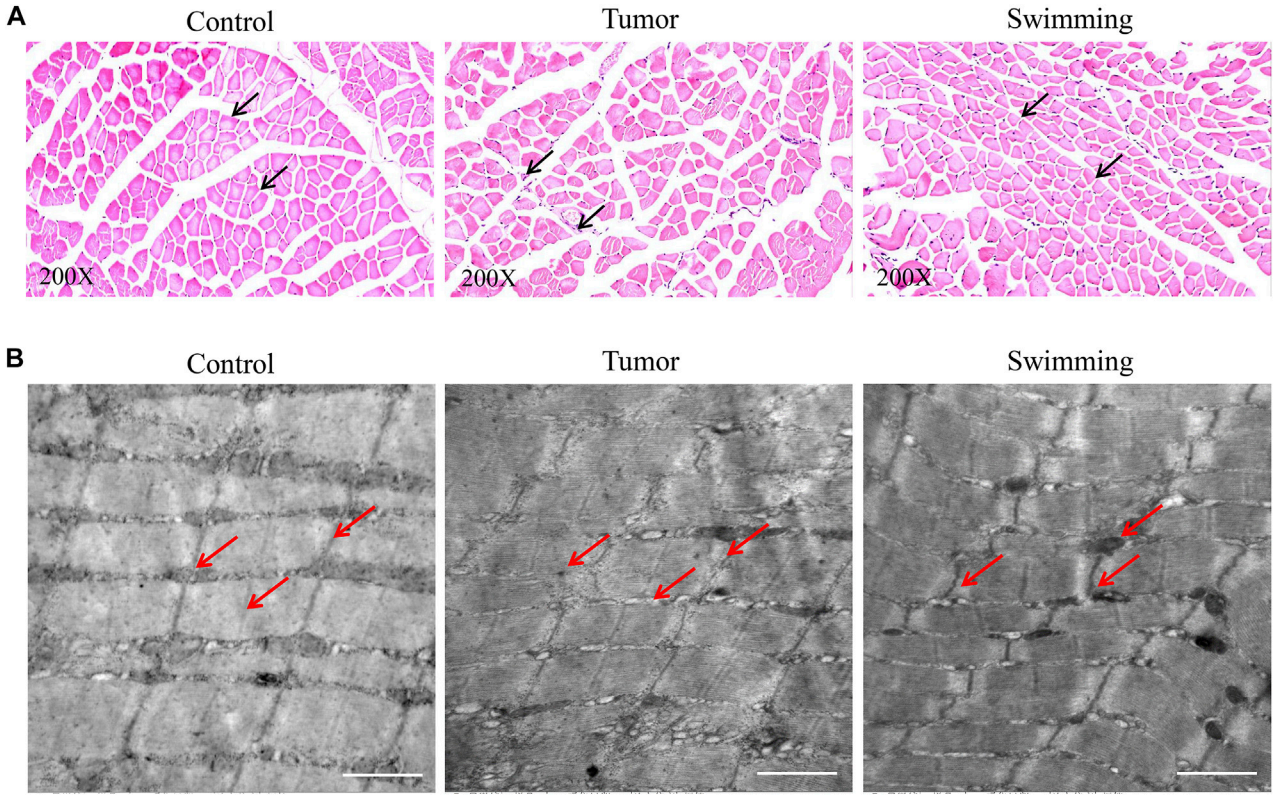

FIGURE 3 | Microscopic observation of muscular tissues in Control, Swimming and Tumor groups. **(A)** HE staining was used to determine and observe the pathological changes of quadriceps muscles from each group. The images were taken at a magnification of ×200 **(B)** Transmission electron microscope was performed to determine and observe the microstructure changes of quadriceps muscles from each group.

good separation between the two groups, indicating regular swim training makes a big difference in the metabolic profile of muscle (**Figure 7A**). The ions with variable importance in the projection (VIP) values > 1.0 were identified as the potential DEMs. Volcano plots exhibited the variation tendency of the metabolites with VIP values > 1.0 and adjusted by p values < 0.05 between two groups (**Figure 7B**). Compared with the Tumor group, a total of 76 DEMs were identified under anion mode (43 up-regulated and 33 down-regulated), and 330 were identified under the cationic mode (179 up-regulated and 151 down-regulated). Among the DEMs, the level of taurochenodeoxycholic acid, taurocholic acid, ascorbic acid and eicosapentaenoic acid in Swimming group were significantly higher compared with the Tumor group (**Figure 8**, all $^{*}p < 0.05$ *vs.* Tumor group).

DISCUSSION

Muscle wasting in cancer cachexia contributes to resistance to treatment, low quality of life and death (Guo et al., 2017). We therefore explored the therapeutic approaches in an attempt to counteract cancer cachexia and improve treatment response, as well as quality of life of cancer patients. Using a tumor-bearing mice of CT-26 cells (Acharyya et al., 2005), the current study confirmed that swimming obviously attenuated the tumor growth, increased muscle strength and autonomic activity of CT-26 cells bearing mice. Moreover, swimming also alleviated muscle wasting, pathological ultrastructure of quadriceps muscles in CT-26 bearing mice. Mechanistic studies revealed that swimming reduced cell apoptosis and down-regulated the protein expression of Bax, up-regulated the protein expression of Bcl-2 in muscle tissues of CT-26 bearing mice, and inhibited the activation of NF-κB and its downstream pro-inflammatory cytokines (including TNF-α, IL-6 and IL-1β) which might be one of its underlying mechanisms. These studies suggest the potential of swimming as a supplementary therapeutic approach for cancer treatment.

More than 80% of patients with advanced cancer develop cancer-associated weight loss, a syndrome characterized by a forfeiture of muscle and a decline in functional status, quality of life, and survival (Doles et al., 2018). Physical activity has proved to be an effective therapeutic strategy due to its effect on both strength and muscle mass (Wang et al., 2021). Moderate exercise could relieve muscle wasting and prevent the loss of muscle strength through reducing the levels of reactive oxygen species (ROS), carbonylated proteins, markers of autophagy, and improving antioxidant capacity (Ballaro et al., 2019).

Due to the difficulty for cancer patients to perform high-intensity exercises, we assessed the effects of swimming (low-intensity exercises) on tumor growth and muscle wasting. As

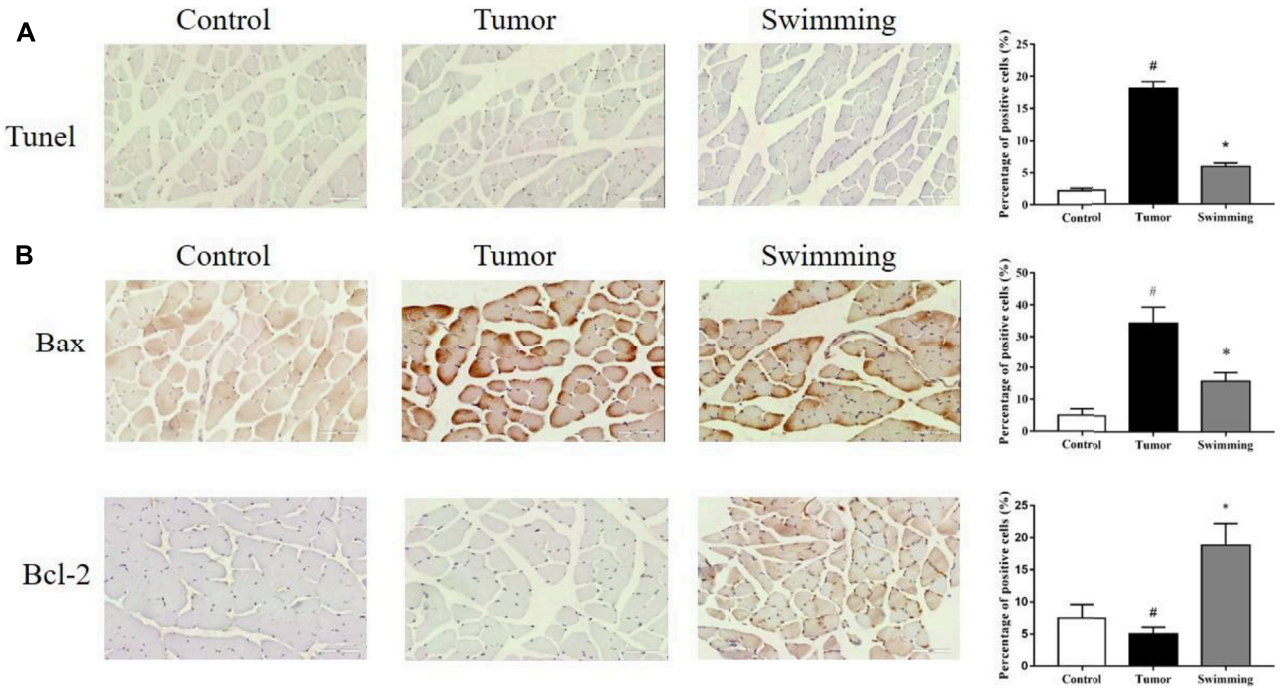

FIGURE 4 | Effects of swimming on cell apoptosis and expression of Bax and Bcl-2 in muscle tissues. **(A)** TUNEL staining was performed determine the cell apoptosis in muscle tissues from each group. The images were taken at a magnification of ×400 by microscopy. The cells with brown nucleus were defined as apoptotic cells. **(B)** The protein levels of Bax and Bcl-2 in quadriceps muscle tissues of mice from each group was determined by IHC analysis. The images were taken at a magnification of ×400 by microscopy. The cells with brown staining were defined as positive stained cells. Data were presented as mean ± SD. #$p < 0.05$ *vs*. Control group; *$p < 0.05$ *vs*. Tumor group.

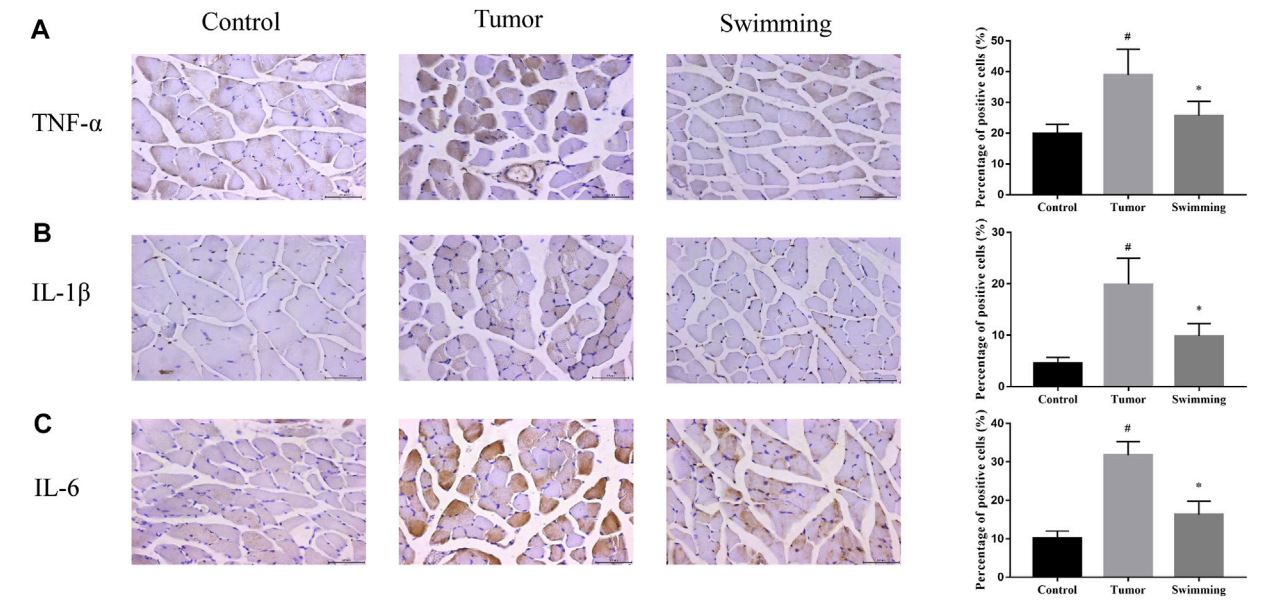

FIGURE 5 | Effects of swimming on level of inflammatory cytokines in quadriceps muscle tissues. The protein levels of **(A)** TNF-α, **(B)** IL-1β and **(C)** IL-6 in quadriceps muscle tissues of mice from each group was determined by IHC analysis. The images were taken at a magnification of ×400 by microscopy. The cells with brown staining were defined as positive stained cells. Data were presented as mean ± SD. #$p < 0.05$ *vs*. Control group; *$p < 0.05$ *vs*. Tumor group.

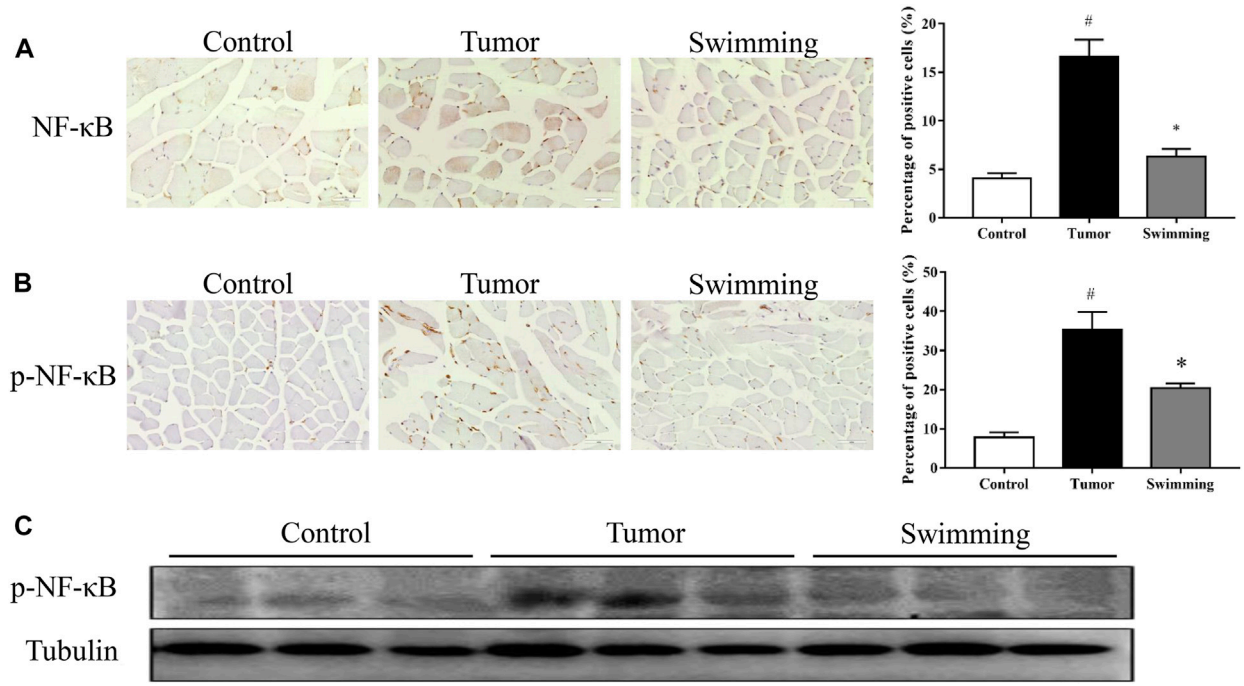

FIGURE 6 | Effects of swimming on NF-κB signaling pathway. The protein levels of **(A)** NF-κB and **(B)** p-NF-κB in quadriceps muscle tissues of mice from each group was determined by IHC analysis. The images were taken at a magnification of ×400 by microscopy. The cells with brown staining were defined as positive stained cells. **(C)** The protein expression of p-NF-κB in quadriceps muscle tissues for each group was determined by Western-blot analysis. The tubulin was used as a loading control. Data were presented as mean ± SD. #$p < 0.05$ *vs*. Control group; *$p < 0.05$ *vs*. Tumor group.

expected, swimming obviously alleviated tumor growth in CT-26 bearing mice, which is consistent with a previous study (Almeida et al., 2009). More importantly, swimming also attenuated the decrease of muscle strength and autonomic activity, as well as muscle fibers and cross-sectional areas of quadriceps muscles in CT-26 bearing mice. These studies suggest swimming might have contributed to the improvement in muscle wasting.

Consistently, TUNEL assay indicated that swimming significantly reduced the number of TUNEL positive staining cells in quadriceps muscles of CT-26 bearing mice. Usually, muscle wasting is accompanied by the apoptosis of a large number of muscle cells, corresponding expression changes of Bcl-2 and Bax also occur in muscle tissue (Murphy et al., 2011). Interestingly, mechanistic study revealed an up-regulation of Bcl-2 and down-regulation of Bax on protein levels in quadriceps muscles of CT-26 bearing mice after swimming, which might be one of the mechanisms of swimming on attenuating cell apoptosis in quadriceps muscles of CT-26 bearing mice. However, the complex mechanism should be further explored by determining the activation of related signaling pathway. Taken together, comparing with other voluntary exercise, swimming exhibits potential on serving as a therapeutic approach for cancer patients, particularly the elderly cancer patients, which might be more acceptable.

The therapeutic potential of swimming on cancer cachexia and muscle wasting encouraged us to further explore its underlying mechanism, due to the essential role of systemic inflammation on cancer induced muscle wasting and cachexia (Pin et al., 2015). Consistent with the previously study (Tisdale 2009), we observed the increase of inflammatory cytokines of TNF-α and IL-6 levels in quadriceps muscles of CT-26 bearing mice, which were significantly reduced after swimming. Increase of inflammation cytokines lead to the activation of transcription factor NF-κB, resulting in promoting muscle wasting and atrophy (Cai et al., 2004). More importantly, NF-κB pathway activation further promotes the synthesis of cytokines, contributing to muscle wasting as described above (Bonetto et al., 2016). Consistently, we found a significant increase of both NF-κB and p-NF-κB expression in quadriceps muscles of CT-26 bearing mice, which were significantly reduced after swimming. These results suggested that the suppression of NF-κB pathway activation and synthesis of inflammation cytokines might be one of the underlying mechanisms for swimming on attenuating cancer induced muscle wasting. However, the translocation of NF-κB should be further assessed both *in vivo* and/or *in vitro* systems.

With the development of metabolomics, metabolism is emerging as one of the key factors among various mechanisms that contribute to the regulation of the signaling pathways leading to apoptosis (Andersen and Kornbluth, 2013; Matsuura et al., 2016) and the development of inflammation in cancer (Gaber et al., 2017). Recently, the results acquired in both experimental

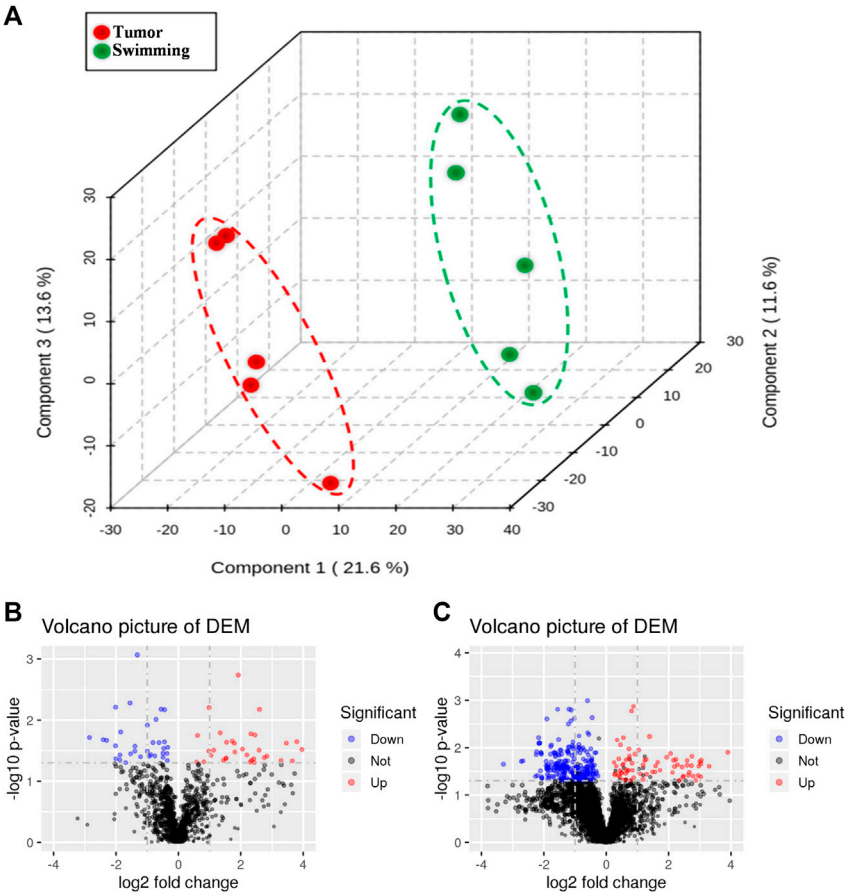

FIGURE 7 | Changes in metabolic profiling after regular swimming training. **(A)** PCA plot was obtained by reduction of dimensionality for LC-MS data of Tumor group and Swimming group (explained variance by Component1 21.6%, Component2 11.6% and Component3 13.6%). The red circle shows the distribution of Tumor group in PCA plot, while the green one displays the location of Swimming group. Distance represents the difference between two groups. Volcano plots of differential metabolite screening between the Tumor group and the Swimming group at both **(B)** positive and **(C)** negative ion modes. The blue and red dots respectively mark down-regulated and up-regulated metabolites after swimming training, whereas the black ones represent no differences between the two groups.

and clinical studies clearly demonstrate that energy and protein dysmetabolism are closely related to muscle wasting during cancer cachexia (Penna et al., 2018). It is becoming increasingly evident that cachexia can be effectively improved by modulating muscle metabolism (Carson et al., 2016; Aversa et al., 2017; Penna et al., 2018).

Therefore, further metabolomic profiling analysis of quadriceps muscles between Swimming and Tumor groups identified a variety of DEMs, including taurochenodeoxycholic acid, taurocholic acid, ascorbic acid and eicosapentaenoic acid, which are closely related to inflammation and immune regulation. Among them, taurfodeoxycholic acid plays an important role in anti-inflammatory effects through the TGR5 receptor-induced cAMP-PKA-CREB signaling pathway, specifically by activating kappa light chain enhancer in B cells, reducing the activities of TNF-α, IL-1β and IL-6 (Qi et al., 2020). Taurocholic acid not only exhibits the effects of anti-inflammation, lowering blood pressure, and reducing the amplitude and frequency of cardiac contraction (Taranto et al., 2001), it is also involved in immune response by directly inhibiting the apoptosis of immune cells at different stages (Schwarz et al., 1975; Rodriguez-Garay et al., 1999). Ascorbic acid is a hexacarboxylate, also known as vitamin C, was involved in inhibiting the growth of HCT116 cells in mice, prolonging the survival rate of CRC patients, enhancing the sensitivity of colorectal cancer to chemotherapy, and reducing the adverse reactions of radiotherapy and chemotherapy (Siegel et al., 2014; Roncucci and Mariani, 2015). Eicosapentaenoic acid is an omega-3 polyunsaturated fatty acid, which has an effect on anti-tumor, anti-inflammatory, anti-oxidative stress, and reduces the risk of cardiovascular disease (Pappalardo et al., 2015; D'Eliseo and Velotti, 2016). In addition, it can also balance metabolism, inhibit proliferation and induce apoptosis (Brinton and Mason, 2017). Combined with our findings, we assumed that reducing the level of muscle inflammation by metabolic compensation in the Swimming

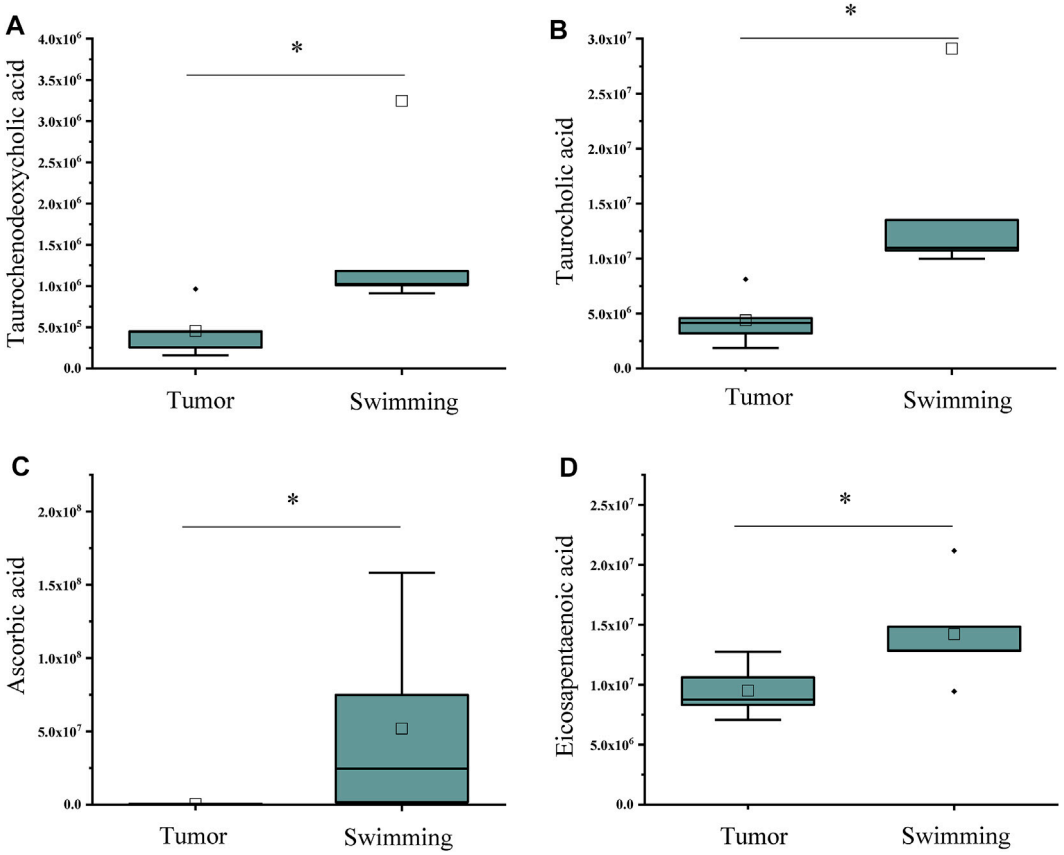

FIGURE 8 | Statistical analysis of differential metabolites associated with inflammation and immune regulation. The levels of **(A)** Taurochenodeoxycholic acid, **(B)** Taurocholic acid, **(C)** Ascorbic acid and **(D)** Eicosapentaenoic acid in muscle tissues between Tumor and Swimming groups. *$p < 0.05$ vs the Tumor group.

group may also be one of the ways to attenuate muscle wasting in CT-26 bearing mice.

CONCLUSION

Swimming maintains the muscle fine structure, attenuates muscle wasting, and improves muscle function, suggesting its potential in serving as a therapeutic approach for cancer patients. Suppression of NF-κB signaling pathway, reduction in the level of inflammatory factors, elevated levels of differential metabolites associated with anti-inflammatory and anti-apoptosis in quadriceps muscles of CT-26 bearing mice might be the important underlying mechanisms contributing to the benefits of swimming on attenuating muscle wasting.

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

The animal study was reviewed and approved by the Committee of Fujian University of Traditional Chinese Medicine.

AUTHOR CONTRIBUTIONS

AS, JP, and HC conceived and designed the experiments. JL, QX, YL, and AS conducted bioinformatics analyses. JL, XZ, YC, XC, and HL conducted data analysis. YL, YC, and YH protein test and draw the images. LJ, ZL conducted morphological experiment. AS, JP, YL, HC, JL wrote and revised the manuscript. All authors read and approved the final version of the manuscript.

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Physical Activity and Cancer Status Among Middle-Aged and Older Chinese: A Population-Based, Cross-Sectional Study

Chunsu Zhu†, Zhiwei Lian†, Ying Chen and Jianmin Wang**

Fujian Medical University Cancer Hospital, Fujian Cancer Hospital, Fuzhou, China

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Edited by:
Yao Lin,
Fujian University of Traditional Chinese Medicine, China

Reviewed by:
Xiaolong Liu,
Mengchao Hepatobiliary Hospital of Fujian Medical University, China
Samuel T. Orange,
Newcastle University, United Kingdom
Ran Wei,
Changzhou University, China

***Correspondence:**
Ying Chen
fjbccy@fjmu.edu.cn
Jianmin Wang
wangjm8605@163.com

† These authors have contributed equally to this work and share first authorship

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Background: The relative contributions of demographic and lifestyle behaviors to the association between physical activity (PA) and cancer are poorly understood. This study assesses the relationship between PA level and cancer status considering the full activity spectrum within a large and representative Chinese population.

Methods: Data were derived from the Chinese Health and Retirement Longitudinal Study (using four-stage stratified probability-proportional-to-size sampling), including 416 cancer survivors and 14,574 individuals without cancer from 28 provinces in China. Cancer status and sites were self-reported, and PA, other health behaviors (e.g., smoking, drinking) and comorbidities (e.g., hypertension, diabetes) were assessed by a questionnaire. The total PA score was calculated using metabolic equivalent (MET) multipliers. Multivariable logistic regression was used to estimate differences in PA levels between cancer survivors and those without a cancer diagnosis, adjusting for age, sex, and other potential confounding factors.

Results: Cancer survivors (416, 2.8%) were more likely to be women than men (65.4 vs. 34.6%). They were older (age ≥65 years, 43.8 vs. 38.9%) and more likely to be overweight (18.3 vs. 13.3%), be depressed (49.5 vs. 37.6%), have quit smoking (17.8 vs. 14.4%), drink less (17.5 vs. 26.6%), sleep less (65.9 vs. 56.8%) and have more chronic comorbidities (≥2 comorbidities, 26.0 vs. 19.2%) than those without cancer. There was a significant associations between cancer status and participation in vigorous-intensity activity for at least 10 min every week, when compared with the inactivity [odds ratio (OR) = 0.56, 95% CI = 0.39–0.80], while no differences were observed in the moderate and light activity groups. Individuals who spent more than half an hour performing moderate or vigorous intensity activity every day were significantly less likely to report a cancer diagnosis than inactive individuals (moderate OR = 0.64, 95% CI = 0.48–0.86; vigorous OR = 0.50, 95% CI = 0.37–0.68). Participants who spent more than 2 h performing light, moderate or vigorous intensity activity reported fewer cancer

cases than their inactive counterparts. In addition, there was an inverse dose-response relationship between the total PA score and cancer status ($P_{-trend} < 0.001$).

Conclusion: Associations between PA and cancer status were independent of demographics, lifestyle confounders, and comorbidities. Cancer survivors are less physically active than those without cancer.

Keywords: physical activity, cancer, epidemiology, cancer survivors, cross-sectional study

INTRODUCTION

Cancer is a leading cause of morbidity and mortality around the world and contributes to one in eight deaths globally (Fitzmaurice et al., 2019). In 2017, there were 24.5 million cancer cases and 9.6 million cancer deaths (Fitzmaurice et al., 2019). New cancer incidence is expected to increase by nearly 70% by 2030 (Fitzmaurice et al., 2019). In China, approximately 4.3 million people were diagnosed with cancer in 2015 (Chen et al., 2016), and the prevalence of this disease is growing rapidly due to the aging population and westernized lifestyle (de Magalhães, 2013). Cancer patients can expect to live for decades thanks to earlier detection, better diagnostic and staging methods, and more effective treatments. For example, during the past decade, the number of cancer survivors in China has increased remarkably, and nearly 40.5% of cancer survivors live for more than 5 years (Zeng et al., 2018). Compared with individuals without cancer, cancer survivors are at increased risk for other chronic diseases, secondary complications, recurrence, and decreased physical function and quality of life (Aziz and Rowland, 2003; Jemal et al., 2007). Thus, attenuating secondary health problems in cancer survivors has become a major public health challenge. However, cancer incidence and recurrence can be prevented with a healthy lifestyle, including regular physical activity (PA), to some extent (Loprinzi et al., 2012; Lahart et al., 2015; Murray et al., 2020). PA is a modifiable behavior that is linked to several cancers and has been shown to be effective in the primary prevention of cancer (Moore et al., 2012; Hojman et al., 2018). Efforts to address modifiable risk factors can provide support for economical options to control cancer.

Previous studies have shown that PA has beneficial effects on the risk of developing certain types of cancer and also has favorable influences on outcomes among cancer survivors (Moore et al., 2016; Kerr et al., 2017; Matthews et al., 2020). There is convincing evidence that PA is associated with a lower risk of death and recurrence in cancer survivors, along with psychosocial wellness and life satisfaction (Lahart et al., 2015; Pudkasam et al., 2018; Van Blarigan et al., 2018). Increasing the level of PA participation can improve body composition, cardiopulmonary function, muscle strength and quality of life in cancer survivors, and it can also reduce the rate of cancer recurrence and cancer-related mortality (Haydon et al., 2006; Meyerhardt et al., 2006; Schmid and Leitzmann, 2014). It is essential that we better understand the prevalence and patterns of PA to promote healthy and proper PA among cancer survivors. The second American College of Sports Medicine Roundtable recommends that cancer survivors avoid inactivity and engage in specific levels of PA to improve common cancer-related outcomes, such as anxiety, depressive symptoms, and fatigue (Campbell et al., 2019). Unfortunately, however, few meet or exceed the PA guidelines for cancer survivors (Mo et al., 2021). Individuals with cancer may be less likely to engage in PA than cancer-free participants as a result of their diagnosis and treatment. However, previous studies on PA levels among cancer survivors and cancer-free controls have shown inconsistent results (Park et al., 2015; Wang et al., 2015; Friis et al., 2018; Morris et al., 2018). For instance, some reported that cancer survivors were more likely to have higher PA levels (Park et al., 2015), while others reported that cancer survivors are either not different from or have lower levels of PA than their cancer-free counterparts (Wang et al., 2015; Mowls et al., 2016; Friis et al., 2018). Although very informative, these mixed studies did not assess the wide range of covariates that we were able to include in our study, and some of these previous studies had small sample sizes, which led to limited generalizability. Moreover, the majority of these studies were carried out in Western populations. To the best of our knowledge, no large-scale population-based study in China to date has assessed the associations between PA level and cancer status while accounting for a wide range of potential confounding factors. Comparisons and assessments of the association between PA level and cancer status may inform the development and implementation of evidence-based PA recommendations for these survivors.

Therefore, the purpose of this study was threefold. First, we wanted to compare PA levels between cancer patients and their cancer-free counterparts while considering a wide range of cofounders within a large, nationally representative Chinese population aged ≥45 years. Second, we wanted to examine the potential dose-response relationship between the total PA score and cancer status. Finally, we wanted to explore whether demographic characteristics, unhealthy lifestyles and chronic comorbidities influenced these associations.

MATERIALS AND METHODS

Participants

The data used in this study were derived from the fourth wave of surveys of the China Health and Longitudinal Study (CHARLS), which is a nationally representative longitudinal survey of household residents aged ≥45 years in mainland China, with 19,752 individuals from 28 provinces, 150 countries/districts, and 450 villages/urban communities surveyed from March, 2018 to March, 2019. In the current study, we excluded those with missing values for cancer status (n = 47), PA (n = 339), age

($n = 76$), body mass index (BMI) ($n = 2901$), depressive symptoms ($n = 1950$), residence ($n = 1603$), and smoking ($n = 3$). Finally, 14,990 adults were included in our analysis. No analysis plan was prespecified or registered for this study.

Physical Activity

A modified version of the International Physical Activity Questionnaire-Short Form (IPAQ-SF) was used to measure PA in terms of vigorous, moderate, and light activity (see **Supplementary Material**; Craig et al., 2003). The participants were asked about the amount of time they spent on different types of PA during a typical week. Vigorous activity was defined as activities requiring hard/high-intensity physical effort and making breath much harder than normal, such as heavy lifting, digging, plowing, aerobics, fast bicycling, and cycling with a heavy load. Moderate activity refers to activities that take moderate physical effort and make breath somewhat harder than normal and may include carrying light loads, bicycling at a regular pace, or mopping the floor. Light activity was defined as walking at work and at home, traveling from place to place and any other walking for recreation, sport, exercise or leisure. The response to the amount of PA in 1 day was indexed as $1 \leq 0.5$ h; 2 = 0.5–2 h; 3 = 2–4 h; and $4 \geq 4$ h (Deng and Paul, 2018). The PA duration score for a week was calculated by multiplying the daily PA duration index for each activity by the number of days. Then, the total PA score was calculated with metabolic equivalent (MET) multipliers as follows: total PA score = 8 × total vigorous activity weekly duration score + 4.0 × total moderate activity weekly duration score + 3.3 × total light activity weekly duration score (Deng and Paul, 2018). The total PA score was classified into four groups according to the 25th percentile, median and 75th percentile: Q1 = $<Q_{25}$, Q2 = Q_{25}–Q_{50}, Q3 = Q_{50}–Q_{75}, and Q4 = $\geq Q_{75}$.

Cancer

Participants were asked the question "Have you been diagnosed with cancer or a malignant tumor (excluding minor skin cancers) by a doctor?" Individuals who reported having a cancer history were further asked about the site or organ of cancer. The sensitivity for self-reported cancer varied from 0.79 to 0.93, using the registry reports of cancer as the standard (Bergmann et al., 1998).

Covariates

Demographic characteristics were self-reported, including age (used as a continuous variable), sex (male, female), marital status (married, separated/divorced/widowed/never married), education level (below high school, high school, and above), and place of residence (rural, urban). Health-related behaviors included smoking (never smoked, quit smoking, current smoker), drinking frequency in the past (never, ≤1/month, >1/month), and sleep duration (7–8 h, <7 h, ≥8 h). Sleep duration was assessed with the question "During the past month, how many hours of actual sleep did you get at night?" BMI was calculated as the weight in kilograms divided by the square of height in meters, and obesity was defined as BMI $\geq$ 28.0 kg/m^2 according to the guidelines for Chinese people. Depressive symptoms (yes/no) were measured using the 10-item version of the Center for Epidemiological Studied Depression Scale (CESD-10), and a total score of at least 10 was classified as clinically elevated depressive symptoms (Chen and Mui, 2014). In this study, chronic physical comorbidities included hypertension, diabetes, heart problems (including heart attack, coronary heart disease, angina, congestive heart failure, or other heart problems), chronic lung diseases (including chronic bronchitis, emphysema, and excluding tumors or cancers), liver diseases (except fatty liver, tumors, or cancers), stroke, kidney disease (except for tumor or cancers), stomach or other digestive disease (except for tumor), and arthritis or rheumatism. Hypertension was defined based on a history of hypertension, intake of antihypertensive drugs, and measurement of blood pressure (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg). Diabetes was diagnosed based on self-reported medical history and blood measurements. The other chronic diseases were defined by a self-reported doctor diagnosis. Physical comorbidities were categorized as 0, 1, or ≥2 according to the number of chronic diseases.

Statistical Analysis

Descriptive statistics of participant characteristics are presented according to the self-reported doctor diagnosis of cancer (yes/no), continuous variables are presented as the means and SD, and categorical variables are shown as the counts and frequency. To compare the characteristics of cancer survivors and participants without cancer, Student's *t* test was adopted for normally distributed continuous variables, and the chi squared test was adopted for categorical variables. To examine whether there were differences in PA between cancer survivors and cancer-free participants, three binary logistic regression models were fitted with cancer status as the dependent variable and PA as the independent variable. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to show the association between cancer and PA. Model 1 was unadjusted to calculate the crude OR and 95% CI. Model 2 was adjusted for demographic characteristics (including age, sex, marital status, education, and place of residence). Model 3 was further adjusted for health-related behaviors (smoking, drinking frequency, sleep duration, and obesity), depressive symptoms and chronic comorbidities (Shi et al., 2017; Cortés-Ibáñez et al., 2020). We retained the covariates, even if they were not statistically significant in changing the association between PA and cancer, to see if PA was independent of these variables. To assess the linear trends between PA and cancer status, Wald tests were performed, considering the total PA score categories as a continuous variable. To assess whether the relationship between being a cancer patient and PA was more prominent in specific subgroups, stratification analyses by all aforementioned variables were carried out, and the chi square-based *Q* test was used to test the heterogeneity. Interaction analyses were further performed by entering the interaction terms into the logistic regression model. In the sensitivity analyses, missing value analyses were conducted to identify the missingness mechanism of missing variables; based on these results, we hypothesized that the data used in this study were missing at random (Siddique et al., 2008). Therefore,

we excluded individuals with missing values less than 10%, variables with more than 10% missing data were imputed with the median or mean (this was the case for BMI), and logistic regression analyses were repeated. R software was used to draw figures, as well as perform subgroup and interaction analyses. The relevant packages and codes are provided in the **Supplementary Material**. Other analyses were conducted using SPSS version 21.0, two-sided *P*-values < 0.05 were considered to be statistically significant for the main analyses. To reduce the false discovery rate, two-sides *P*-value < 0.01 was used for subgroup analyses.

RESULTS

Participants' Characteristics

The characteristics of the participants are presented in **Table 1**. A total of 14,990 individuals were included, with 416 (2.8%) reporting a history of cancer. Among these cancer survivors, 12.5% had breast cancer, 7.2% had lung cancer, 15.4% had cervix cancer, 5.5% had liver cancer, 7.0% had endometrium cancer, 6.3% had colon or rectum cancer, 6.3% had stomach cancer, 4.1% had thyroid cancer, 2.9% had brain cancer, 3.4% had esophagus cancer, 2.4% had kidney cancer, 2.6% had ovary cancer, 18.5% had cancer of another organ, 6.0% had cancer of an unknown site. The cancer survivors were older than those without cancer (mean age 63.3 ± 9.1 vs. 61.9 ± 9.6 years), and were more likely to be women ($p < 0.001$), live in an urban area ($p = 0.006$), be obese ($p = 0.003$), quit smoking ($p < 0.001$), drink less ($p < 0.001$), sleep less ($p < 0.001$), have depressive symptoms ($p < 0.001$), have physical comorbidities ($p < 0.001$), and be physically inactive ($p < 0.001$) (**Table 1** and **Supplementary Table 1**).

Compared with cancer-free participants, cancer survivors were more often women than men (OR = 1.63, 95% CI = 1.18–2.24) and were more likely to quit smoking (OR = 1.60, 95% CI = 1.13–2.27), sleep less than 8 h (OR = 0.66, 95% CI = 0.47–0.92), and have depressive symptoms (OR = 1.46, 95% CI = 1.19–1.79) or chronic comorbidities (1 chronic disease: OR = 1.42, 95% CI = 1.13–1.79, ≥2 chronic diseases: OR = 1.41, 95% CI = 1.09–1.84) (**Supplementary Table 2**).

Logistic Regression Models to Describe the Association Between Physical Activity and Cancer

Table 2 presents the multivariate logistic regression results for the associations between PA level and cancer. In model 1, there was a significant association between cancer and taking part in vigorous activity for at least 10 min in a usual week, compared with inactivity (OR = 0.48, 95% CI = 0.34–0.68), and no significant differences were observed between groups in terms of moderate or light activity ($p > 0.05$). Participants with cancer diagnoses were less likely to spend more than 30 min (light OR = 0.75, 95% CI = 0.58–0.99; moderate: OR = 0.64, 95% CI = 0.48–0.85; vigorous: OR = 0.43, 95% CI = 0.32–0.58) or 2 h per day (light: OR = 0.71, 95% CI = 0.53–0.94; moderate: OR = 0.58, 95% CI = 0.42–0.80; vigorously: OR = 0.40, 95% CI = 0.30–0.54) on light, moderate and vigorous-intensity PA

TABLE 1 | Characteristics of study participants in the fourth wave of CHARLS.

Characteristic	Total (*N* = 14,990)	Cancer survivors (*N* = 416)	Non-cancer (*N* = 14,574)	*P*-value
Age, year, mean (SD)	61.9 (9.6)	63.3 (9.1)	61.9 (9.6)	0.045
Sex, n (%)				<0.001
Male	7015 (46.8)	144 (34.6)	6871 (47.1)	
Female	7975 (53.2)	272 (65.4)	7703 (52.9)	
Education, n (%)				0.690
Below high school	13245 (88.4)	365 (87.7)	12880 (88.4)	
High school and above	1745 (11.6)	51 (12.1)	1694 (11.6)	
Marital status, n (%)				0.404
Married	12928 (86.2)	353 (84.9)	12575 (86.3)	
Separated/divorce/widowed/never married	2062 (13.8)	63 (15.1)	1999 (13.7)	
Place of residency, n (%)				0.006
Rural	11208 (74.8)	287 (69.0)	10921 (74.9)	
Urban	3782 (25.2)	129 (31.0)	3653 (25.1)	
BMI, kg/m_2, n (%)				0.003
<27.9 (not obesity)	12976 (86.6)	340 (81.7)	12636 (86.7)	
≥28.0 (obesity)	2014 (13.4)	76 (18.3)	1938 (13.3)	
Smoking status, n (%)				<0.001
Never	8780 (58.6)	274 (65.9)	8506 (58.4)	
Quit	2170 (14.5)	74 (17.8)	2096 (14.4)	
Current smoker	4040 (27.0)	68 (16.3)	3972 (27.3)	
Drinking frequency, n (%)				<0.001
Never	9921 (66.2)	307 (73.8)	9614 (66.0)	
Less than once a month	1114 (7.4)	36 (8.7)	1078 (7.4)	
More than once a month	3955 (26.4)	73 (17.5)	3882 (26.6)	
Sleep duration, hours, n (%)				<0.001
7–8	2495 (16.6)	69 (16.6)	2426 (16.6)	
<7	8548 (57.0)	274 (65.9)	8274 (56.8)	
≥8	3947 (26.3)	73 (17.5)	3874 (26.6)	
Depressive symptoms, n (%)				<0.001
No	9311 (62.1)	210 (50.5)	9101 (62.4)	
Yes	5679 (37.9)	206 (49.5)	5473 (37.6)	
Comorbidities, n (%)				<0.001
0	7176 (47.9)	151 (36.3)	7025 (48.2)	
1	4904 (32.7)	157 (37.7)	4747 (32.6)	
≥2	2910 (19.4)	108 (26.0)	2802 (19.2)	
Cancer site, n (%)				
Brain	12 (0.08)	12 (2.9)		
Lung	30 (0.20)	30 (7.2)		
Thyroid	17 (0.11)	17 (4.1)		
Breast	52 (0.35)	52 (12.5)		
Esophagus	14 (0.09)	14 (3.4)		
Stomach	26 (0.17)	26 (6.3)		

(Continued)

TABLE 1 | (Continued)

Characteristic	Total (*N* = 14,990)	Cancer survivors (*N* = 416)	Non-cancer (*N* = 14,574)	*P*-value
Liver	23 (0.15)	23 (5.5)		
Kidney	10 (0.07)	10 (2.4)		
Ovary	11 (0.07)	11 (2.6)		
Cervix	64 (0.43)	64 (15.4)		
Endometrium	29 (0.19)	29 (7.0)		
Colon or rectum	26 (0.17)	26 (6.3)		
Other organs	77 (5.1)	77 (18.5)		
Unknown site	25 (0.17)	25 (6.0)		

SD, standard deviation. Chi square test and t-test were used as appropriate.

than the cancer-free participants. In addition, an inverse dose-response relationship was detected between the total PA score and cancer status ($p_{-trend} < 0.001$). In model 2, after adjusting for demographic characteristics, the associations between PA patterns and cancer were slightly altered, but the statistical significance remained similar. In model 3, after full adjustment, the associations between PA level and cancer were slightly lower. Individuals with cancer status were less likely to perform vigorous PA for at least 10 min every week than cancer-free individuals (OR = 0.56, 95% CI = 0.39–0.80). Nevertheless, the differences between groups were not significant across light and moderate PA ($p > 0.05$). Adults with cancer status were less likely to participate in light, moderate or vigorous PA for more than 30 min or 2 h. In addition, there was a significant dose-response relationship between the total PA score and cancer ($p_{-trend} < 0.001$).

Subgroup Analyses

Stratification analyses for the associations between PA and cancer were then conducted by all aforementioned variables. In Supplementary Material (**Supplementary Tables 3–13**), the associations between cancer status and PA were more prominent in individuals aged ≥65 years, males, those living in urban areas, those who were married, those with a lower education

TABLE 2 | Logistic regression analyses of associations between different physical activity levels and cancer status.

Variables	Total n (%)	Model 1		Model 2		Model 3	
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Taking part in activity more than 10 min a week							
Inactive	1337 (8.9)	1		1		1	
Light	5034 (33.6)	0.90 (0.65, 1.25)	0.539	0.92 (0.66, 1.27)	0.613	0.96 (0.69, 1.33)	0.803
Moderate	4311 (28.8)	0.73 (0.53, 1.02)	0.069	0.70 (0.50, 0.99)	0.041	0.75 (0.53, 1.05)	0.091
Vigorous	4308 (28.7)	0.48 (0.34, 0.68)	<0.001	0.53 (0.37, 0.76)	<0.001	0.56 (0.39, 0.80)	0.001
Time usually spend doing activity ≥30 min 1 day							
Inactive	2224 (14.8)	1		1		1	
Light	4880 (32.6)	0.75 (0.58, 0.99)	0.040	0.76 (0.58, 0.99)	0.044	0.79 (0.60, 1.04)	0.087
Moderate	3737 (24.9)	0.64 (0.48, 0.85)	0.002	0.61 (0.46, 0.82)	0.001	0.64 (0.48, 0.86)	0.003
Vigorous	4149 (27.7)	0.43 (0.32, 0.58)	<0.001	0.48 (0.35, 0.64)	<0.001	0.50 (0.37, 0.68)	<0.001
Time usually spend doing activity ≥2 h 1 day							
Inactive	6576 (43.9)	1		1		1	
Light	3958 (26.4)	0.71 (0.53, 0.94)	0.016	0.71 (0.54, 0.95)	0.019	0.73 (0.55, 0.97)	0.029
Moderate	2134 (14.2)	0.58 (0.42, 0.80)	0.001	0.58 (0.42, 0.80)	0.001	0.60 (0.44, 0.83)	0.002
Vigorous	2322 (15.5)	0.40 (0.30, 0.54)	<0.001	0.45 (0.33, 0.60)	<0.001	0.46 (0.34, 0.62)	<0.001
Time usually spend doing activity ≥4 h 1 day							
Inactive	9999 (66.7)	1		1		1	
Light	2797 (18.7)	0.65 (0.42, 1.01)	0.056	0.68 (0.44, 1.05)	0.082	0.70 (0.45, 1.09)	0.112
Moderate	1157 (7.7)	0.75 (0.51, 1.11)	0.145	0.79 (0.53, 1.18)	0.249	0.81 (0.54, 1.20)	0.291
Vigorous	1037 (6.9)	0.49 (0.36, 0.68)	<0.001	0.57 (0.41, 0.78)	0.001	0.58 (0.42, 0.81)	0.001
Total PA score							
Q1	2869 (19.1)	1		1		1	
Q2	4626 (30.9)	0.79 (0.62, 1.02)	0.075	0.80 (0.62, 1.03)	0.086	0.83 (0.64, 1.07)	0.142
Q3	3747 (25.0)	0.65 (0.49, 0.85)	0.002	0.64 (0.48, 0.85)	0.002	0.66 (0.50, 0.88)	0.005
Q4	3748 (25.0)	0.48 (0.35, 0.65)	<0.001	0.53 (0.39, 0.73)	<0.001	0.56 (0.41, 0.76)	<0.001
P-trend		<0.001		<0.001		<0.001	

OR, odds ratio; CI, confidence interval. Model 1 was unadjusted; Model 2 was adjusted for age, sex, education, marital status, place of residence; Model 3 was further adjusted for smoking, drinking, obesity, sleep duration, depressive symptoms, and chronic comorbidity.

level, those with BMI < 28.0 kg/m^2, those who were depressed, former smokers, those whose sleep duration was <7 h, those with chronic comorbidities. However, heterogeneity among the strata was only significant for sleep duration ($p = 0.006$).

Then, statistically significant multiplicative interactions between PA and sex and depressive symptoms were identified (**Figure 1**, $p_{-\text{interaction}} < 0.05$). Females with cancer status were less likely to spend more than 30 min performing vigorous PA every day than males (OR = 0.54, 95% CI = 0.37–0.80), after adjusting for age, marital status, education level, place of residence, BMI, smoking, drinking, sleep duration, depressive symptoms, and physical comorbidities. Compared with cancer-free individuals, those with cancer were more likely to be physically inactive and have depressive symptoms (OR = 0.37, 95% CI = 0.25–0.55).

Sensitivity Analyses

In sensitivity analyses, after the missing BMI values were replaced with the median, the logistic regression analysis results showed robustness. Individuals who were diagnosed with cancer tended to be more physically inactive than their cancer-free counterparts after full adjustment (OR = 0.54, 95% CI = 0.38–0.76). Those who had cancer diagnoses were less likely to spend more than 30 min or 2 h every day performing PA than cancer-free individuals, regardless of the PA intensity. Moreover, a significant dose-response relationship was observed between the total PA score and cancer status ($p_{-trend} < 0.001$) (**Supplementary Figure 1**).

DISCUSSION

In this cross-sectional analysis, we compared the PA levels of 14,574 individuals without cancer and 416 cancer survivors from 28 provinces in mainland China. We found that participants with cancer were more likely to be physically inactive than their cancer-free counterparts in both the unadjusted and adjusted analyses. Individuals with cancer diagnoses were less likely to spend more than 10 min performing vigorous activity each week than cancer-free individuals, while no significant differences existed between groups that performed light or moderate activity, independent of demographic characteristics, health-related lifestyle factors, depressive symptoms and chronic comorbidities. Cancer patients had a lower tendency to perform PA for more than 30 min or 2 h every day than cancer-free individuals, regardless of the PA intensity. An inverse dose-response association was also observed between the total PA score and cancer. In addition, the interaction between sex and depressive symptoms and PA significantly affected cancer status.

Our analyses found that cancer survivors were more likely to perform less PA than those who had no cancer diagnoses. This finding may be explained by the fact that some cancer survivors are currently undergoing treatment or recovering from surgery (e.g., adjuvant radiation therapy or chemotherapy), which poses limitations to their physical function and thus prevents or discourages PA. The previous comparison of PA levels between cancer survivors and individuals without cancer remains controversial. The results of the Canadian Community Health Survey (CCHS) are consistent with our results. The CCHS found that respondents with cancer were more likely to be inactive than those who had never had cancer (OR = 1.39, 95% CI = 1.21–1.58 inactive vs. active) (Neil et al., 2014). However, the Lifelines cohort study conducted in a Dutch population contrasted with our study, as the Lifelines study found that cancer survivors were significantly more likely to be physically active than people without a history of cancer (Cortés-Ibáñez et al., 2020). These conflicting findings might be attributed to differences in cancer types, current treatment status, types of treatment, and cancer stages. Researchers in the United States carried out a secondary data analysis to compare PA levels between 31,078 cancer survivors with a history of single-site cancer diagnosis and participants without cancer, and the results demonstrated that higher PA levels were present among prostate cancer survivors, while lower PA levels were present among cervical and endometrial cancer survivors (Kwon et al., 2012). Although evidence has shown that PA throughout treatment is conducive to improving survival and reducing mortality (Demark-Wahnefried et al., 2007; Speck et al., 2010), some cancer treatments can make PA challenging due to their distinct side effects, and even those who participate in PA during treatment may do so at a lower intensity. Future investigations to identify parameters that influence PA among cancer survivors are warranted.

A notable pattern of differences was observed between sexes in the current study, with female cancer patients being less likely to participate in vigorous activity than male cancer patients. A previous study, which found that female cancer survivors were approximately 30% less likely to meet the PA recommendation than male cancer survivors, was in line with our results (LeMasters et al., 2014). This phenomenon may be attributed to the masculinity-femininity theory that a man's health practices are influenced by his desire to comply with the dominant masculine ideals shaped by cultural norms (e.g., engaging in vigorous PA, smoking, heavy alcohol drinking), while women are more likely to manage their weight through diet adjustment (Wardle et al., 2004; Gough and Conner, 2006). Another theory that may explain the differences in PA levels between sexes is the Health Belief Model, which describes the relationship between an individual's perception of risk and the corresponding health practices (Cummings et al., 1978). For example, cardiovascular disease is the leading cause of mortality for both men and women in the United States; however, it has been regarded as a "man's disease," and only half of women recognize it as a leading cause of death among women (Mosca et al., 2010). Therefore, the lack of perception of risk for developing cardiovascular disease may lead to less PA among females than males. In addition, female cancer survivors seemed to be more vulnerable to mental health declines and sleep disturbance than male cancer survivors, which may also lower their PA levels (LeMasters et al., 2013).

The association between PA level and cancer was more prominent in depressed participants, and cancer survivors with depressive symptoms were less likely to engage in moderate- and vigorous-intensity PA. Researchers found that a depressed mood is negatively related to PA levels among cancer survivors, which may explain the differences observed in depressive status (Galiano-Castillo et al., 2014). We also found an inverse dose-response relationship between the total PA score and cancer status, which might provide new insight into the amount of

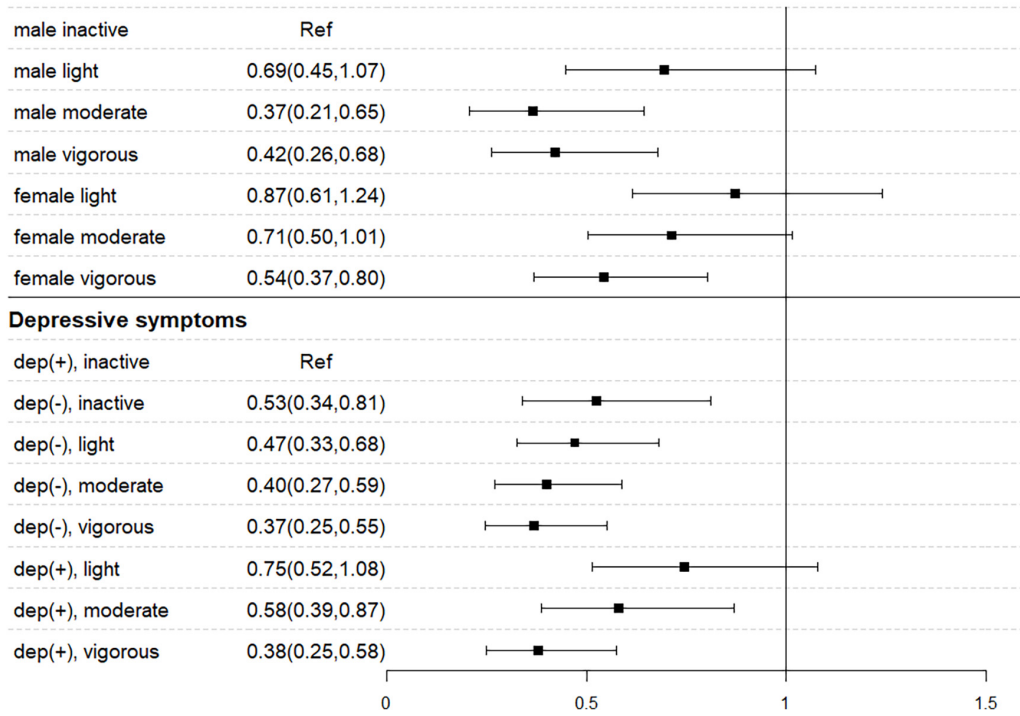

FIGURE 1 | Interaction analysis between sex, depressive symptoms, and physical activity. OR, odds ratio; CI, confidence interval. Model was adjusted for age, sex, education, marital status, place of residence, smoking, drinking, obesity, sleep duration, depressive symptoms, and chronic comorbidities.

PA needed for cancer survivors. The finding of significant associations for vigorous activity for more than 10 min weekly but not for light or moderate activity, along with significant relationships for activity for more than 30 min despite PA intensity, suggests that there is a minimum threshold of PA intensity and duration for cancer survivors.

Strengths and Limitations

The strengths of this study are as follows. First, relatively recent data from a representative middle-aged and older Chinese sample were utilized in this analysis, which improves the generalizability and validity of our findings. Unlike previous studies involving a single intensity of PA, the current study examined different PA intensities and durations in cancer survivors and those without cancer. Moreover, a variety of covariates, including demographic characteristics, health-related lifestyle factors and chronic comorbidities that previous studies failed to fully adjust for, were included in our analysis. Adjustments for these confounders may have resulted in more reliable conclusions since cancer survivors can differ from cancer-free individuals in diverse factors, such as age, sex, BMI, and education levels (Naik et al., 2016).

However, this study also has several limitations. First, the study relied on self-reported PA levels, which may overestimate or underestimate the amount of time spent performing PA compared with objectively measured PA levels. Previous findings suggested that measurement methods may have a significant influence on observed PA levels, and participants may answer questions according to what they think is socially desirable (Prince et al., 2008; Troiano et al., 2008). Nevertheless, a previous study suggested that such bias could potentially be overcome with a large sample size (e.g., CHARLS) (Celis-Morales et al., 2012). Detailed cancer-related medical information, such as cancer diagnosis time, treatment status, and treatment methods, was unavailable in the CHARLS data, which may have limited the interpretation of our results. We only described the sites of cancer because the sample size for each specific cancer was relatively small, which restricted further analyses, as no firm results could be generated. In addition, asking cancer survivors about the levels of PA a few years before their diagnosis was not reliable because of recall bias; thus, PA levels before diagnosis were not measured. Finally, due to the cross-sectional design, it is difficult to know which came first, the physically inactive behavior or the cancer occurrence. The time sequence between PA and cancer was not ascertained; thus, the causality associations between PA and cancer could not be established. Further prospective surveys are needed.

Implications

Participating in PA is an inexpensive and non-pharmacologic intervention for cancer patients with numerous benefits, such as potential improvements in survival, physical function, quality

of life and mortality (Li et al., 2016). The current study found that cancer survivors were less likely to be physically active than individuals without cancer, especially females and those with depressive symptoms.

This may support the introduction of PA into the management of cancer survivors to some extent. Studies have suggested that the period after cancer diagnosis is a "teachable moment," in which patients are likely to change their lifestyles to improve health outcomes (Blanchard et al., 2003). Therefore, in clinical practice, health care practitioners, especially nurses, can play an important role in monitoring PA levels among cancer survivors. As nurses are close to cancer survivors, they can inform survivors of the beneficial effects of PA and provide valuable advice on recommended PA levels. Moreover, in circumstances where accelerometers are available, nurses and survivors can monitor both PA duration and intensity objectively.

Currently, China houses the world's largest population with 1.4 billion people, and it is transforming into an aging country (Zeng, 2012; Fang et al., 2015). It is expected that there will be up to 400 million people aged more than 65 years in China by 2030, accounting for 26.9% of the total population (Zeng, 2012). With the rapid growth of the elderly population, the incidence of cancer is also growing rapidly, and significant advancements in medical care and treatment methods have been made (Yang et al., 2008; Chen et al., 2016). Thus, the cancer population is increasing in a parallel manner, and China is encountering formidable healthcare challenges brought about by the problem of cancer survivors. Healthy lifestyle behaviors, including PA, have an important role in both preventing cancer and improving survival and quality of life among cancer survivors and are now receiving attention. Providing training for nurses and establishing multidisciplinary teams (e.g., oncologists, nutritionists, physiotherapists, and nurses) should be considered.

CONCLUSION

We found significantly lower PA levels among cancer survivors than among cancer-free individuals after adjustment for demographic characteristics, health-related behaviors, and chronic physical comorbidities. Furthermore, female and depressed cancer survivors were less likely to be physically active and should be given more attention. Our findings demonstrate the need for more prospective studies investigating different PA levels in Chinese cancer survivors.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: http://charls.pku.edu.cn/index.html.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board at Peking University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CZ was in charge of the conception and design of the study, as well as the writing of original draft. ZL was responsible for the review and editing of the manuscript. JW and YC reviewed, edited, and supervised this study. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.812290/full#supplementary-material

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Combined Effects of Exercise Training and Nutritional Supplementation in Cancer Patients in the Context of the COVID-19: A Perspective Study

Mahdieh Molanouri Shamsi[1], Alieh Vahed[1,2], AmirHossin Ahmadi Hekmatikar[1,2] and Katsuhiko Suzuki[1,3]**

[1] Department of Physical Education and Sport Sciences, Faculty of Humanities, Tarbiat Modares University, Tehran, Iran, [2] Faculty of Humanities, Tarbiat Modares University, Tehran, Iran, [3] Faculty of Sport Sciences, Waseda University, Tokorozawa, Japan

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***Correspondence:**
Mahdieh Molanouri Shamsi
molanouri@modares.ac.ir
Katsuhiko Suzuki
katsu.suzu@waseda.jp

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The 2019 coronavirus (COVID-19) epidemic, has caused unprecedented global social and economic impacts and many deaths. Many risk factors have been identified in the progression of COVID-19 to severe and critical stages, and it is shown that the coronavirus appears more severely in people with cancer. Pro-inflammatory status and weakened immune system due to cancer-related treatments can be determinants in the immune system's response to the coronavirus in these patients. Higher physical activity levels are associated with lower hospitalization rates and mortality in COVID-19. Also, regular exercise training can improve immune system responses, modulate inflammatory responses, and improve psychological parameters in cancer patients. The interactive effects of nutritional supplements on immune responses and anti-inflammatory status have been shown in some studies. The purpose of this perspective article was to investigate the interaction between dietary supplementation and regular physical exercise in controlling risk factors associated with coronavirus in cancer patients. In addition to appropriate dietary habits, some nutritional supplements, especially vitamin D, have been shown to improve the immune system's response against COVID-19 and cancer. Using lifestyle strategies such as regular physical activity and intake of functional compounds as supplements can be effective in treatment outcomes, quality of life, and overall survival in cancer patients. We proposed that combining dietary supplements and exercise training in cancer patients can boost immune responses against COVID-19 and probably improve vaccine responses. Angiotensin (ANG)-(1-7) Mas receptor axis can probably activate following exercise training and vitamin D combination. And can prevent pulmonary injury, hematological alterations, and hyperinflammatory state in COVID-19.

Keywords: aerobic exercise training, cancer, immune response, vitamin D, coronavirus

INTRODUCTION

Humans are exposed to different viruses; and the human body is rapidly involved in an immune response to eradicate the virus, with a pattern of detection and the production of memory cells (1). Coronavirus (COVID-19) has been observed in China since 2019 and has become an epidemic (2). The virus is caused by acute respiratory syndrome (SARS) Coronavirus 2 (SARS-CoV-2). According to the statistics published as of September 21, 2021, the number of infected people worldwide was approximately 230 million; and the number of victims was about 4.7 million, which continues so far, and 14% of patients experiences acute and severe conditions (3). Coronavirus can cause uncontrolled releases of pro-inflammatory cytokines, which leads to cytokine release syndrome (CRS) or "cytokine storm" (4). Activation of CRS can worsen acute respiratory syndrome and lead to multiple organ dysfunction (4). Evidence suggests that patients with SARS-CoV-2, who previously suffered from rheumatological immune diseases and other inflammatory diseases are more fragile to an acute respiratory syndrome caused by CRS (5). Despite global vaccinations to tackle this pandemic, we can see new infections and the prevalence of new variants. Still observing social distancing and staying at home is one of the main ways to keep the COVID-19 under control (6, 7).

In particular, cancer patients appear to be a high-risk category to experience COVID-19 with more severe symptoms, especially due to damage to immune defenses and the consequences of anti-neoplastic treatments (8). Chronic cancer-related inflammation can create an immunosuppressive tumor microenvironment to help the tumor to escape immune monitoring (9). Also, cancer is associated with over-expression of immunosuppressive cytokines, decreased proinflammatory risk signals, and increased populations of functional immunosuppressive leukocytes, which weaken the immune responses and increase the risk of infectious complications (10, 11). Therefore, these patients cannot show effective immune responses when exposed to the virus.

In addition to regular cancer treatments, appropriate dietary habits and regular physical exercise have been considered in these patients. Regular physical exercise training can induce positive changes in anxiety, depression, immune and physiological responses in cancer (12). Moreover, some dietary supplements have always been considered to boost immune responses in cancer patients (13). Higher physical activity levels are associated with lower hospitalization rates and mortality in COVID-19 (14). Positive effects of acute or chronic physical exercise were observed on innate and acquired immune responses and modulation of inflammatory status (15). Also, the effects of regular physical exercise on angiotensin-converting enzyme 2 (ACE2) as an effective agent in the pathogenesis of COVID-19 are suggested in some studies (16). In addition, the effects of physical exercise on nitric oxide and oxidative stress have been proposed as possible effective mechanisms in the prevention and recovery of the COVID-19 (17). Impaired immune responses, inflammatory status, and oxidative stress have been observed in cancer patients (18). Also, some studies showed different expressions of ACE2 in cancer patients, which makes them more susceptible to SARS-CoV-2 (19). The modulatory effects of regular physical exercise on ACE2 and its receptors may be effective in preventing severe cases of COVID-19 in cancer patients.

In addition, the positive effects of various dietary supplements such as probiotics, omega-3 fatty acids, multivitamins, or vitamin D supplements in COVID-19 are suggested in some studies (20). Considering the observed effects of dietary supplements and physical exercise in cancer and COVID-19, synergic effects of combining regular physical exercise and supplementation can improve the immune system responses in cancer patients against coronavirus. The possible effects of combining regular physical exercise and dietary supplementation in cancer and COVID-19 were discussed following a systematic search in Scopus, Web of Science, ISC, and Pub-Med databases. And the results were divided into two main topics 1) Physical Exercise in Cancer and COVID-19, 2) Nutritional Supplements in Cancer and COVID-19.

PHYSICAL EXERCISE IN CANCER AND COVID-19

Angiotensin-converting enzyme 2 (ACE2) is one of the causes of SARS-CoV-2 entry and infection (21). It has recently been found that ACE2 receptors are also more common in cancer patients (19). Once CoV-2 entry into the target cell, the host response is determinant of the severity of the ensuing pathogenesis (22). The immune system plays a critical role in COVID-19, and in addition to the genetic profile, environmental indices can be effective on the immune responses (23).

The first immune system responses are when the COVID-19 enters the body through the innate immune system. Inflammation is the body increases when a person becomes infected with COVID-19. As seen in patients with SARS-CoV, Viral infection and proliferation in airway epithelial cells can cause high levels of virus-associated pyroptosis associated with vascular leakage (24). During inflammation, type I interferons (IFNs) are activated by the innate immune system, and the presence of type I interferons can regulate myeloid cell activation and migration (25). Rapid and appropriate activation of type I IFNs can effectively limit virus replication and reduce immune pathological damage. It also limits the hyperactive inflammatory response (26). Also, in the innate immune system, natural killer cells (NK) play a vital role in infectious diseases. NK cells decreased in patients with COVID-19 (27). Activation of NK cells lead to cytotoxic degranulation and production of inflammatory cytokines and destroys target cells (28). In addition, the adaptive immune system would activate to help innate immunity for further controlling the infection. The three critical components of the adaptive immune system against SARS-CoV-2 are B cells, $CD4^+$ T cells, and $CD8^+$ T cells (29). $CD4^+$ T cell responses are more prominent than $CD8^+$ T cell responses and play a role in controlling primary infection earlier (30). It has also been shown that the function of B cells is very important in controlling viral infections. But the critical point is that COVID-19 can disrupt the phenotype of T and B cells and reduce their function (29).

Regular exercise throughout life is effective in cardiovascular fitness and self-reported mood, anxiety, and depressive symptoms. In addition to the physiological effects of regular physical exercise, it can improve immune responses. Moderate intensity training is directly associated with a lower upper respiratory tract infection (URTI) (31). Regular physical exercise can improve innate and acquired immune system responses (32). In particular, secondary antibody responses to some traditional vaccines approved after exercise training, especially in elderly people (33).

Moreover, it is shown that physical exercise has anti-inflammatory effects, especially in chronic diseases and obesity (34). Modulation of adipose tissue macrophages, the release of anti-inflammatory cytokines after acute physical exercise, and interaction of skeletal muscle and immune system can be effective in exercise-induced anti-inflammatory effects (34). The anti-inflammatory effects of exercise and changes in various indicators of the innate immune system following acute physical exercise can improve the innate immune system responses to infections. In addition, regular physical exercise training creates effective responses in the acquired immune system especially in elderly people (35). Physical exercise has been shown to reduce mortality from illnesses such as the flu, and higher levels of physical activity have reduced the incidence of severe cases, hospitalization rates, and mortality in COVID-19 (36, 37).

Cancer-related inflammation is a prominent feature of cancer (38). Cancer-related inflammation, which in different stages of tumorigenesis, contributes to genomic instability, epigenetic modification, induction of cancer cell proliferation, strengthening cancer anti-apoptosis pathways, stimulating angiogenesis, and ultimately spreading cancer (38). In the early stages of tumor growth, cytotoxic immune cells such as NK cells and $T^{+}CD8$ cells detect and destroy cancer cells (39). In addition, the interaction between NK cells, effective T cells, and antitumor macrophages by secreting IFN-γ and tumor necrosis factor-alpha (TNF-α) at the tumor site increases the cytotoxic ability of NK cells (39). When tumors escape primary anti-tumor immunity, they undergo different strategies that shift the balance toward immune tolerance, as they reduce the effect of inherently compatible immune cells at different levels and through different mechanisms. Tumor cells escape immune attacks using two main strategies; avoid immune detection and stimulate an immunosuppressive environment. Firstly, cancer cells may lose expression of tumor antigens on the cell surface, thus preventing detection by cytotoxic T cells. In this sense, mutations and deletions may lead to low regulation of the antigen-providing device and possibly show resistance to effective T-cell molecules such as TNF-α and IFN-γ (40). Also, agents derived from cancer cells stimulate the expression of inhibitory inspection molecules such as programmed death-ligand 1 (PD-L1), CTLA β-4, and Tregs expression by tumor-derived chemokines of the immune-resistant environment by tumor-derived chemokines (18). Overall, these strategies lead to a complex and efficient system for safe escape.

Adjuvant therapies such as physical exercise that makes changes in different aspects of the immune system in cancer may improve the efficacy of current immunotherapy. The diagnosis of cancer and the treatments that cancer patients undergo have a significant impact on their mental and physical health. Some studies showed that regular physical exercise is effective in body weight and body mass index controlling, and improving patients' quality of life and sleep quality (41). It also seems that regular exercise through myocyte secretion, decreased inflammatory factors within the tumor, decreased tumor angiogenesis, and increased expression of factors involved in apoptosis can slow tumor growth (42). Regular exercise and physical activity can prevent the disease from recurring by affecting sex steroid hormones, metabolic hormones, inflammatory markers, cytokines and adipokines, myokines, and stress hormones (43). There are also observed effects of regular and long-term exercise on immune responses to diseases such as cancer (44). Prolonged exercise promotes anti-inflammatory effects and improves immune responses in cancer patients. In addition, recent studies have shown the positive effects of physical exercise on antitumor immunity that can affect tumor growth (45). Tumor induces physiological changes in its environment such as changes in acidity and metabolism in order to suppress antitumor immunity. Physiological responses following physical exercise can increase the infiltration of macrophages, neutrophils, NK cells, and regulatory and cytosolic T lymphocytes to the tumor microenvironment, which can be effective in tumor suppression (46).

Unfortunately, cancer patients are among the groups most at risk for severe cases of COVID-19. The psychological, physiological, and especially immunological effects of physical exercise can help these patients respond better to infection and possibly even better immune responses to the vaccine. However, the effects of exercise training have always been considered along with nutritional factors. We discussed the effects of nutritional supplements on the immune system in cancer patients to prevent severe cases of COVID-19, we also discussed the combined effects of exercise and supplementation.

NUTRITIONAL SUPPLEMENTS IN CANCER AND COVID-19

Many studies have approved that proper nutrition and some nutritional supplements can improve immune system function and reduce infection (47, 48). Long-term use of foods and supplements rich in antioxidants such as tart cherry juice, pomegranate juice, beetroot juice, creatine, omega-3 polyunsaturated fatty acids, and vitamin D3, watermelon juice has been associated with effective immune responses (49). In addition, the effect of ginger and some of its compounds against inflammation and improving immune responses have been reported in some studies (50–52).

Among vitamins, vitamin E is one of the most effective nutrients known to modulate the function of the immune system (53). There are reports of improved immune systems in human and animal specimens by taking this supplement. Improving immune function with vitamin E is clinically important because, in addition to allergic diseases such as asthma and the flu, it also affects infectious diseases such as respiratory infections

(54). Also, evidence showed that vitamin E supplementation for 30 days increased Th1 immune responses, improved lung damage, and reduced influenza infection in mice (55).

In addition, Vitamin D is a fat-soluble steroid that plays an important role in regulating calcium and phosphorus levels. Vitamin D receptors are located on immune cells, and all leukocytes can synthesize the active metabolite of vitamin D. Moreover, vitamin D can act autonomously, boosting the innate immune response and inhibiting the acquired immune response (56). Low concentrations of vitamin D have been reported to be associated with upper respiratory tract infections and allergic asthma (57). Vitamin D enhances chemotaxis, antimicrobial peptides, and macrophage differentiation. It can also inhibit the maturation of DCs, the differentiation of Th1 and Th17, and boost the functions of regulatory T cells (57). Moreover, vitamin D has been reported to play an important role in influenza virus infection (58).

The American Cancer Research Institute (AICR) has always recommended a low-fat diet, high in fruits, vegetables, and whole-grain products for cancer survivors. It also considers adequate levels of major macronutrients and various vitamins and minerals necessary to maintain health (59). During cancer treatments with chemotherapy or radiotherapy, patients often experience nausea, vomiting, diarrhea, and loss of appetite, leading to fewer food combinations and weight loss (60). Supplementation with essential vitamins and minerals may seem desirable, but it may not always. Food interactions with treatment may affect the outcome of treatment. Of particular concern here are dietary supplements with antioxidant properties, but supplements without antioxidant properties may also affect the effectiveness of cancer treatment (61). Many non-oxidant supplements are widely consumed by cancer patients, although their effects on the effectiveness of chemotherapy treatments are controversial. Vitamin D is one of the non-oxidant vitamins that is probably useful in cancer treatment (62). It has been observed that vitamin D receptor (VDR) is expressed significantly in the immune system, raising the possibility that vitamin D and similar may have immunomodulating activity (63). Cell studies state that vitamin D modulates the activity of various defense and immune cells including blood monocytes, macrophages, antigen-providing cells, and activated $CD4^+$ T cells or epithelial cells (64, 65). In general, vitamin D metabolites have significant anti-neoplastic activity in clinical models. The immune system is a suitable target for the anti-neoplastic effects of vitamin D. Vitamin D receptor (VDR) is expressed in different immune cells. Vitamin D can have inhibitory effects on chronic inflammation, resulting in the proliferation of immune cells. Also, it is suggested that some types of cancer may be more sensitive to the effects of vitamin D supplementation than others (66).

Another supplement that has been considered in cancer research is selenium (67). Selenium is an essential component in several major metabolic pathways, including the antioxidant defense system and the immune system. Selenium is incorporated into 30 different selenoproteins (68). Selenoproteins play an important role in antioxidant and DNA stability and may have anti-cancer effects. Also, selenium is involved in cell proliferation and apoptotic cell death in healthy and malignant cells. Low selenium levels are associated with a high prevalence of several different types of cancer and cancer mortality (69). Also, the effects of foods and supplements such as omega 3, vitamins E and C on the immune system responses in cancer have been specifically considered and confirmed. However, due to the stage and treatments related to cancer, the use of various supplements has always been cautious. Antioxidants are not always effective during cancer treatment, and also higher doses of some supplements may cause side effects. In the following, we discussed the possible effects of some supplements in combination with exercise training as a possible modulator and the possible effects in the COVID-19.

DISCUSSION

Limited studies have been performed on the combined effects of dietary supplementation and regular physical exercise on the prevention and treatment of COVID-19. It seems that regular physical exercise and dietary intake of functional compounds as two main parts of a proper lifestyle that can prevent severe cases of this disease. Also, it is shown that patients with chronic disease are susceptible to severe cases of COVID-19, and a combination of exercise and proper nutrition have always been effective in reducing some chronic diseases (70). So, one of the main considerations would be lifestyle changes, including increasing physical exercise and intake of functional compounds to improve immune function and induce antiviral effects. Cancer patients as one of the chronic diseases are involved in more severe cases of COVID-19 (71). It is probable that some dietary supplements especially in high doses with anti-inflammatory and antioxidant effects can interfere with cancer treatment. Combining exercise training with dietary supplements can be effective in modulating the effects of supplements in these patients and improve immune system's response to infectious diseases, especially COVID-19.

In the athletes, nutritional supplements such as probiotics, glutamine, a variety of vitamins and minerals, selenium, etc., have always been considered to improve the immune system responses and prevent upper respiratory system infections (72). It has also been shown that the combined effects of exercise with a proper diet can be effective in the reduction of inflammation, leukocytes adhesion, and chemotaxis capacity, and creating anti-oxidative effects in metabolic syndrome and obesity. This has been especially true for the elderly due to immunosenescence (73). Regular physical exercise and dietary intake of functional compounds can induce immune-boosting and decrease the adverse effects of age-related immune dysfunction.

Cancer survivors are often highly motivated to seek about dietary choices, physical activity, and dietary supplements to improve their treatment outcomes, quality of life, and overall survival. Many dietary supplements contain levels of antioxidants that are significantly higher than the recommended dietary intake. Research has shown that taking high doses of vitamin D or selenium supplements can improve cancer and reduce tumor volume (74). On the other hand, it has been suggested that taking high doses of supplements with antioxidant activity during chemotherapy or radiation therapy may not be wise, as

antioxidants can potentially reduce cellular oxidative damage to cancer cells, which contributes to the effectiveness of these therapies (75). Recent studies have suggested the possible role of exercise training in reducing the side effects of high doses of antioxidant supplementation in breast cancer tumors. The results of studying the use of high-dose vitamin D with exercise training in women with breast cancer have shown that a combination of exercise training and a high dose of vitamin D can modulate leukocytes' cell survival-related gene expression in breast cancer survivors (76). It is suggested that response to vitamin D supplementation in cancer modulated *via* vitamin D receptor (77). The effects of physical exercise on the increase of vitamin D receptors in various tissues have been observed in some studies (78). Also, Lithgow et al. (79) showed that moderate aerobic exercise increases T-cell vitamin D receptor expression in vitamin D-deficient men (79). Changes in vitamin D receptors in various tissues, including the immune system following physical exercise, can be used as a mechanism to enhance the immune system's

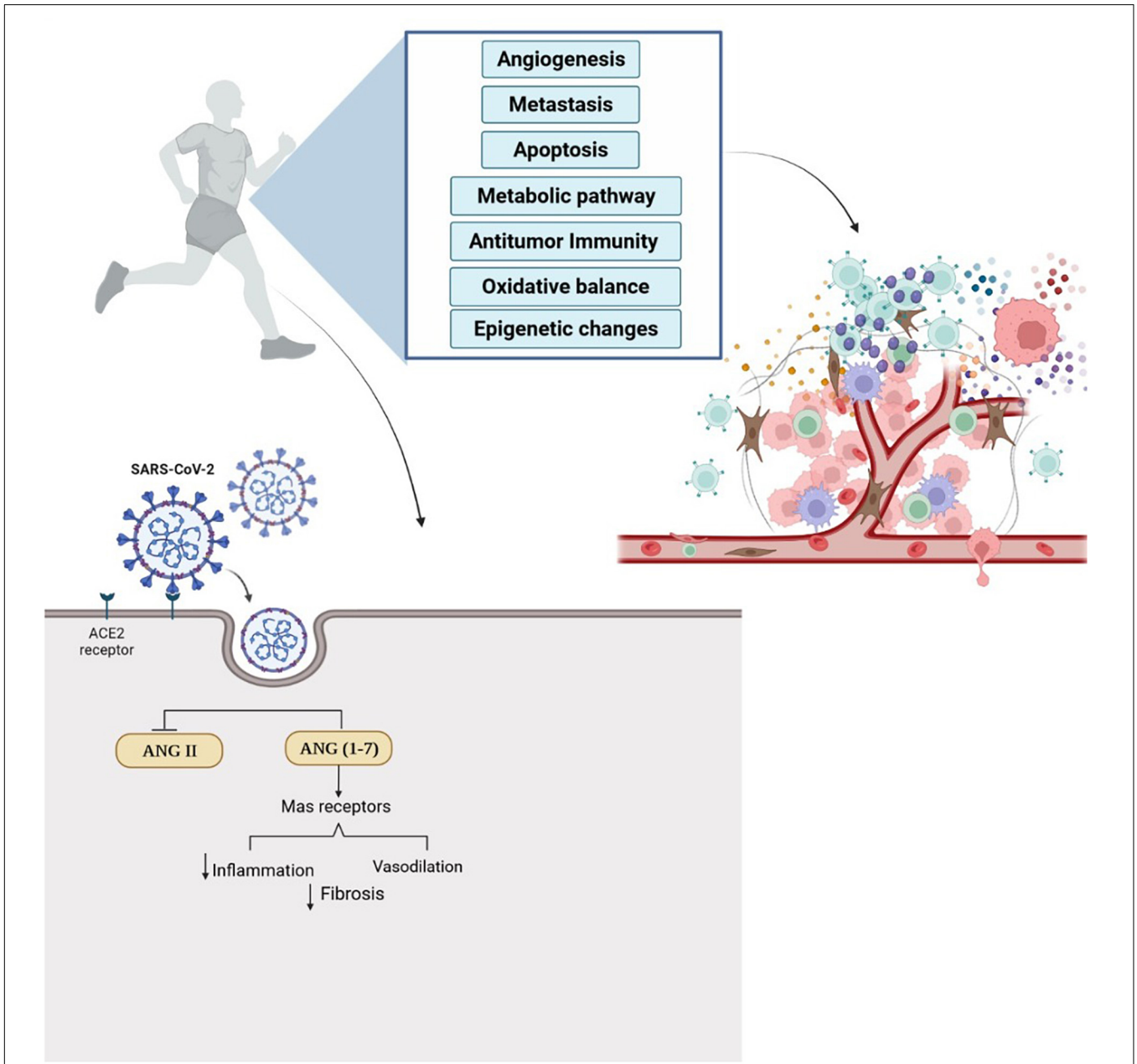

FIGURE 1 | Regular physical exercise can affect tumor microenvironment and induce anti-tumor immunity. Also, exercise training augments the expression of ACE2/ANG-(1-7) Mas receptor and activate anti-inflammatory pathways. Physical exercise simultaneously inhibits the ACE-Ang II-AT1 receptor pathway, inflammatory molecules, and oxidative stress in different tissues and can decrease the possibility of infection with COVID-19. The effects of regular physical exercise on antitumor immunity and ACE2 receptors can probably help cancer patients in the prevention and treatment of COVID-19.

response to vitamin D supplementation. However, more studies are needed in this area.

It has also been suggested that daily doses of 100–200 mcg of selenium consumption, inhibit genetic damage and cancer development in humans (80). About 400 mcg of selenium per day is considered the upper limit. Higher doses of RDA are needed to inhibit genetic damage and cancer. However, it is assumed that taking excessive selenium may cause oxidative damage and lead to genomic instability (81). A study in mice with cancer confirmed the effects of using selenium nanoparticles on cancer-induced cachexia (82). Concomitant use of exercise while improving the immune response, including T cells and antitumor immunity, prevented cachexia in animals (83). It seems that the modulating effects of exercise on selenium-induced responses in cancer can be effective in enhancing antitumor immunity.

The effects of physical exercise on cancer cells have been proven, including activation of invasion and metastasis, escaping growth inhibitors, reducing cell death, inhibiting cancer inflammatory cells, normalizing vessels, escaping immune damage, and reprogramming energy metabolism. Lactic acid is the final metabolite in the anaerobic glycolysis pathway. Increasing aerobic glycolysis in cancer cells can produce a large amount of lactic acid, which reduces pH in cancer cells (84). Proliferation, invasion, and metastasis of cancer cells are associated with angiogenesis, which is related to low pH levels in the tumor microenvironment. Also, lactic acid accumulation in cancer suppresses the immune responses, inhibits the T-cell response, and prevents lactic acid from leaving the T-cell, thereby disrupting the metabolic pathway. It has been stated that moderate-intensity exercise can reduce lactate levels in cells. Therefore, the metabolic process plays a role in the inhibition of anaerobic glycolysis in cancer metabolism (85). As a result, exercise can affect the reprogramming of cancer metabolism by improving the internal blood flow of cancer, angiogenesis, and cancer hypoxia. And rearranging in cancer cells metabolism and taking supplements that are effective in curing cancer, such as selenium and vitamin D, can show a synergistic effect on curing cancer and reducing tumor volume. Using a combination of selenium and other vitamins along with regular physical exercise also seems to be a promising approach to controlling genetic damage, and cancer development. Also, physical exercise can mitigate the side effects of high doses of antioxidants (86).

In addition, combining regular physical exercise and dietary supplementation can probably improve innate and acquired immune system responses (48, 87–89) and protect cancer patients against COVID-19. COVID-19 hyper-inflammatory responses and effects on the respiratory system are mediated by the ACE2, which ultimately leads to effects on other organs (90). Two receptors in the renin-angiotensin system by two opposite arms included: one classical composed by ACE/Angiotensin (Ang) II AT1 receptor (AT1R); and the alternative arm comprising ACE2/Ang-(1-7)/Mas receptor that have anti-inflammatory, vasodilatory, antiproliferative, cardioprotective, and renoprotective actions (91, 92). It is shown that COVID-19 is activated with renin-angiotensin system imbalance, which activates the classical arm (ACE/Ang II/AT1R) and leads to pulmonary injury, hematological alterations, and hyper-inflammatory state. Dysfunction of ACE2/Ang-(1-7)/Mas receptor has been observed in some cancers (91). Exercise training can activate the ACE2-Ang1-7-Mas receptor to induce an anti-inflammatory effect; and lead to inhibiting the ACE-Ang II-AT1 receptor pathway, inflammatory molecules, and oxidative stress in different tissues (93). **Figure 1** summarizes the possible effects of exercise training in cancer and COVID-19.

In addition, the effects of supplementation in COVID-19 can probably be discussed in more detail concerning the ACE2. Although there is conflicting information about the effects of this supplement on the prevention and treatment of COVID-19, some studies have suggested an active form of Vitamin D improved immune system response against COVID-19 (64). Also, it can probably induce ACE2/Ang-(1-7)/MasR axis activity and inhibits renin and the ACE/Ang II/AT1R axis (94). Vitamin D hydroxylate in the kidney yield 1,25(OH)2D3, and it binds to the VDR to activate vitamin D response elements within target genes (95). Considering the effects of exercise training on VDR, the combination of exercise training and vitamin D supplementation can also be effective in regulating the effects of ACE2 and preventing severe cases of COVID-19 in cancer patients (96). However, more studies are needed, especially in cancer patients with different cancers and at different stages of cancer.

Social distance and vaccination are still the best way to prevent severe cases of COVID-19. Despite the observed effects on immune responses to traditional vaccines, especially in older people (97), there is no information on the possible effects of physical exercise or dietary supplements on the COVID-19 vaccines. More studies with assessment of the combination of exercise training and dietary supplements on the potential efficacy of COVID-19 vaccines may be on the agenda of future studies.

CONCLUSION

COVID-19 is spreading with new variants, and cancer patients are prone to severe cases of COVID-19. However, using lifestyle strategies such as regular physical activity and intake of functional compounds as supplements can be effective in treatment outcomes, quality of life, and overall survival. In addition, we cannot ignore the role of regular physical exercise due to metabolic, physiological, and psychological effects. Exercise training and supplementation can improve immune system responses. Combining these two factors can be an important strategy for improving immune responses against COVID-19 and probably improving vaccine responses. However, more studies are needed on nutrition, exercise, and their combined effects on cancer and improving immune responses to infectious diseases in cancer.

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.

AUTHOR CONTRIBUTIONS

MM and KS conceived the review, drafted, and approved the final version of the manuscript. AH and AV made some additions to the text, revised the manuscript, and approved the final version. All authors contributed to the article and approved the submitted version.

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Effect of Exercise on Breast Cancer: A Systematic Review and Meta-analysis of Animal Experiments

Yuxi Li[1†], Xili Xiao[2†], Yue Zhang[1†], Wenjing Tang[1], Dongling Zhong[1], Tianyu Liu[3], Yuanyuan Zhu[4], Juan Li[1,4] and Rongjiang Jin[1,4*]*

[1]School of Health Preservation and Rehabilitation, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Department of Ophthalmology, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]School of Sports, Chengdu University of Traditional Chinese Medicine, Chengdu, China, [4]Affiliated Hospital of Panzhihua University Panzhihua, Panzhihua, China

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Edited by:
Yao Lin,
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***Correspondence:**
Juan Li
785939016@qq.com
Rongjiang Jin
cdzyydxjrj@126.com

[†]These authors have contributed equally to this work and share first authorship

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Objective: Exercise is reported to be beneficial for breast cancer. However, the results seem inconsistent. We conducted this systematic review and meta-analysis of animal experimental studies to fully understand the effect of exercise on breast cancer in animal model.

Methods: We searched databases from inception to April 2022 and manually searched related references to retrieve eligible studies. We screened eligible studies and extracted related data. We assessed the risk of bias and reporting quality using the SYstematic Review Centre for Laboratory animal Experimentation Risk of Bias tool and the Animal Research: Reporting of *In Vivo* Experiments guidelines 2.0, respectively. We summarized the study characteristics and findings of included studies and conducted meta-analysis with RevMan software. Subgroup analysis and sensitivity analysis were also performed.

Results: We identified 537 potential literatures and included 47 articles for analysis. According to the results of risk of bias assessment, only selective outcome reporting was in low risk of bias. Items of sequence generation, random outcome assessment, and incomplete outcome data were rated as high risk of bias. Most of other items were rated unclear risk of bias. In reporting quality assessment, all included articles reported grouping method and experimental procedures. However, no study provided information of the study protocol registration. Meta-analysis showed that, compared with sedentary lifestyle, exercise reduced more tumor weight (MD = −0.76, 95%CI −0.88 to −0.63, p = 0.85, I^2 = 0%) and tumor number per animal (MD = −0.61, 95%CI −0.91 to −0.31, p = 0.34, I^2 = 8%). Exercise decreased more tumor incidence than sedentary lifestyle both in motorized wheel/high-intensity (OR = 0.22, 95%CI 0.11 to 0.46, p = 0.09, I^2 = 41%) and free wheel/low-intensity treadmill running (OR = 0.45, 95%CI 0.14 to 1.44, p = 0.04, I^2 = 60%). Sensitivity analysis showed that the results were robust.

Conclusion: Exercise could reduce tumor weight, number of tumors per animal, and incidence of tumor in breast cancer model of mice and rats. However, the risk of bias items and reporting guidelines in preclinical studies should be concerned. Future research

should consider standards of conducting and reporting preclinical studies and choose suitable exercise protocol for higher quality evidence of exercise for breast cancer.

Keywords: exercise, breast cancer, systematic review, meta-analysis, animal experiment

INTRODUCTION

Breast cancer is the main malignant tumor in females and is the leading cause of female cancer death worldwide (Fitzmaurice et al., 2019; Sharma, 2019). Globally, breast cancer has surpassed lung cancer as the most common cancer, with an estimated 2.3 million new cases and 6.9% mortality rate of them (Sung et al., 2021). Early detection, advanced treatment and an active lifestyle can improve breast cancer survival rates (Miller et al., 2019). The American Cancer Society recently issued guideline that recommended exercise for breast cancer prevention (Rock et al., 2020). Statistics also showed that, compared with inactivity, adults achieved 150–300 min of moderate-intensity exercise (or 75–150 min of vigorous-intensity exercise) per week could reduce 25–30% risk in breast cancer (Kushi et al., 2012). Currently, increasing studies have investigated whether exercise is beneficial for breast cancer during and after cancer treatment (Dieli-Conwright et al., 2018; Odynets et al., 2019; Rangel et al., 2019). However, the results are inconsistent and the relationship between exercise and breast cancer remains to be understood.

Experimental studies using animal models to mimic human disease can detail the onset, promotion, or progression of disease and identify the potential biological pathways (Hoffman-Goetz, 2003; Ashcraft et al., 2016), yet the current results of animal studies in exercise on tumor or the intensity of exercise effect were heterogeneous. Some studies found exercises were effective to slow tumor growth (Shalamzari et al., 2014; Alizadeh et al., 2018) and decrease tumor cell number of breast cancer (Alvarado et al., 2017), while other studies reported exercises did not inhibit tumor initiation (Steiner et al., 2013) or have no effect on the tumor volume (Garritson et al., 2019). The reason may be related with the different cancer phenotypes, the different model established methods of mammary adenoma and different exercise schemes (type, duration, intensity, and frequency of exercise).

To fully understand the effects of exercise on breast cancer in animal experiments, we retrieved preclinical studies focusing on the effect of exercise on breast cancer to comprehensively assess the risk of bias and reporting quality and conduct systematic review and meta-analysis.

METHODS

This meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page et al., 2021). The full PRISMA checklist is presented in **Supplementary Table S1**.

Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (Fitzmaurice et al., 2019) animal experiments investigating the effects of exercise on breast cancer; (Sharma, 2019) rats or mice model; (Sung et al., 2021) control group were set as sedentary control or other activity control; (Miller et al., 2019) outcomes included characteristics of tumor (including tumor volume, tumor weight, tumor number, tumor cell number, tumor incidence, and tumor growth rate); and (Rock et al., 2020) the language was limited to English and Chinese. The exclusion criteria included: (Fitzmaurice et al., 2019) studies with exercise combined diet, chemical therapy, or other therapies; (Sharma, 2019) duplicate studies; (Sung et al., 2021) studies reported outcomes of other tumors.

Database and Search

We searched the literature from the following databases: PubMed, Embase, China National Knowledge Infrastructure (CNKI), Chinese Science and Technology Periodical Database (VIP), Wan Fang database and Chinese Biomedical Literature Database (CBM). We searched the literature from inception to 14 April 2022. Search terms combined breast cancer, exercise, and animal. The detailed search strategy is presented in **Supplementary Table S2**. We also searched websites, reference list from included articles and consulted experts to obtain possible eligible studies.

Studies Selection

We used Endnote X9 to manage all records and identify duplicates. Two reviewers (YXL and YZ) independently screened titles and abstracts to select potential eligible studies according to inclusion criteria. Then, they read full texts of potential eligible studies to identify the final included literature. Any disagreement was resolved by consulting a third reviewer (RJJ).

Data Extraction

Two reviewers (YXL and YZ) independently extracted data using an extraction table designed in advance. We extracted several literatures in advance and discussed the extraction results to ensure data consistency. We extracted the following data: 1) study characteristics; 2) methods of establishing animal model; 3) route of administration; 4) exercise design features; 5) tumor outcomes. Any disagreement was resolved through consensus or discussion with the third reviewer (JL).

Risk of Bias Assessment

Two reviewers (XXL and DLZ) independently assessed the risk of bias using the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE)'s Risk of Bias (RoB) tool. The SYRCLE's RoB tool specifically assesses the risk of bias in animal intervention studies (Hooijmans et al., 2014). The tool contains 10 entries in six aspects: selection bias (items 1–3), performance bias (items 4–5), detection bias (items 6–7), attrition bias (items 8), reporting bias (items 9) and other biases (items 10).

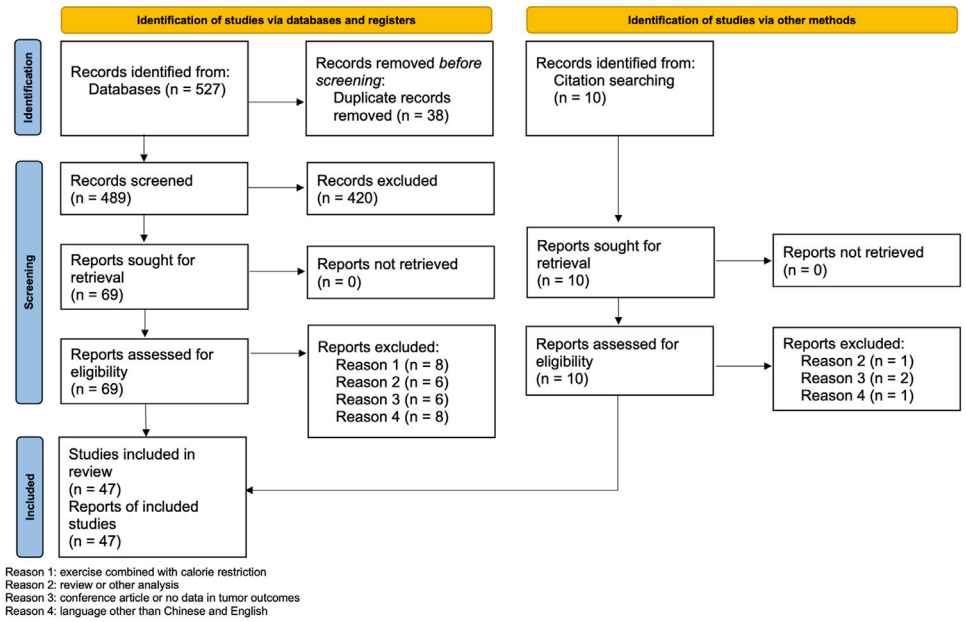

FIGURE 1 | PRISMA 2020 flow diagram.

Each item is rated as "yes" (low risk of bias), "no" (high risk of bias) and "unclear" (if insufficient details are obtained). Any disagreement was resolved through discussion or by consulting a third reviewer (YYZ).

Reporting Quality Assessment

Two reviewers (XXL and DLZ) independently assessed reporting quality using the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines 2.0, respectively. The ARRIVE 2.0 consists of 21 items, which divided the items into 2 sets, the "ARRIVE Essential 10" which constitutes the minimum requirement, and the "Recommended Set," which describes the research context. "ARRIVE Essential 10" contains detailed information on the study design, the sample size, measures to reduce subjective bias, outcome measures, statistical methods, the animals, experimental procedures, and results. "Recommended Set" includes detailed information on the abstract, background, objectives, ethical statement, housing and husbandry, animal care and monitoring, interpretation/scientific implications, generalisability/translation, protocol registration, data access, and declaration of interests. Each item is judged as "Yes", "No", and "Partial Yes". Any disagreement was resolved through discussion or by consulting a third reviewer (YYZ).

Data Analysis

We summarized the study characteristic and findings of included literatures and presented the results in tables. We used diagram and tables to summarize the results of risk of bias and reporting quality assessment. RevMan software (version 5.3.5) was utilized to conduct the data analysis. The MD (Mean difference) was utilized for data measurement of continuous outcomes, and OR (Odds ratio) was for dichotomous variable. All of them were expressed with a 95% confidence interval (CI). We assessed the heterogeneity between studies using Cochrane's Q test and I^2 test. We conducted sensitivity analysis using Stata/SE 15.1 software to explore the robustness of results. We also performed subgroup analysis according to intensity of exercise. Funnel plot was used to evaluate publication bias. $p < 0.05$ was considered significant. For those outcomes with high heterogeneity, we conducted descriptive analysis.

RESULTS

We identified 537 potential literatures. After removal of duplicates and initial screening, we excluded 420 articles, and downloaded 79 full texts for secondary screening. Finally, we included 47 articles for analysis. The excluded studies and the reasons for exclusion are listed in **Supplementary Table S3**. **Figure 1** shows the flow chart of the selection process.

Study Characteristics

The characteristics of studies are presented in **Supplementary Table S1**. The methods of establishing tumor models include: carcinogenic agent 1- methyl-1-nitrosourea (MNU) injection (n = 15, 31.9%), 4T1 breast tumor cells injection (n = 8, 17.0%), 7, 12-dimethylbenz(a)anthracene (DMBA) oral or injection (n = 6, 12.8%), MC4-L2 cells injection (n = 6, 12.8%), MDA-MB-231 breast carcinoma cells implantation (n = 3, 6.4%), EO771 breast or B16-F10 melanoma tumor cells inoculation (n = 2, 4.3%), transgenic mice (n = 1, 2.1%), BCAP-37 breast cancer cells inoculation (n = 1, 2.1%), and breast adenocarcinoma cells inoculation without mention of tumor cell lines (n = 1, 2.1%), and transgenic mice with spontaneous breast cancer (n = 1, 2.2%). Types of exercise are as follows: treadmill/wheel running (n = 43,

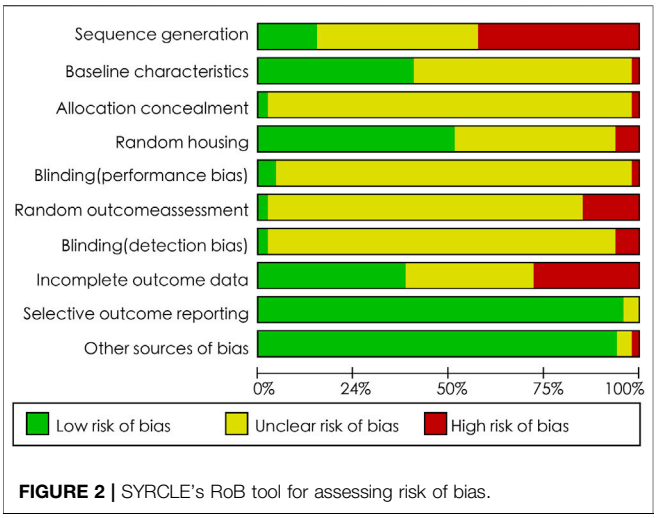

FIGURE 2 | SYRCLE's RoB tool for assessing risk of bias.

91.5%), including voluntary and motorized running, swim training (n = 3, 6.4%), and interval aerobic training (n = 1, 2.1%). The duration of exercise ranges from 2 to 36 weeks. Control groups include tumor/non-trained, sedentary, locked running wheels, immobile treadmill and shallow water pool. The animal sample sizes varies from 12 to 150.

Risk of Bias Assessment

Figure 2 shows the results of the risk of bias assessment. Item 9 "selective outcome reporting" and item 10 "other sources of bias" presented low risk of bias, with rate of 95.7 and 93.6%, respectively. Item 1 "sequence generation", item 6 "random outcome assessment", and item 8 "incomplete outcome data" were rated with high risk of bias, with rate of 42.6, 14.9 and 27.7%, respectively. Item 2 "baseline characteristics" and item 4 "random housing" showed unclear and low risk of bias, respectively. In the remaining items, unclear risk of bias was observed in most articles.

Reporting Quality Assessment

As shown in **Table 1**, all studies reported the most adequate information in grouping method in study design section (1a) and what and how was done in experimental procedures (9a), with a frequency of 100%. Details of experimental animals (8a), when and how often the experimental was done (9b), descriptive statistics for each experimental group (10a) were reported in 44 (95.7%) studies. Definition of outcome measures (6a), statistical methods (7a) and background of the study (12a) were reported in 43 (93.5%) studies. None of the 47 studies provided information on the study protocol registration.

Meta-Analysis

Tumor Weight

We included 7 studies for meta-analysis, which showed tumor weight in exercise group reduced more than control group (MD = −0.52, 95%CI −0.91 to −0.12, $p < 0.00001$, $I^2 = 86\%$). By exploring heterogeneity, we found the duration of exercise of Faustino 2016 (Faustino-Rocha et al., 2016) and Faustino 2017 (Faustino-Rocha et al., 2017) were 35 weeks, Woods 1994 (Woods et al., 1994) was 2 weeks, while exercise time in other studies ranged from 4 to 16 weeks. After removing these 3 studies, the I^2 dropped to 0%, which indicated there was no heterogeneity, and the result remained the same (MD = −0.76, 95%CI −0.88 to −0.63, $p < 0.00001$, $I^2 = 0\%$) (**Figure 3**).

Tumor Number

Pooled data from 3 studies revealed that wheel running decreased the number of tumors per animal (MD = −0.61, 95%CI −0.91 to −0.31, $p < 0.0001$, $I^2 = 8\%$) (**Figure 4**). Murphy 2011 (Murphy et al., 2011), Thompson 2010 (Thompson et al., 2010) and Zhu 2008 (Zhu et al., 2008) also reported running decreased the number of tumors in animal. However, when we synthesized the data, we found that the heterogeneity was too high after analyzing their study characteristics, we found the animal model and exercise protocol may be the reasons of heterogeneity.

Tumor Incidence

As for tumor incidence, we separated data based on different intensity of exercise to conduct subgroup meta-analysis. Based on the exercise protocol of original studies, we grouped motorized wheel and high-intensity treadmill running together, while free wheel and low-intensity treadmill running studies were grouped in another group. Compared with sedentary group, both motorized wheel/high-intensity (OR = 0.22, 95%CI 0.11 to 0.46, $p < 0.0001$, $I^2 = 41\%$) and free wheel/low-intensity treadmill running (OR = 0.45, 95%CI 0.14 to 1.44, $p = 0.18$, $I^2 = 60\%$) could decrease tumor incidence (**Figure 5**). The asymmetric funnel plot showed publication bias might exist (**Figure 6**).

Sensitivity Analysis

We performed sensitivity analysis for outcomes of meta-analysis to test the robustness of the results. We found the result of each study did not have any important impacts on the overall findings (**Supplementary Table S4**).

Descriptive Analysis

Among the included studies, 21 studies (Steiner et al., 2013; Shalamzari et al., 2014; Alvarado et al., 2017; Alizadeh et al., 2018; Murphy et al., 2011; Aveseh et al., 2015; Leila et al., 2015; Malicka et al., 2015; Isanejad et al., 2016; Cui, 2017; Nasiri et al., 2017; Lyv Di et al., 2021; Qi et al., 2013; Siewierska et al., 2018; Siewierska et al., 2020; Wen et al., 2010; Gholamian et al., 2020; Vulczak et al., 2020; Wennerberg et al., 2020) reported that exercise could decrease the tumor volume, 1 (Faustino-Rocha et al., 2016) reported negative effects of exercise, and 2 (Smeda et al., 2017; Garritson et al., 2019) reported that exercise had no effect on the tumor volume.

3 studies (Cohen et al., 1993; Thompson et al., 1995; Zhu et al., 2008) reported that exercise reduced the tumor multiplicity, while one study (Colbert et al., 2009) found multiplicity of mammary carcinomas increased in wheel running animals.

TABLE 1 | Reporting rate of ARRIVE guidelines 2.0 for included studies.

Domain/Number		Item	Reported (Number, %)		
		The ARRIVE Essential 10	**Y**	**N**	**NA**
Study design	1a	The groups being compared, including control groups. If no control group has been used, the rationale should be stated	100		
	1b	The experimental unit (e.g., a single animal, litter, or cage of animals)	89.4	8.5	2.1
Sample size	2a	Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used	68.1	29.8	2.1
	2b	Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done	2.1	93.6	4.3
Inclusion and exclusion criteria	3a	Describe any criteria used for including or excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly	21.3	76.6	2.12
	3b	For each experimental group, report any animals, experimental units, or data points not included in the analysis and explain why. If there were no exclusions, state so	19.1	78.7	2.1
	3c	For each analysis, report the exact value of n in each experimental group	38.3	57.4	4.3
Randomisation	4a	State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence	17	74.5	8.5
	4b	Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly	6.4	93.6	
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis)	2.1	95.7	2.1
Outcome measures	6a	Clearly define all outcome measures assessed (e.g., cell death, molecular markers, or behavioural changes)	93.6	4.3	2.1
	6b	For hypothesis-testing studies, specify the primary outcome measure, i.e., the outcome measure that was used to determine the sample size	74.5	21.3	4.3
Statistical methods	7a	Provide details of the statistical methods used for each analysis, including software used	93.6	6.4	
	7b	Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	23.4	74.5	2.1
Experimental animals	8a	Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight	95.7	2.1	2.1
	8b	Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures	70.2	27.7	2.1
Experimental procedures	9a	What was done, how it was done, and what was used	100		
	9b	When and how often	95.7	4.3	
	9c	Where (including detail of any acclimatization periods)	76.6	23.4	
	9d	Why (provide rationale for procedures)	17	83	
Results	10a	Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g., mean and SD, or median and range)	95.7	4.3	
	10b	If applicable, the effect size with a confidence interval	19.1	38.3	42.6
The recommended set					
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions	91.5	8.5	
Background	12a	Include sufficient scientific background to understand the rationale and context for the study and explain the experimental approach	93.6	6.4	
	12b	Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology	21.3	66	12.8
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested	89.4	4.3	6.4
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study and any relevant license or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification	70.2	27.7	2.1
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment	76.6	21.3	2.1
Animal care and monitoring	16a	Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering, and distress	34	63.8	2.1
	16b	Report any expected or unexpected adverse events	12.8	87.2	
	16c	Describe the humane endpoints established for the study, the signs that were monitored, and the frequency of monitoring. If the study did not set humane endpoints, state this	40.4	55.3	4.3
Interpretation/scientific implications	17a	Interpret the results, taking into account the study objectives and hypotheses, current theory, and other relevant studies in the literature	89.4	10.6	
	17b	Comment on the study limitations, including potential sources of bias, limitations of the animal model, and imprecision associated with the results	17	83	
Generalisability/translation	18	Comment on whether, and how, the findings of this study are likely to generalize to other species or experimental conditions, including any relevance to human biology (where appropriate)	21.3	74.5	4.3
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered		100	

(Continued on following page)

TABLE 1 | (*Continued*) Reporting rate of ARRIVE guidelines 2.0 for included studies.

Domain/Number		Item	Reported (Number, %)		
		The ARRIVE Essential 10	**Y**	**N**	**NA**
Data access	20	Provide a statement describing if and where study data are available	10.6	89.4	
Declaration of interests	21a	Declare any potential conflicts of interest, including financial and nonfinancial. If none exist, this should be stated	31.9	68.1	
	21b	List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis, and reporting of the study	40.4	59.6	

AbbreviationsY = yes; N = no; NA, not available.

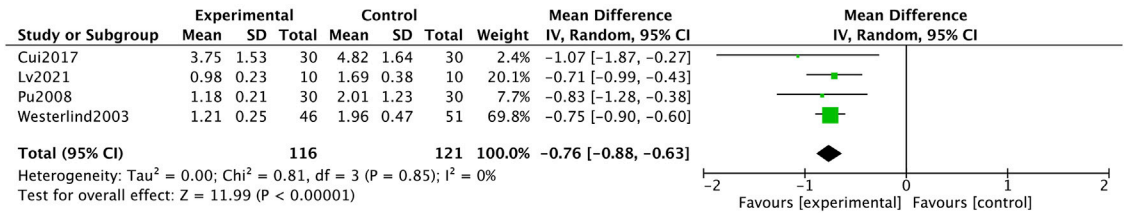

FIGURE 3 | Forest plot of the influence of exercise vs sedentary control on tumor weight.

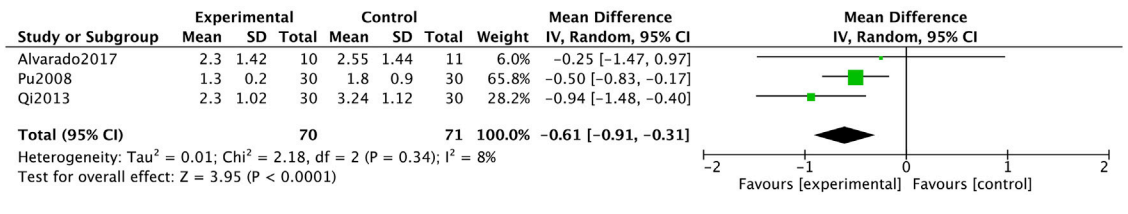

FIGURE 4 | Forest plot of the influence of exercise vs sedentary control on tumor number.

8 studies (Jones et al., 1985; Welsch et al., 1995; Westerlind et al., 2003; Steiner et al., 2013; Shalamzari et al., 2014; Bianco et al., 2017; Nasiri et al., 2017; Vulczak et al., 2020) reported exercise delayed tumor growth rate, while Buss (Buss et al., 2020) and da Costa (da Costa et al., 2021) found exercise did not affect tumor growth rate.

1 study (Alvarado et al., 2017) reported that exercised mice did not develop any metastasis, while 2 pulmonary metastases were observed in the sedentary group. In Goh's study (Goh et al., 2013), no difference in tumor growth was observed between runners and non-runners.

As for other outcomes: 3 studies (Cohen et al., 1993; Pu et al., 2008; Faustino-Rocha et al., 2016) reported exercise increased the tumor latency; 2 studies (Jones et al., 1985; Faustino-Rocha et al., 2016) reported exercise training could enhance vascularization.

DISCUSSION

The results of this study indicated that exercise could reduce tumor weight, number of tumors per animal, and incidence of tumor in breast cancer model of mice and rats. However, we found most of included studies failed to report some items in ARRIVE guideline, such as sample size calculation, randomization, blinding methods, which also led to unclear risk of bias in SYRCLE assessment.

Our study found that exercise reduced tumor incidence. In contrast, Cohen (Cohen et al., 1993) reported no effect on overall tumor incidence in exercise group animals, Woods (Woods et al., 1994) and Colbert (Colbert et al., 2009) found exercise might increase tumor incidence. Different animal models and exercise protocols may be the reasons for inconsistent findings.

Our study showed exercise could decrease tumor weight. Although, the results in tumor volume were too heterogeneous to be synthesized, 21 studies reported beneficial effects of exercise in tumor volume. Smeda's study (Smeda et al., 2017) demonstrated that spontaneous voluntary wheel running had no effect on the volume and size of primary breast tumor. In Faustino's research (Faustino-Rocha et al., 2016), the tumors' weight and volume were higher in exercised animals compared with sedentary ones. The author explained that this might be related with the enhancement of blood perfusion.

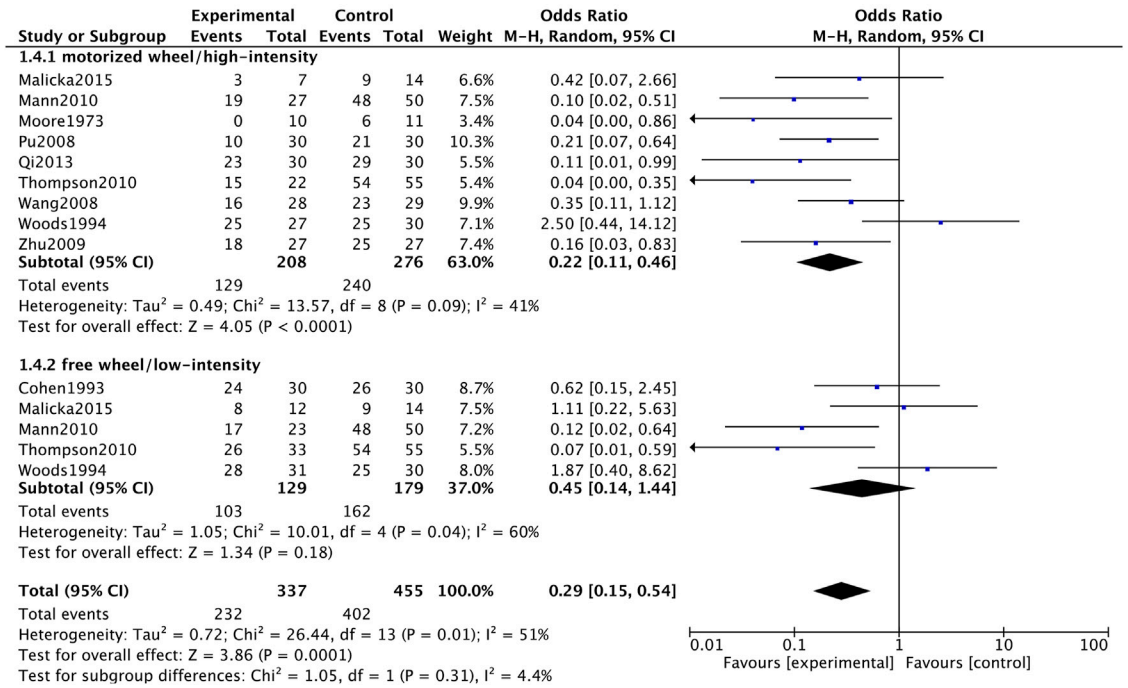

FIGURE 5 | Forest plot of the influence of exercise vs control on tumor incidence.

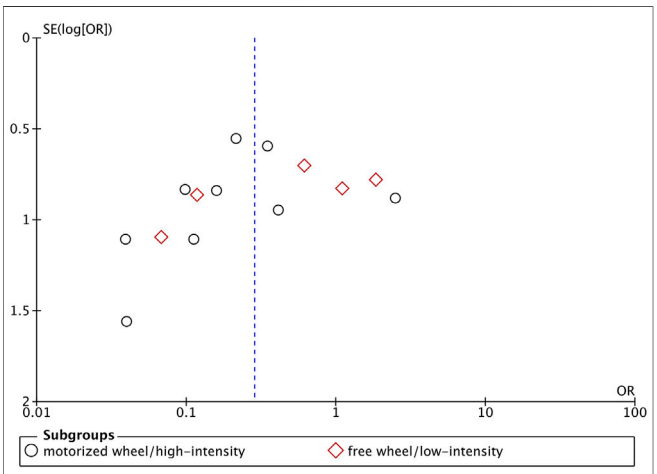

FIGURE 6 | Funnel plot of the influence of exercise vs control on tumor incidence.

Significant reduction in tumor number in exercised animals was noted in our study. Among included studies, Steiner's study showed that voluntary wheel running was associated with an increased number of tumors developing in mice. This negative effect of exercise in tumor number may attribute to the different animal model they used. The model in Steiner's study was the representative, triple-negative C3 (1)/SV40Tag transgenic mouse model. The author interpreted that voluntary exercise might not overcome the highly tumorigenic phenotype induced by the inactivation of two primary tumor suppressors, p53 and pRb (Green et al., 2000).

Malicka (Malicka et al., 2015) reported tumor incidence increased in low intensity exercise group, while dropped in moderate and high intensity exercise group. Our meta-analysis also came up with the same results, except for the result about low intensity exercise on tumor incidence due to limited research data. A previous review pointed out that as exercise intensity increased, it is more likely that physical activity would inhibit carcinogenesis (Thompson, 1994). The present results also demonstrated that different exercise protocols may be associated with different influences on tumor outcomes. Negative effects were more likely to be found in voluntary or low-intensity exercises, whilst forced or moderate, high-intensity exercises studies appeared to have better results. However, previous correlation analysis revealed that benefits were associated with low-intensity exercise, and voluntary exercise appeared to have more positive influence on the incidence, multiplicity and weight of tumors than forced exercise (Figueira et al., 2018).

Our results showed that most of included studies were assessed as unclear risk of bias, and they rarely followed the reporting guidelines. In 2002, the Lancet published an influential commentary (Sandercock and Roberts, 2002) mentioned the importance of risk of bias of animal studies. Since then, the awareness of risk of bias of preclinical studies had been increasing. Several practical guidelines were issued to facilitate well-informed decision-making evidence from animal studies (Hooijmans et al., 2012; Vesterinen et al., 2014; Soliman et al., 2020). The implementation of risk of bias tool and reporting guideline will enhance reliability and robustness of evidence from animal studies. More than that, this may subsequently improve the transformation of preclinical results into clinical experiments.

In the field of exercise-oncology research, there are several risk of bias items and reporting issues should be concerned: (Fitzmaurice et al., 2019) state criteria for including or excluding animals; (Sharma, 2019) describe randomization and allocation of animals; (Sung et al., 2021) describe blinding methods of each stage of experiment; (Miller et al., 2019) report the implantation methods of tumor cell lines or induction of orthotopic tumors; (Rock et al., 2020) mention protocol of exercise intervention (forced or voluntary, exercise intensity, duration, frequency); (Kushi et al., 2012) design and report a prior-registered study protocol.

Limitations

There are few limitations in our study. First, we were unable to do meta-analysis for some of the findings, as the heterogeneity among the included studies allowed us to only summarize and describe their results. Second, unclear risk of bias and relatively high risk of bias may affect the results. Third, we only included studies published in Chinese and English, which language bias was inevitable.

CONCLUSION

Exercise could reduce tumor weight, number of tumors per animal, and incidence of tumor in breast cancer model of mice and rats. However, the risk of bias items and reporting issues in preclinical studies should be concerned. Future research should consider standards of conducting and reporting preclinical studies and choose suitable exercise protocol for higher quality evidence in exercise for breast cancer.

AUTHOR CONTRIBUTIONS

RJJ and JL designed the study. YXL and YZ drafted the manuscript. DLZ and TYL revised the manuscript. XLX, WJT, and YYZ analyzed the data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2022.843810/full#supplementary-material

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High-Intensity Aerobic Exercise Suppresses Cancer Growth by Regulating Skeletal Muscle-Derived Oncogenes and Tumor Suppressors

Hyunseok Jee[1,2]*, *Eunmi Park*[2], *Kyunghoon Hur*[3], *Minjeong Kang*[3] and *Yoosik Kim*[3,4]*

[1]School of Kinesiology, Yeungnam University, Gyeongsan, South Korea, [2]College of Life Science and Nano Technology, Hannam University, Daejeon, South Korea, [3]Department of Chemical and Biomolecular Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea, [4]KAIST Institute for Health Science and Technology (KIHST), KAIST, Daejeon, South Korea

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University of Valladolid, Spain
Vikas Singh,
The Rockefeller University, United States

***Correspondence:**
Hyunseok Jee
jeehs@ynu.ac.kr
Yoosik Kim
ysyoosik@kaist.ac.kr

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High-intensity aerobic exercise (90% of the maximal heart rate) can effectively suppress cancer cell proliferation *in vivo*. However, the molecular effects of exercise and its relevance to cancer prevention remain uninvestigated. In this study, mice with colorectal cancer were subjected to high-intensity aerobic exercise, and mRNA-seq analysis was performed on the heart, lungs, and skeletal muscle tissues to analyze the genome-wide molecular effects of exercise. The skeletal muscle-derived genes with exercise-dependent differential expression were further evaluated for their effects on colorectal cancer cell viability. Compared to the results obtained for the control groups (healthy and cancer with no exercise), the regular and high-intensity aerobic physical activity in the mice produced positive results in comprehensive parameters (i.e., food intake, weight gain, and survival rate). A heatmap of differentially expressed genes revealed markedly different gene expression patterns among the groups. RNA-seq analysis of 23,282 genes expressed in the skeletal muscle yielded several anticancer effector genes (e.g., *Trim63*, *Fos*, *Col1a1*, and *Six2*). Knockdown and overexpression of selected anticancer genes repressed CT26 murine colorectal carcinoma cell proliferation by 20% ($p < 0.05$). Our findings, based on the aerobic exercise cancer mouse model, suggest that high-intensity aerobic exercise results in a comprehensive change in the expression patterns of genes, particularly those that can affect cancer cell viability. Such an approach may identify key exercise-regulated genes that can help the body combat cancer.

Keywords: exercise, cancer, RNA-seq, skeletal muscle, tumor suppressors

Abbreviations: Asb, ankyrin repeat and SOCs box containing; Col1a1, collagen type 1 alpha 1 chain; CT26, colon tumor 26; ILs, interleukins; mRNA seq, messenger ribonucleic acid sequencing; Maff, MAF BZIP transcription factor F; Osr, odd-skipped related transcription factor; pcDNA, plasmid cloning deoxyribonucleic acid; siRNA, small interfering RNA; Trim63, tripartite motif-containing 63; Tnnc, troponin c; Ifrd, interferon-related developmental regulator.

1 INTRODUCTION

Exercise is a positive effector of physical fitness, which contributes to improved health. The effects of exercise involve muscular functions that affect the overall well-being of the body, including mental and emotional health (Solberg et al., 2013; Izquierdo et al., 2020). However, exercise can also have adverse effects on health, depending on exposure to exercise stimulation. A customized exercise regime can be established by selecting optimized conditions, such as time, intensity, frequency, and style of exercise. This principle can be applied in cancer prevention and treatment. Cancer-related outcomes such as anxiety, depression, fatigue, and quality of life can also be improved by specific evidence-based exercise modalities by optimizing FITT (frequency, intensity, time, type) (Campbell et al., 2019). Different modes of exercise can be ideal for different types of cancer. Previous studies have shown that proper exercise can positively serve individuals with cancer (Newton and Galvao, 2008; Jee and Kim, 2019; Schmitz et al., 2019). For example, a recent microarray-based study on the effect of low-intensity resistance training on human subjects showed that the accurate mode and extent of exercise resulted in the upregulation of microRNAs, such as miR-630 and miR-5703, and myokines (fractalkine/CX3CL1) among 42 pathways and 12 cytokines/myokines, which subsequently suppressed tumor growth by inducing tumor-specific cytotoxic T cells (Hashida et al., 2021). In addition, the ladder climbing resistant training exercise on preclinical tumor-bearing animals also mitigated muscle atrophy, tumor growth, and cancer malignancy (Padilha et al., 2019; Padilha et al., 2021). However, the molecular mechanisms underlying the anticancer effects of exercise remain largely unknown.

The muscle is one of the most important organs required for physical performance (Park et al., 2012). Muscle tissues are classified as slow or fast types based on their myosin heavy chain isoforms, and the ratio of these two muscle types affects athletic performance. The different ratios of the muscle types may be related to the heterogeneous expression of specific genes (Cohen et al., 2015). The gastrocnemius muscle, considered in this study, is a fast-type skeletal muscle and consists of a balanced ratio of fast (78%~95%) and slow (5%~22%) fibers (Jee et al., 2009; Jee et al., 2016b). Such muscle is chosen to offset the possibility of having one-sided muscle fibers, which may influence gene expression. By secreting compounds such as myokines, metabolites, and exosomes, the muscle tissue can function as an endocrine organ and affects the entire body in response to exercise (Raschke et al., 2013; Hartwig et al., 2014). Currently, it is unknown how the gene expression pattern of skeletal muscles is affected by high-intensity aerobic exercise and how such changes may help the body combat cancer.

Every day, cells in the human body experience mutations that may induce jeopardizing and uncontrolled growth. This is the basic notion of cancer. Cancers can develop in various tissues such as the oral cavity, digestive, respiratory, reproductive, derma, blood, and more. In addition, different kinds of cancer have unique etiology, such as human papillomavirus causing cervical cancer (Yang and Jee, 2021). Carcinogenesis in the muscle, however, is extremely rare even though sarcomas do exist. In fact, there is no cancer in the cardiac muscle; it is the colonized cells in the myocyte that develop into cancer (Willis, 1952). Therefore, we hypothesized that muscle-specific factors could suppress cancer cell attachment and that muscle contraction-driven exercise may affect this phenomenon.

Several studies have shown the benefits of exercise, particularly in enhancing the immune system to prevent cancer. Exercise affects body composition, endocrine secretion, systematic inflammation, and immune cell function (Sitlinger et al., 2020). In particular, exercise can lead to an influx of immune cells that result in a 60% reduction in tumor incidence and growth (Pedersen et al., 2016). Acute aerobic exercise rapidly increases immune cell counts in blood and results in interleukin-6 dependent redistribution of NK cells, ultimately suppressing the tumor growth (Karre et al., 1986; Walsh et al., 2011; Pedersen et al., 2016). However, only a limited number of studies have explored the molecular effects of exercise (Koelwyn et al., 2015; Idorn and Thor Straten, 2017). These studies focused on the epigenetic effects of aerobic exercise and the potential effects on the expression of specific downstream genes such as interleukins (ILs), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFNγ), and transforming growth factor. Other studies evaluated the effect of aerobic and resistance exercise on the expression of skeletal muscle genes using mRNA-seq (Popov et al., 2018; Pillon et al., 2020). However, they failed to identify the key genes that could mediate the anticancer effects of exercise. This is the key rationale of our study that we combined the aerobic exercise and cancer mouse models to unravel the anticancer genes regulated by aerobic exercise in a genome-wide manner.

In this study, we used mRNA-seq to profile gene expression changes following high-intensity aerobic exercise in mice. In particular, we analyzed the gastrocnemius muscle, heart, and lungs to identify genes potentially mediating the anticancer effects of exercise. By comparing the effects of exercise in normal and colorectal cancer-bearing mice, we analyzed the genetic changes stimulated by exercise and the presence of cancer. Furthermore, we identified genes that are regulated both by exercise and cancer, but opposite in direction. By modulating the expression of these genes *in vitro*, we analyzed their potential anticancer effects. These findings provide evidence of colorectal cancer-related gene expression changes *in vivo* and suggest potential molecular effectors of exercise that may have anticancer activity in this colorectal cancer animal model.

2 MATERIALS AND METHODS

2.1 Animals and Exercise Protocol

Forty pathogen-free male CDF1 mice, weighing 18–20 g, at 4 weeks of age were purchased from the Central Animal Laboratory (Seoul, Korea). The mice were randomly divided into no-exercise non-cancer healthy control group [N = 10; E(−)T(−)], no exercise with cancer group [N = 10; E(−)T(+)], high-intensity aerobic exercise healthy group [N = 10; E(+) T(−)], and high-intensity aerobic exercise with cancer group [N = 10;

E(+)T(+)]. All animals were bred in our pathogen-free animal facilities and allowed access to standard chow and water *ad libitum*. They were housed in a sterile room maintained at 22–24°C with a 12:12 h light-dark cycle. For the treadmill exercise, a modification of a published protocol was used (Zogaib and Monte-Alto-Costa, 2011). The mice were made to run on a four-lane-motorized treadmill with a light shield (45 min with 0° slope, 09:00~12:00 p.m.) once every 2 days in order to minimize the effect of biorhythms and overwork. High-intensity aerobic exercise was conducted at 1.0 km/h (90% of the maximal heart rate). Animals were sacrificed at the end of the experiments, on day 19, by exsanguination under anesthesia induced by a mixture of tiletamine, zolazepam, and xylazine (40 mg/kg body mass). Tissues, including the right gastrocnemius muscles, lungs, and hearts of each group, were isolated for further analyses.

Animal use and maintenance protocols were approved by the Yeungnam University Medical Centre Institutional Animal Care and Use Committee (YUMC-AEC2020-008). The evaluation criteria were as follows: rationale and purpose of the proposed animal use; justification of species and number of animals requested; unnecessary duplication of tests or experiments; availability or appropriateness of the use of minimally invasive procedures; adequacy of training and personnel experience; multiple major surgical procedures conducted; unusual housing and husbandry requirements; appropriate sedation, analgesia, and anesthesia; method of euthanasia or disposition of animals; timely intervention criteria and procedures or euthanasia, if required; and safety of the working environment for personnel.

2.2 Study Design

The gastrocnemius muscles, lungs, and hearts of the four different groups of mice were analyzed through mRNA-seq. In total, 23,282 genes were identified. Of these, 40 genes (*Col3a1*, *Col5a2*, *IL15*, *Tnnc1*, *Col1a1*, *Osr2*, *Ifrd1*, *Trim63*, *Asb2*, *Maff*, *Myl6b*, *Six2*, *Fos*, *etc.*) were selected based on at least two-fold changes and normalized read count of four in all three-way comparisons 1) the effect of high-intensity aerobic exercise [E(+)T(−) vs. E(−)T(−)], 2) cancer effect [E(−)T(+) vs. E(−)T(−)], and 3) the effect of exercise on cancer-bearing mice [E(+)T(+) vs. E(−)T(+)] groups in the gastrocnemius muscle. The expression of these genes was further verified *via* quantitative reverse transcription-polymerase chain reaction (RT-qPCR) analysis of samples from littermates of the original four groups. The selected genes were used for the Kyoto Encyclopedia of Genes and Genomes (KEGG) mapper (https://www.genome.jp/kegg/mapper.html). Based on the mRNA-seq, RT-qPCR validation, and KEGG analysis data, four genes (*Fos*, *Trim63*, *Six2*, and *Col1a1*) were examined for their potential effects on cancer cell viability.

2.3 Establishing the Mouse Cancer Model

Approximately 1×10^5 CT26 murine colorectal cancer cells (purchased from Korean Cell Bank, Seoul, Korea) were injected into each mouse *via* the tail vein to establish the cancer mouse model. The mice were then returned to their cages. They were periodically examined with the VISQUE InVivo Smart imaging system (Vieworks, Chayon, Korea), following the injection of MMPSense680 reagent (PerkinElmer, Waltham, MA, United States) *via* the tail vein to examine cancer cell metastasis.

2.4 Tissue-to-Body Weight Ratio, Body Weight Change, and Food Intake

Bodyweight and food intake were monitored to assess the quality of life. The lung, heart, and gastrocnemius muscles were isolated at the end of the study. The weights of these tissues were determined, and the weight differences among the groups were compared (**Table 1**).

2.5 RNA Extraction

Total RNA from each sample was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer's instructions. The quality of the extracted RNA was assessed using a model 2100 bioanalyzer equipped with an RNA 6000 Nano Chip (Agilent Technologies, Santa Clara, CA, United States). RNA quantification was performed using an ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States).

2.6 Library Preparation and Sequencing

The sequencing library was constructed using the QuantiSeq 3′ mRNA-Seq Library Prep Kit (Lexogen Inc., Vienna, Austria) according to the manufacturer's instructions. Total RNA (500 ng) was extracted, and an Illumina platform-compatible sequence containing an oligo-dT primer was hybridized for reverse transcription. An Illumina-compatible linker sequence containing random sequences was used as the posterior procedure for degradation of the RNA template. Magnetic beads were used to purify the double-stranded sequencing library. Complete adapter sequences for cluster generation were added to amplify the library. The high-throughput sequencing procedure (as the single-end 75 sequences) was then performed using the NextSeq 500 device (Illumina Inc., San Diego, CA, United States). mRNA-seq analysis identified 23,282 genes. Changes in their expression levels were calculated as $\log_2$ values for genes with higher than normalized read count of four.

2.7 Cell Culture and Transfection

CT26 cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 mg/ml streptomycin in a 5% CO_2/95% air humidified atmosphere. Cells were passaged every 3–4 days and maintained at a dilution of 1.5×10^5 cells per well (90 mm in diameter) via trypsinization until the cells reached approximately 70%–80% confluency. For transfection, Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, United States) was used according to the manufacturer's instructions. The sequences of the small interfering RNAs (siRNAs) used in this study are

TABLE 1 | Tissue weight data.

		Normal control	Cancer control	Cancer exercise	Normal exercise
Spleen		75.50 ± 3.69	181.00 ± 19.86	184.00 ± 30.82*	78.33 ± 5.75
Diaphragm		70.33 ± 4.03	269.25 ± 122.65	568.00 ± 130.19	65.29 ± 5.83
Plantaris muscle	R	11.67 ± 0.99	10.00 ± 0.69	13.17 ± 0.83	15.29 ± 0.68 Ω °°°°
	L	12.83 ± 0.60	8.28 ± 0.52##	11.00 ± 1.02$^{\nabla\nabla}$	15.42 ± 0.43°°°°
Gastrocnemius muscle	R	113.17 ± 5.96	85.29 ± 3.76##	88.71 ± 6.09*$^{\nabla\nabla}$	127.17 ± 3.75°°°°
	L	111.33 ± 4.92	82.43 ± 4.59##	93.00 ± 5.54$^{\nabla\nabla}$	128.33 ± 3.77°°°°

N = 4–10; Values are mean ± S.D. Note that Diaphragm muscle showed remarkably increased weight in the cancer groups even though there are no significant differences. Only the right of right hindlimb plantaris muscle shows the statistical difference between control and exercise; *p *< 0.05: statistical significance between control and cancer exercise;* $^{\Omega}$p *< 0.05: statistical significance between control and exercise;* ##p *< 0.01: statistical significance between control and cancer;* °°°°p *< 0.01: statistical significance between exercise and cancer;* $^{\nabla\nabla}$p *< 0.01: statistical significance between exercise and cancer exercise.*

provided in **Supplementary Table S1**. For overexpression of a target gene, pcDNA3 plasmid vectors encoding the complementary DNA of the gene were transfected into the cells using Lipofectamine 3000. Prior to every experiment, the cells were cultured for at least 18 h for stabilization.

2.8 RT-qPCR

Approximately 1 μg of total RNA was reverse transcribed with RevertAid reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, United States) to synthesize cDNA. RT-qPCR was performed using SYBR Green to analyze changes in gene expression. The primer sequences used in this study are listed in **Supplementary Table S2**.

2.9 Cell Viability Assay

For adherent cell lines, 4.0×10^4 cells were seeded per well in a 24-well plate and stabilized for 18 h. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay (CyQUANT™ MTT Cell Viability Assay, Thermo Fisher Scientific, Waltham, MA, United States) was performed, following the manufacturer's instructions, after transfection and incubation of siRNA and cDNA plasmids for 72 h. Cell viability was determined by measuring the absorbance at 580 nm using a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, Waltham, MA, United States).

2.10 Western Blotting

CT26 cell lysates were prepared by suspending the cell pellets in lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.5% NP-40, 10% glycerol, and 1 mM DTT), supplemented with a protease inhibitor cocktail (Merck, Darmstadt, Germany), and incubating on ice for 10 min. Complete lysis was achieved by sonication. Protein samples (40 μg) were separated *via* sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a poly(vinylidene fluoride) membrane (Merck, Darmstadt, Germany) using an Amersham semi-dry transfer system. The primary antibodies used in this study were as follows: Fos (Cell Signaling Technology, Danvers, MA, United States, 4384T), Col1a1 (Cell Signaling Technology, Danvers, MA, United States, 91144S), Six2 (LSBio, Seattle, WA, United States, LS-C386068), Trim63 (R&D Systems, Minneapolis, MN, United States, AF5366), and β-tubulin (Cell Signalling Technology, Danvers, MA, United States, 86298S).

2.11 Data and Statistical Analyses

Bowtie2 sequence alignment was performed using QuantSeq 3′ mRNA-Seq reads (Langmead and Salzberg, 2012). Genome assembly sequence or the representative transcript sequence derived from the Bowtie2 indices was generated to map the sequencing reads to the genome. The aligned files for assembling transcripts estimated their abundance and detected the differential expression of genes. Differentially expressed genes (DEGs) were determined using coverage in Bedtools derived counts (Quinlan and Hall, 2010). Using Bioconductor (Gentleman et al., 2004), read count data were processed according to the quantile normalization method using EdgeR within R (Law et al., 2016). DAVID (http://david.abcc.ncifcrf.gov/) was used for gene ontology analysis of DEGs.

All data are presented as mean ± standard error of the mean. Two-way repeated measure ANOVA was used to calculate the *p*-values for the daily food intake and weight of the mice. *p*-values for the tissue weight data in **Table 1** were determined *via* a one-way ANOVA with a one-sided Dunnett's test by comparing the experimental groups to the normal control group. A student's t-test was used to determine *p*-values for the data presented in **Figure 6**.

3 RESULTS

3.1 High-Intensity Aerobic Exercise Prevents Cancer Development *In Vivo*

We applied increasing physical fitness to imitate regular physical activity in a cancer mouse model to examine the molecular effects of exercise on cancer development. To induce cancer, we introduced CT26 murine colorectal cancer cells in mice *via* tail vein injection, which resulted in the dissemination of cancer throughout the body, including the colon and lungs. Throughout the study, we compared four groups of mice (**Figure 1A**: 1) healthy mice without exercise [Normal control; E(−)T(−)], 2) cancer-bearing mice without exercise [Cancer control; E(−)T(+)], 3) cancer-bearing mice with high-intensity aerobic exercise [Cancer exercise; E(+)T(+)], and 4) healthy mice with high-intensity aerobic exercise [Normal exercise; E(+)T(−)]. High-intensity aerobic exercise was performed on a motorized treadmill with a maximal heart rate of 90%, which is a standard

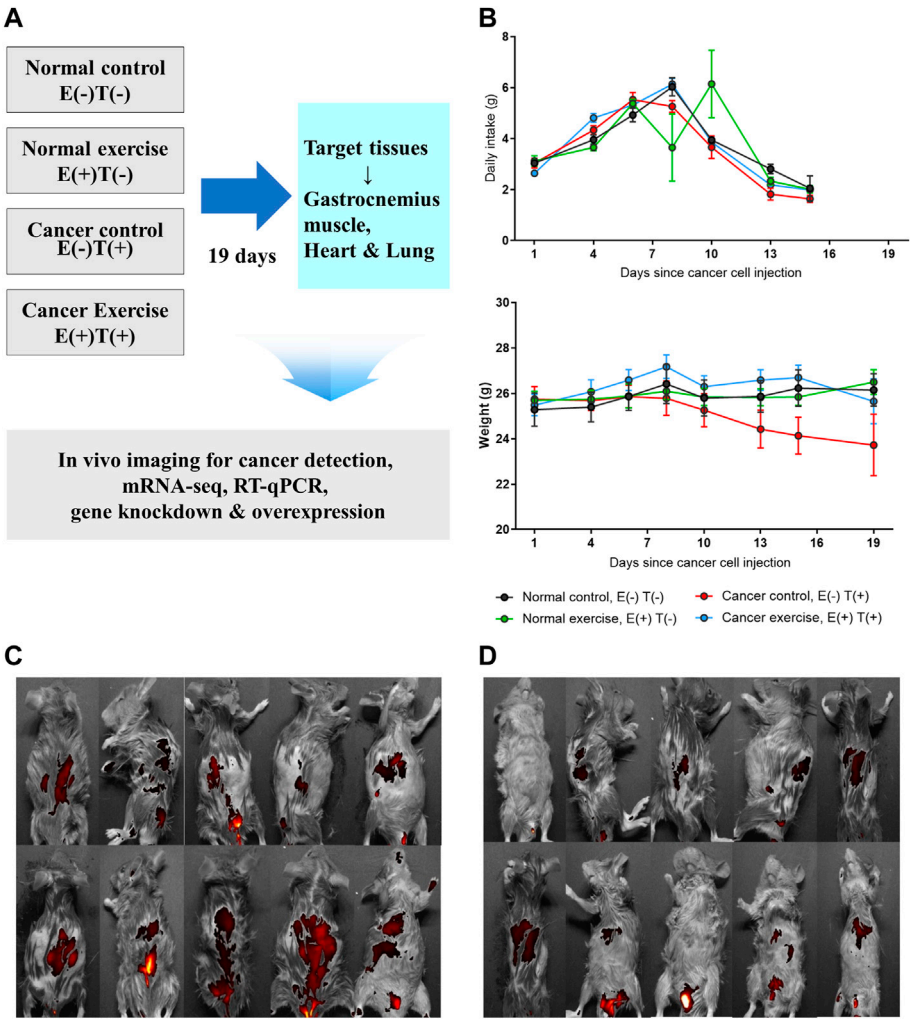

FIGURE 1 | *In vivo* animal model to study the molecular effect of high-intensity aerobic exercise. **(A)** In the current study, 40 mice were divided into four groups of ten mice each (normal control, normal exercise, cancer control, and cancer exercise). The changes of daily intake and weight of each group were traced for 19 days. After 19 days, *in vivo* images for detecting CT-26-derived cancer metastasis were taken for cancer-bearing mice, and tissues were isolated for detecting proteins, mRNA-seq, RT-qPCR, *in vitro* gene downregulation and/or overexpression analyses. **(B)** Average daily food intake and weight changes of mice from each group during the 19 days of the experiment. Statistical differences ($p = 0.007$ for food intake and $p = 0.001$ for weight changes) in the interaction between the number of days and the group are shown. **(C,D)** *In vivo* images showing that cancer metastasized the whole mice in cancer control **(C)** and cancer exercise mice used in this study **(D)**.

load for the high-intensity aerobic exercise based on the estimated maximum oxygen consumption (Zogaib and Monte-Alto-Costa, 2011). We monitored the changes in food intake and weight to analyze the effects of cancer and high-intensity aerobic exercise (**Figure 1B**). Significant differences were observed in body weights and food intake among the four groups during the exercise intervention ($p < 0.01$). Mice in the cancer control group [E(−)T(+)] showed a dramatic weight loss over the course of the experiment. In contrast, mice in the high-intensity aerobic exercise group maintained their weight regardless of cancer development. In addition, we observed significantly different interactions with food intake among the four groups over time ($p < 0.01$) (**Figure 1B**). Metastasis was confirmed in cancer-induced mice; however, the degree of tumor growth was significantly reduced in the high-intensity aerobic exercise group (**Figure 1C**). The images of all cancer-bearing mice, with or without high-intensity aerobic exercise, are shown in **Figures 1C,D**.

To further analyze the molecular effects of high-intensity aerobic exercise, specifically to identify the cancer-preventive effectors of exercise, we sacrificed the mice at the end of the experiment (19 days after the vein injection) and examined tissues obtained from the spleen, diaphragm, plantaris, and gastrocnemius muscle (**Figure 1A**; **Table 1**). The weight of spleen tissue in the cancer exercise group was approximately 2.44 times higher than that in the control group ($p < 0.05$). Examination of the distal part of the right and left skeletal muscles (plantaris and gastrocnemius muscles) revealed that hypertrophy

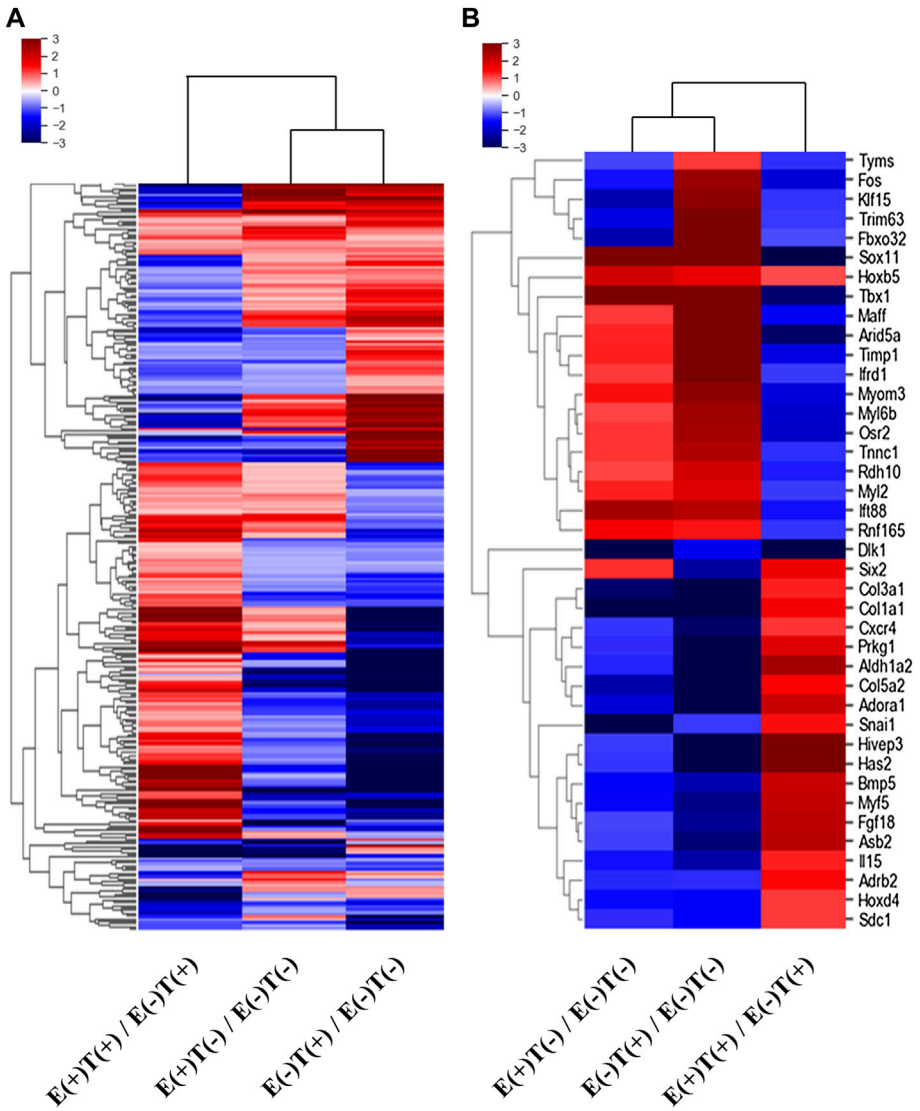

FIGURE 2 | Heatmaps of DEGs of skeletal muscle-related genes. The differentially expressed skeletal muscle-related genes were selected according to the fold changes and normalized read counts from the output of mRNA-seq of skeletal muscles (gastrocnemius muscle). E, exercise; T, tumor injection; (+), execution; (–), non-execution. **(A)** A heatmap of top 285 DEGs (at least 1.25-fold change in expression in any one of the three-way comparisons shown) from 998 skeletal muscle-related genes. **(B)** A heatmap of top 40 DEGs (at least two-fold change in expression in all three-way comparisons) from 998 skeletal muscle-related genes. Remarkably different expressional patterns of the gastrocnemius muscle-derived genes are shown.

was induced to a greater extent in the high-intensity aerobic exercise group than in the no-exercise group ($p < 0.01$). This result indicated cancer-induced atrophy in the gastrocnemius muscle rather than in the plantaris muscle. While both legs showed a significant reduction of the gastrocnemius muscle, only the plantaris muscle of the left leg decreased significantly ($p < 0.01$). Exercise did not influence hypertrophy in the cancer groups [E(−)T(+) vs. E(+)T(+)]. However, hypertrophy of the diaphragm in the cancer control mice was approximately 3.84 times more than that in the healthy control mice (**Table 1**). Hypertrophy of the diaphragm in cancer with high-intensity aerobic exercise groups was about eight times greater than that in the healthy control group (**Table 1**).

3.2 Changes in Gene Expression Patterns Induced by High-Intensity Aerobic Exercise

We isolated the gastrocnemius muscles from each group and extracted total RNA after homogenization. We then constructed mRNA-seq libraries using oligo-dT beads and analyzed the gene expression patterns *via* high-throughput sequencing. Each sample was analyzed using ExDEGA, including gene ontology (GO), which defines skeletal muscle-related genes from a pool of 23,282 genes. Skeletal muscle-related genes were identified using the QuickGO annotation list, which yielded 16 skeletal muscle-related terms defining a total of 336,468 annotations (https://www.ebi.ac.uk/QuickGO/). This analytical method yielded the

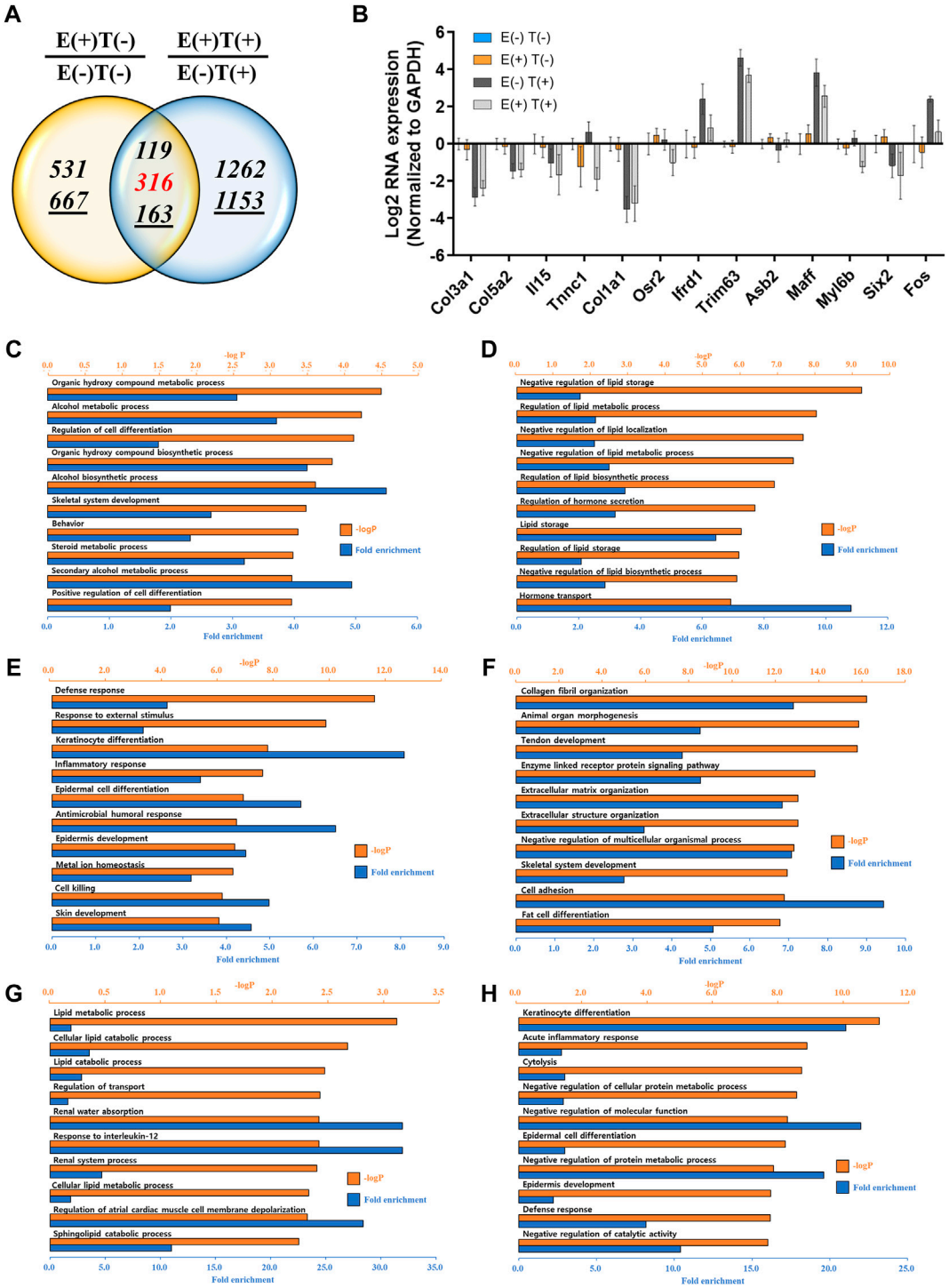

FIGURE 3 | mRNA-seq analysis of skeletal (gastrocnemius) muscle tissue. **(A)** Venn diagram of the number of DEGs for the effect of exercise for normal [E(+)T(–)/E(–)T(–)] and tumor-bearing [E(+)T(+)/E(–)T(+)] mice. The high-intensity aerobic exercise resulted in 1,796 up- or downregulated genes by at least two-fold, while tumor-bearing mice resulted in a change in the 3,013 genetic expressions by at least two-fold. The italicized number indicates the number of upregulated genes while the italicized and underlined number indicates the number of downregulated genes. Those with opposite effects between the two groups (contra-regulated) are shown in red. The DEGs were defined as genes with a fold change of two and a normalized read count of four. **(B)** Bar graph of log2 fold change of selected genes in the contra-regulated group using RT-qPCR of the littermates. The fold change was calculated by normalizing the data to that of the housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase, *GAPDH*) and then to the level in the normal control group. A consistent change compared to the mRNA-seq analysis was observed. **(C,D)** GO analysis of the upregulated **(C)** or downregulated DEGs **(D)** comparing E(+)T(–) and E(–)T(–) mice. The significant enriched terms include biosynthetic hormone

(*Continued*)

FIGURE 3 | secretion and lipid-related processes. **(E,F)** GO analysis of the upregulated **(E)** or downregulated DEGs **(F)** comparing E(–)T(+) and E(–)T(–) mice. This result indicates that the presence of cancer results in the systemic modulation of immune function. **(G,H)** GO analysis of the upregulated **(G)** or downregulated DEGs **(H)** comparing E(+)T(+) and E(–)T(+) mice. Enrichment of immune-related terms is clear, suggesting that exercise may successfully prevent the global gene expression changes induced by cancer, particularly those related to immune function. For all GO analyses, the top 300 DEGs were used, and the top ten pathways ranked by –logP and fold enrichment are shown.

expression patterns of 998 skeletal muscle-related genes. Clustering analysis was performed on these 998 genes, and a heatmap was constructed (**Figure 2**). The left column describes the expression pattern of the top 285 from the 998 genes selected with a minimum normalized read count of four and 1.25 fold change against that of the control group (**Figure 2A**). The red indicates upregulated genes, and the blue indicates downregulated genes. Similarly, the list was narrowed down by selecting genes that showed at least 2-fold changes in all three-way pairwise comparisons (**Figure 2B**).

3.3 Gene Expression Patterns in Tissues From the Exercise Intervention Mouse Cancer Model

The gastrocnemius muscle was obtained from a representative mouse from each of the four groups, and the gene expression profiles were analyzed *via* mRNA-seq. We first analyzed the effect of high-intensity aerobic exercise on gene expression [E(+)T(−) vs. E(−)T(−)] and the effect of exercise on gene expression in cancer-bearing mice [E(+)T(+) vs. E(−)T(+)]. We found that high-intensity aerobic exercise resulted in up- or downregulation of 1,796 genes by at least two-fold, while in the cancer-bearing mice, exercise resulted in a change in the expression of 3,013 genes by at least two-fold (**Figure 3A**). Considering that no tumor growth was observed in the gastrocnemius muscle tissue of the cancer exercise mouse, bearing cancer results in a remarkable change in gene expression throughout the body.

Among these DEGs, we focused on the genes that showed a negative correlation between high-intensity aerobic exercise and the presence of tumors (shown in red in **Figure 3A**). These genes showed differential expression by more than two-fold when the mice underwent high-intensity aerobic exercise, but showed a reverse change when cancer was induced. In other words, these genes may represent the molecular factors under which exercise resulted in a cancer-preventive effect by reversing the changes induced by the presence of cancer. A few examples of genes with up- or downregulation and a negative correlation between exercise and cancer are shown in **Figure 3B**. We further validated the expression patterns of these genes by analyzing their expression in littermates using RT-qPCR, which showed a consistent change as in our mRNA-seq analysis (**Figure 3B**).

Next, we performed GO analysis on the DEGs in each pair. First, we compared gene expression in the healthy control mice and high-intensity aerobic exercise mice (**Figures 3C,D**). The significantly enriched terms for upregulated genes were related to the biosynthetic process and hormone transport, indicating that high-intensity aerobic exercise may induce systemic effects by increasing biosynthetic hormone secretion from the skeletal muscle (**Figure 3C**). In addition, the lipid-related process term was downregulated in mice undergoing high-intensity aerobic exercise (**Figure 3D**). Next, we compared the effect of cancer by comparing gene expression in healthy controls with that in cancer-bearing mice (**Figures 3E,F**). Significantly enriched terms included defense response, cell killing, and skin-related responses such as keratinocyte differentiation and skin development. This result indicates that the presence of cancer results in the systemic modulation of immune function. Lastly, when we compared gene expression in the cancer control mice with that in cancer and high-intensity aerobic exercise mice, the observed enrichment of immune-related terms are shown in the downregulated genes, suggesting that exercise may successfully prevent the global gene expression changes induced by cancer, particularly those related to immune function (**Figures 3G,H**).

We performed a similar analysis by examining gene expression profiles in other organs, such as the lungs and heart (**Figures 4**, **5**). For the lungs, 810 up or downregulated genes were detected by filtering genes with a fold change of two and a normalized read count of four, owing to the high-intensity aerobic exercise effect, while 5,702 genes showed altered expression due to the effect of exercise in the cancer model (**Figure 4A**). Under the same conditions as in the lungs, the expression of 806 genes in E(+)T(−) vs. E(−)T(−) and 1,668 genes in E(+)T(+) vs. E(−)T(+) was altered in the heart tissue (**Figure 5A**). The RT-qPCR validation using the littermates for a selected DEGs (*Cflar*, *Cacna1s*, *Actn3*, *Myoz2*, *Il15*, *Acta1*, and *Ar* for the lungs; *Cflar*, *Myoc*, *Trim63*, *Acta1*, and *Ar* for the heart) from red-labeled contra-regulated genes (198 for the lungs and 96 for the heart) are shown in each figure (**Figures 4B**, **5B**). Some genes from the heart and lungs were commonly regulated (i.e., *Cflar*, *Ar*, and *Acta1*).

GO analysis was also performed in the lungs and heart tissues as done for the gastrocnemius muscle. In the lungs, significantly enriched terms for upregulated genes by exercise were associated with cell adhesion and protein folding while downregulated genes by exercise were associated with the phosphorus metabolic process (**Figures 4C,D**). The presence of cancer resulted in the upregulation of genes related to cell division while genes related to cell adhesion and T cell activation were downregulated (**Figures 4E,F**). Notably, exercise in cancer-bearing mice resulted in the upregulation of genes related to blood cell differentiation and cell activation while decreased genes associated with organ development (**Figures 4G,H**). In the heart, exercise triggered the expression of genes related to protein folding and matrix reorganization while decreased genes associated with DNA replication and cell-cell adhesion (**Figures 5C,D**). At the same time, the presence of cancer resulted in inflammatory response and chemotaxis while decreased genes related to transport (**Figures 5E,F**). Lastly, exercise in the cancer-bearing mice upregulated genes involved in detoxification and

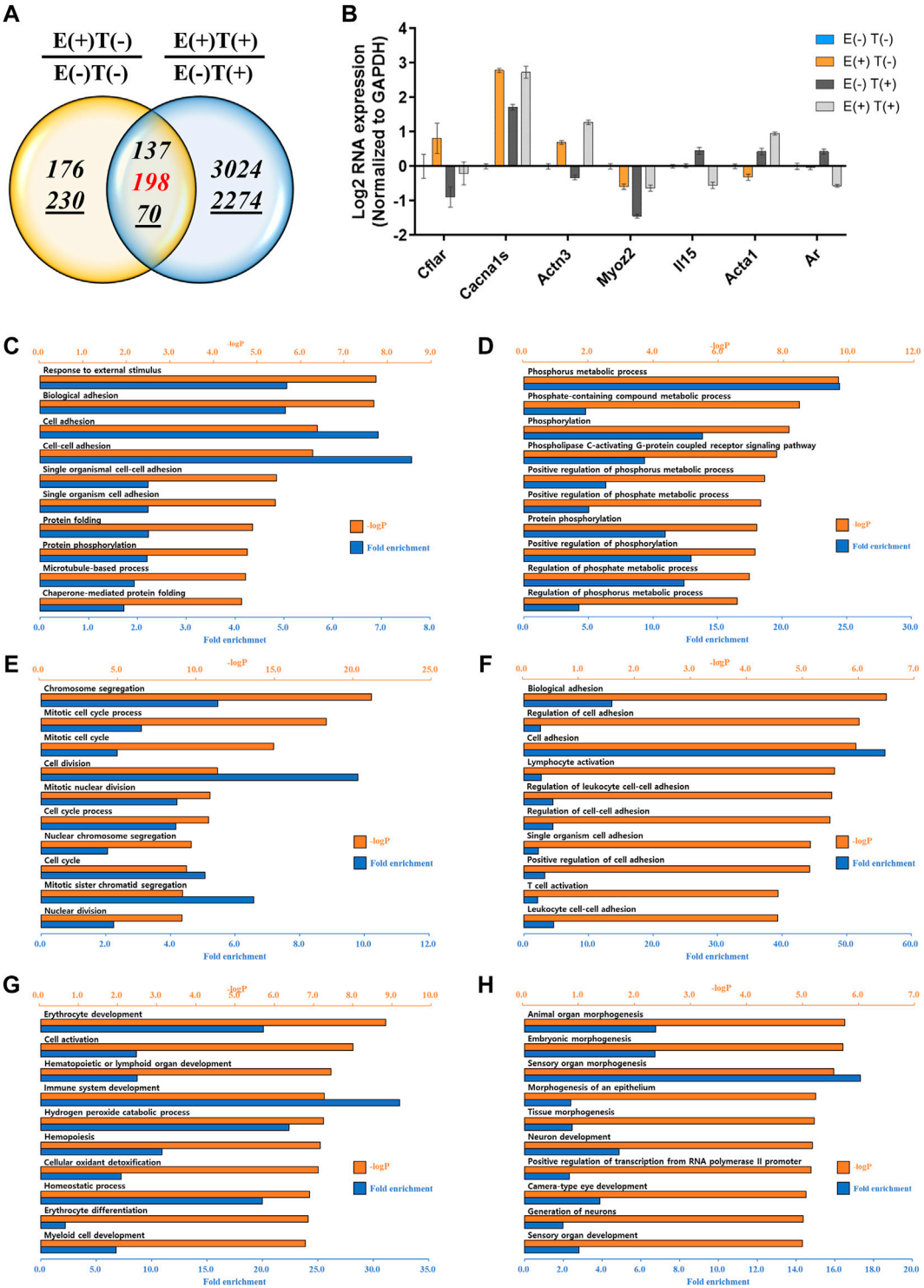

FIGURE 4 | mRNA-seq analysis of the lungs. **(A)** Venn diagram of the number of DEGs for the effect of exercise for normal [E(+)T(–)/E(–)T(–)] and tumor-bearing [E(+)T(+)/E(–)T(+)] mice. The italicized number indicates the number of upregulated genes while the italicized and underlined number indicates the number of downregulated genes. Those with opposite effects between the two groups (contra-regulated) are shown in red. The DEGs were defined as genes with a fold change of two and a normalized read count of four. **(B)** Bar graph of log2 fold change of selected genes in the contra-regulated group using RT-qPCR of the littermates. The fold change was calculated by normalizing the data to that of *GAPDH* and then to the level in the normal control group. A consistent change compared to the mRNA-seq analysis was observed. **(C,D)** GO analysis of the upregulated **(C)** or downregulated DEGs **(D)** comparing E(+)T(–) and E(–)T(–) mice. Significantly enriched terms include cell adhesion, protein folding, and phosphorus metabolic process. **(E,F)** GO analysis of the upregulated **(E)** or downregulated DEGs **(F)** comparing E(–)T(+) and E(–)T(–)

(*Continued*)

FIGURE 4 | mice. The presence of cancer resulted in the upregulation of genes related to cell division while genes related to cell adhesion and T cell activation were downregulated. **(G,H)** GO analysis of the upregulated **(G)** or downregulated DEGs **(H)** comparing E(+)T(+) and E(–)T(+) mice. Exercise in cancer-bearing mice resulted in the upregulation of genes related to blood cell differentiation and cell activation while decreased genes associated with organ development. For all GO analyses, the top 300 DEGs were used, and the top ten pathways ranked by –logP and fold enrichment are shown.

blood cell differentiation and downregulated genes involved in tissue morphogenesis and fibril organization (**Figures 5G,H**). Based on the GO terms analyzed above, the observed systematic responses were differently enriched according to the cancer state, type of tissue, and exercise execution.

While analyzing our data, we noticed that *IL15* was commonly present in the contra-regulated group for gastrocnemius muscle and lungs, C*flar*, *Acta1*, and *Ar* were commonly present for lungs and heart, while *Trim63* was commonly present between gastrocnemius muscle and heart. In addition, among the DEGs, we found that *Rdh11*, *1700048E18Rik*, *Car3*, *Hcar1*, *Nup98*, and *Timp1* were commonly present in all three tissues.

3.4 Modulating the Expression of Exercise-Regulated Genes Affects Cancer Cell Viability

Further, we performed functional annotation analysis of the 18 RT-qPCR validated DEGs using KEGG Mapper annotations (*Col3a1*, *Col5a2*, *Ifrd1*, *Fos*, *Asb2*, *Tnnc1*, *Osr2*, *Maff*, *Six2*, *Trim63*, *Il15*, *Cflar*, *MyoC*, *Acta1*, *Ar*, *Cacna1s*, *Actn3*, and *Myoz2*). Their functions were then identified using the *Mus musculus* database. Overrepresented pathways involved ten genes: *Cflar*, *Fos*, *Actn3*, *Ar*, *Cacna1s*, *Col1a1*, *Col3a1*, *Col5a2*, *Il15*, and *Tnnc1*. Among these genes, all, except *Actn3* and *Tnnc3*, were relevant in signaling pathways related to cancer and the immune system, such as the nuclear factor-kappa B, tumor necrosis factor, mitogen-activated protein kinase (MAPK), Toll-like receptor, B- and T-cell receptor signaling, and PI3K-AKT signaling pathways (**Supplementary Data S1**).

We also performed KEGG pathway analysis on 285 skeletal muscle-related DEGs. Out of these 285 genes, 34 of them participate in major pathways in cancer, and 26 are involved in the metabolic pathways. When we focused on the differential expression induced by exercise [E(−)T(−) vs. E(+)T(−)], we found that many of the genes involved in the MAPK pathway are downregulated (**Supplementary Data S2**). Moreover, increased p53 which activates the apoptotic pathway, is also evident (**Supplementary Data S2**). Analysis using the STRING database (http://string-db.org/) revealed annotated interactions between 40 selected DEGs with other genes at the protein level (**Supplementary Data S3**). However, these connections were not evident in any of the pathways obtained in the KEGG Mapper annotations.

We further examined the effects of modulating the expression of *Fos* and *Col1a1* genes in an *in vitro* cell line system. Using siRNAs, we knocked down the expression of *Fos* in CT26 cells and examined its effect on cell proliferation. We targeted *Fos* expression because our mRNA-seq analysis revealed that *Fos* expression was significantly decreased in mice that underwent high-intensity aerobic exercise, while its expression was increased in mice with cancer. Therefore, we investigated whether the downregulation of *Fos* expression might partly mimic the effect of exercise in terms of preventing cancer development. The results confirmed that siRNA transfection caused over 80% reduction in target mRNA expression and a remarkable decrease in protein levels (**Figures 6A,B**). Under these conditions, we examined cancer cell proliferation using the MTT assay. Downregulation of *Fos* reduced murine colorectal cancer cell proliferation by approximately 20% (**Figure 6C**, p = 1.8e-5).

In addition to *Fos,* we also investigated the effect of high-intensity aerobic exercise on *Col1a1* expression. *Col1a1* is related to the PI3K-AKT pathway and proteoglycans in cancer signaling pathways (**Supplementary Data S1**). For *Col1a1*, we performed an analogous experiment, but this time, we examined the effect of overexpression of *Col1a1* as its expression was increased in mice that underwent high-intensity aerobic exercise and decreased in cancer-bearing mice. We confirmed that the expression of a plasmid encoding *Col1a1* resulted in increased *Col1a1* mRNA and protein expression (**Figures 6D,E**). Moreover, the overexpression of *Col1a1* resulted in an approximately 20% reduction in CT26 cell viability (**Figure 6F**, p = 0.02).

Although they are not associated with immune-related signaling pathways, we also examined the expression of *Trim63* and *Six2*, based on the negative correlation observed in expression upon the induction of high-intensity aerobic exercise and cancer. Similar to *Fos*, *Trim63* showed decreased expression in the exercised mice, while it showed increased expression in the cancer-bearing mice. *Six2* also showed a negative correlation, but the opposite was observed in direction. We downregulated *Trim63* using siRNA and confirmed the knockdown efficiency using RT-qPCR and western blotting (**Figures 6A,B**). Similar to *Fos* knockdown, downregulation of *Trim63* also resulted in a significant reduction in CT26 cell viability (**Figure 6C**, $p = 6.5^{-4}$). Similarly, we overexpressed *Six2* and confirmed the increased expression using RT-qPCR and western blotting (**Figures 6D,E**). Overexpression of *Six2* also resulted in an approximately 20% decrease in cell viability (**Figure 6F**, p = 0.02).

Finally, we analyzed the clinical significance of the four exercise-regulated genes for rectum adenocarcinomas using the Kaplan–Meier Plotter (Nagy et al., 2021). With the exception of *Fos*, we found that genes regulated by exercise were related to the overall survival of cancer patients. Patients with increased expression of *Trim63* showed an inferior overall survival rate (**Figure 6G**). Notably, *Col1a1* and *Six2* showed the opposite associations. We found that patients with low expression of *Col1a1* and *Six2* showed inferior overall survival rates (**Figure 6G**). These data were consistent with our CT26 cell viability analysis. Collectively, our analyses confirmed that the genes whose expression showed a negative correlation between

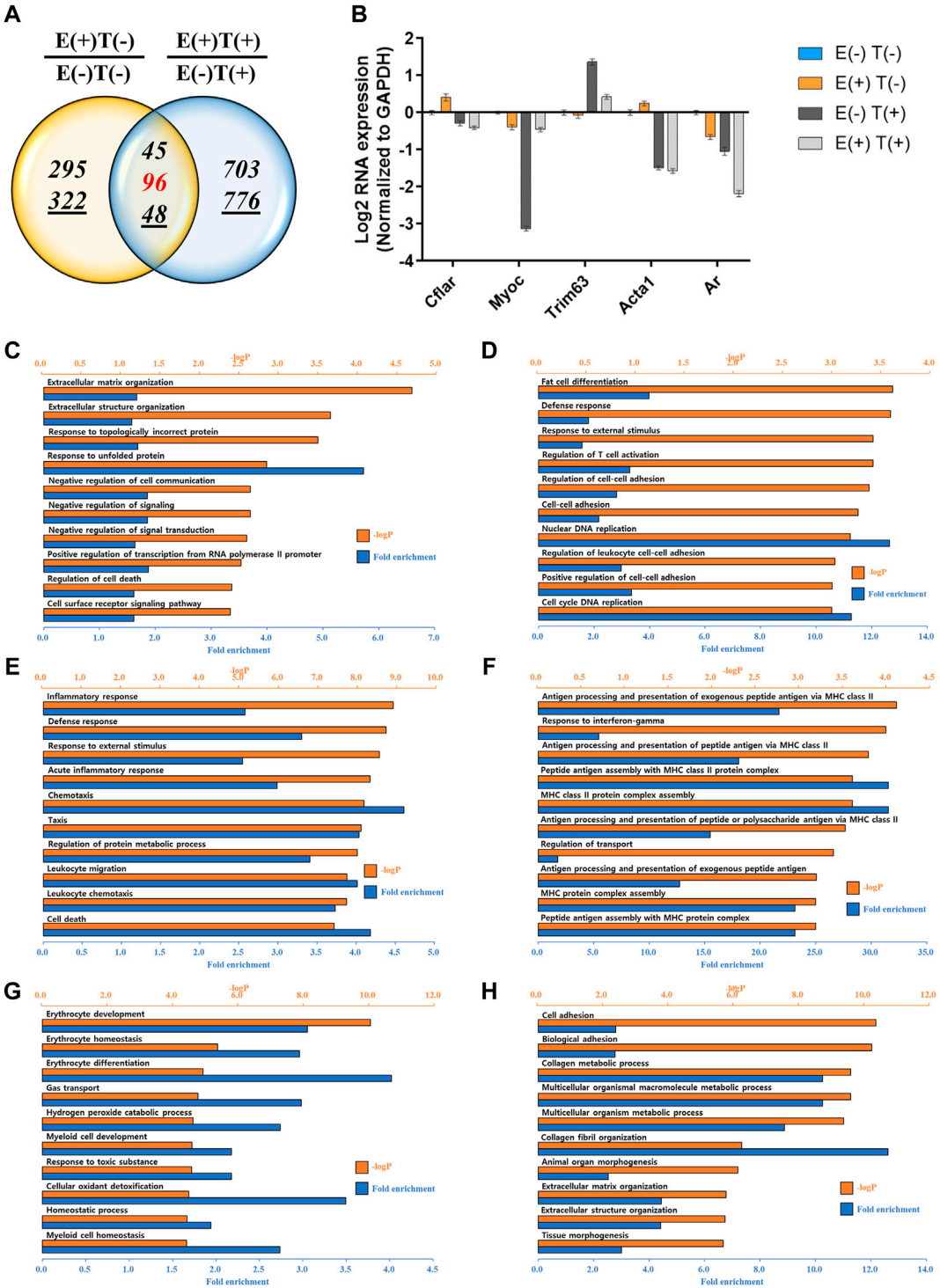

FIGURE 5 | mRNA-seq analysis of the heart. **(A)** Venn diagram of the number of DEGs for the effect of exercise for normal [E(+)T(–)/E(–)T(–)] and tumor-bearing [E(+)T(+)/E(–)T(+)] mice. The italicized number indicates the number of upregulated genes while the italicized and underlined number indicates the number of downregulated genes. Those with opposite effects between the two groups (contra-regulated) are shown in red. The DEGs were defined as genes with a fold change of two and a normalized read count of four. **(B)** Bar graph of log2 fold change of selected genes in the contra-regulated group using RT-qPCR of the littermates. The fold change was calculated by normalizing the data to that of *GAPDH* and then to the level in the normal control group. A consistent change compared to the mRNA-seq analysis was observed. **(C,D)** GO analysis of the upregulated **(C)** or downregulated DEGs **(D)** comparing E(+)T(–) and E(–)T(–) mice. Exercise triggered the expression of genes related to protein folding and matrix reorganization while decreased genes associated with DNA replication and cell-cell adhesion. **(E,F)** GO analysis of the upregulated **(E)** or downregulated DEGs **(F)** comparing E(–)T(+) and E(–)T(–) mice. The presence of cancer resulted in inflammatory response and chemotaxis while decreased genes related to transport. **(G,H)** GO analysis of the upregulated **(G)** or downregulated DEGs **(H)** comparing E(+)T(+) and E(–)T(+) mice. Exercise in the cancer-bearing mice upregulated genes involved in detoxification and blood cell differentiation and downregulated genes involved in tissue morphogenesis and fibril organization. For all GO analyses, the top 300 DEGs were used, and the top ten pathways ranked by –logP and fold enrichment are shown.

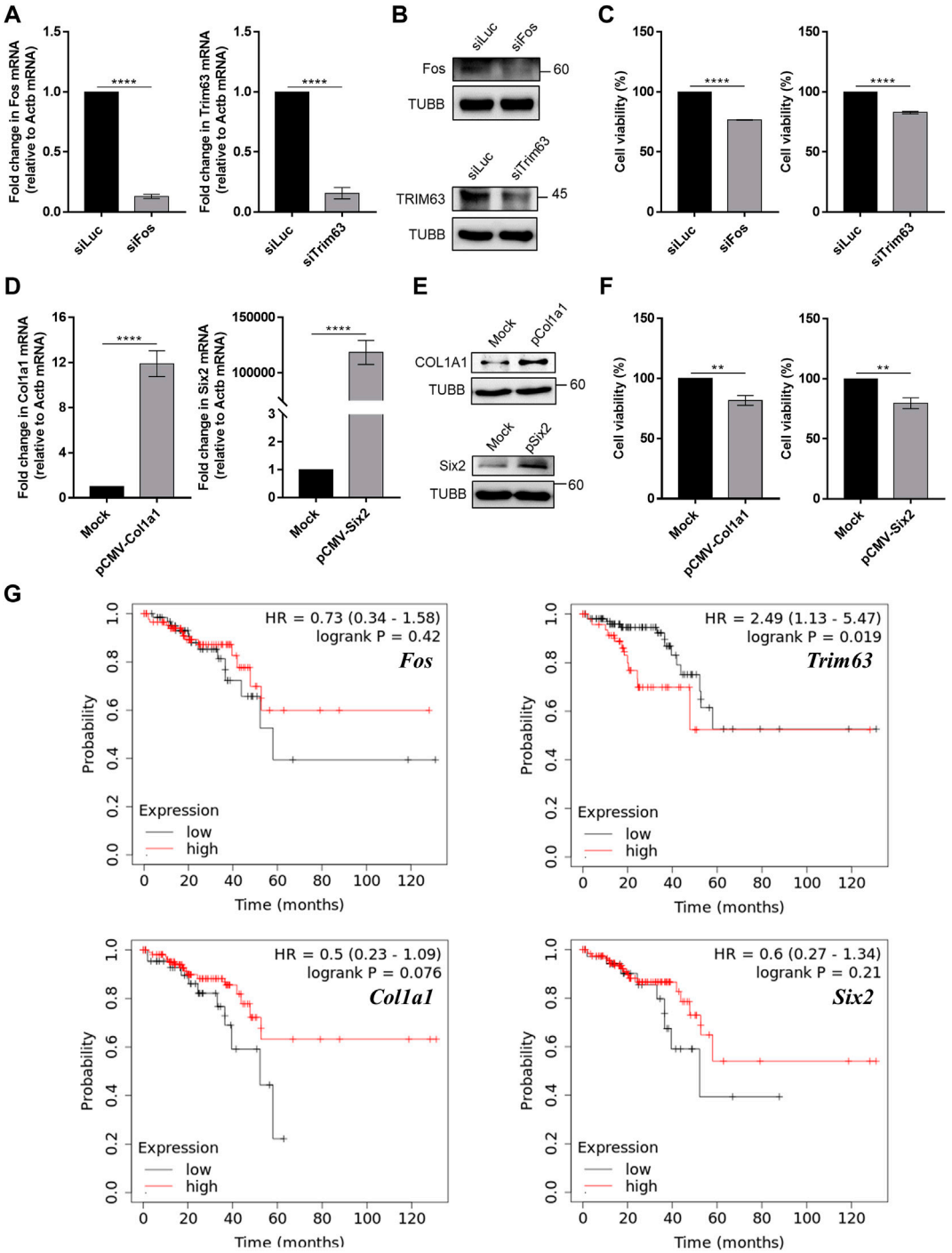

FIGURE 6 | Modulation of skeletal muscle gene expression affects cancer cell viability *in vitro*. **(A)** Knockdown efficiency of siRNAs against *Fos* and *Trim63* in CT26 cells was confirmed using RT-qPCR. **(B)** The protein expression of Fos and Trim63 in siRNA-transfected cells. **(C)** Cell viability of CT26 cells with knockdown of skeletal muscle genes. **(D)** Overexpression of *Col1a1* and *Six2* resulted in increased mRNA expression, as confirmed using RT-qPCR. **(E)** Increased mRNA expression resulted in increased protein levels. **(F)** Viability of CT26 cells when *Col1a1* or *Six2* was overexpressed. The overexpression of *Col1a1* resulted in an about 20% reduction in CT26 cell viability. **(G)** Plots of Kaplan–Meier estimates for the overall survival rate of rectum adenocarcinoma patients based on the expression of skeletal muscle genes. With the exception of *Fos,* our anticancer candidate genes showed an expected association with the overall survival rate. For RT-qPCR and cell viability data, the average of three biological replicates is shown, with error bars indicating the standard error of the mean. For statistical analysis, the student's t-test was performed against the control (siLuc or Mock), and the statistical significance was indicated with a number of *. **p-value < 0.01, ***p-value < 0.001, ****p-value < 0.0001.

exercise and cancer might be responsible for the cancer-preventive effect of high-intensity aerobic exercise.

4 DISCUSSION

In this study, we used a mouse cancer model to investigate changes in the gene expression profiles upon high-intensity aerobic exercise and the molecular effects of exercise on cancer prevention. Consistent with the results of a previous study (Jee et al., 2016a), high-intensity aerobic exercise increased the survival rate, quality of life, and muscle hypertrophy in a mouse cancer model. We employed high-throughput sequencing to examine DEGs in high-intensity aerobic exercise and cancer-bearing mice. More importantly, we identified genes (e.g., *Trim63*, *Fos*, *Col1a1*, and *Six2*) that were regulated by high-intensity aerobic exercise, which adversely affected colorectal cancer development, and confirmed their effects *in vitro* using knockdown and overexpression systems.

4.1 Suppressive Effect of Skeletal Muscle-Derived Genes on Cancer Growth

We hypothesized that unknown skeletal muscle-derived factors play a role in suppressing cancer growth and metastasis. These factors could be regulated by physical activities, which may account for the preventive effect of exercise on carcinogenesis. Our previous study, using mouse cancer models, revealed the positive effects of high-intensity aerobic exercise rather than moderate-intensity aerobic exercise on various parameters, including a 100% survival rate (Jee et al., 2016a). The present study extended these observations by analyzing the genes regulated by high-intensity aerobic exercise. Through analysis of mRNA transcriptome, we identified 23,282 genes expressed in the gastrocnemius muscle, lungs, and heart. Of these genes, those with significantly altered expression were identified. Among them, we confirmed that 11 genes from the skeletal muscle (*Col3a1*, *Col5a2*, *Tnnc1*, *Col1a1*, *Osr2*, *Ifrd1*, *Trim63*, *Asb2*, *Maff*, *Myl6b*, and *Fos*), five from the lungs (*Cflar*, *Actn3*, *Myoz2*, *Il15*, and *Acta1*), and two from the heart (*Myoc*, and *Trim63*) showed reverse expression changes between high-intensity aerobic exercise and cancer-bearing. We focused on identifying epigenetically regulated genes in the skeletal muscle and selected a few candidate genes that might account for the cancer-preventive effects of aerobic exercise. These included *Fos*, *Trim63*, *Col1a1*, and *Six2*, two of which were downregulated by high-intensity aerobic exercise, while the other two were upregulated by high-intensity aerobic exercise. We also examined the effects of modulating their gene expression in cancer and observed significantly opposing effects on cancer cell viability by approximately 20%.

Fos and *Trim63* were selected for the knockdown experiments. Tripartite motif (Trim)-related families are renowned as muscle atrophying effector genes (Yang et al., 2014). *Trim24* has been identified as an oncogene in colorectal cancer using lentivirus-mediated RNA interference knockdown in HCT116 human colorectal cancer cells, which significantly decreased cell proliferation (Cohen et al., 2012; Wang J. et al., 2014). *Trim63*, also known as *Murf1*, maintains muscle protein homeostasis by acting on sarcomere-related proteins, such as microtubules and myosin heavy chain (McElhinny et al., 2002; Chen et al., 2012). It is involved in the atrophy of skeletal muscles and myocardium (Tan et al., 2017). Trim63 protein localizes to the Z-line and M-line lattice of myofibrils and interacts with numerous signaling pathways, such as the microtubule-dependent signaling pathway in muscle or cancer regulating SUMO-related pathways (Centner et al., 2001; Hu and Jiang, 2019). It is also suppressed by the Wnt/β-catenin signaling pathway in breast cancer and affects cell proliferation and migration (Bowen et al., 2017).

In addition to *Trim63,* our results on *Fos* are also consistent with previous reports, where the inhibition of *Fos* suppressed colon carcinoma tumor growth in athymic mice as well as the progression of ovarian and breast cancer (Pandey et al., 2012; Oliveira-Ferrer et al., 2014; Li et al., 2019). Bioinformatic studies have also shown that, regardless of the tumor type, *Col1a1* acts as a cancer suppressor (Lu et al., 2005). Lastly, the transcription factor *Six2*, which is involved in kidney development, controls E-cadherin and promotes stemness in the lung and breast cancer cells (Li et al., 2020; Weber et al., 2008; Wang C.-A. et al., 2014).

4.2 Relationship Between the Skeletal Muscle-Derived Factors Identified in This Study and Cancer Suppression

In the current study, along with a significant impact on hormone and protein secretion, exercise resulted in a more remarkable effect on the regulation of the skeletal muscle than that of the lungs or heart. In particular, the expression of secretion-related genes (e.g., *BNDF, BRSK1*, and *Ccnd1*) in the gastrocnemius muscle during high-intensity aerobic exercise suggests their potential role in cancer suppression. This is consistent with previous studies that reported the importance of muscle-derived secretion factors and their effects on other tissues throughout the body (Hojman et al., 2011; Hamrick, 2012; Hou et al., 2019). Our study also revealed differential expression of growth factor-related genes (*Fibroblast growth factor 2/6/10/18, Fibroblast growth factor receptor 2, Epidermal growth factor-containing fibulin-like extracellular matrix protein 2, Insulin-like growth factor 1, Insulin-like growth factor binding protein 5,* and *Platelet-derived growth factor*), interleukin-related gene (IL15), and other myokine or cytokine-related genes (*interferon-related developmental regulator1*, and *Myostatin*). In the future, it will be interesting to investigate how high-intensity aerobic exercise affects the body through the secretion of these factors.

In addition to the four genes (*Fos*, *Col1a1*, *Trim63*, and *Six2*), our study identified other potential molecular factors of high-intensity aerobic exercise, which may prevent cancer proliferation. A key factor is *IL15*. Studies on the effects of myokines, such as interferons and interleukins, on cancer reported that the muscle-derived gene *IL15* triggers the immune system by forming the IL15+IL15R complex. It then stimulates the spleen cells and activates the natural killer (NK) T, $CD8^+$ T, and $CD4^+$ T cells (Carson and Caligiuri, 1998; Pedersen

and Hojman, 2012; Idorn and Hojman, 2016). Moreover, the IL15+IL15R complex increases the number of effectors and central memory cells, which are anticancer immune cells involved in inducing metabolic differentiation of T lymphocyte-related subsets in the spleen (Yang et al., 2011; Klebanoff et al., 2016; Thi et al., 2019). Although IL15 alone is not involved in any major cancer signaling pathways, the IL15+IL15R complex can suppress CT26 colon cancer cells through NK cells (Thi et al., 2019), which is consistent with the findings on the upregulation of *IL15* by intensive aerobic exercise in the current study.

4.3 Effect of Exercise on Improving Body Wellness in Cancer Patients, and the Type of Exercise That Can Effectively Suppress Cancer

In an epidemiologic study with 500,000 participants, those who engaged in moderate-intensity exercise three to four times a week (men) and once or twice a week (women) showed a significantly decreased incidence rate of colon cancer (by 54% and 47%, respectively) (Jee and Kim, 2019). The findings of this epidemiological study on human subjects suggest that colon cancer-targeted effects vary depending on sex and the amount of exercise. Our previous (Jee et al., 2016a) and current studies also showed the cancer-suppressive effect of high-intensity aerobic exercise. Other studies demonstrated that resistant exercise on the animal cancer model mitigates tumor growth, tumor grade, viable tumor area, tumor cell proliferation, and myofiber atrophy-causing cancer cachexia *via* attenuating some key markers such as TNF-α, IL-6, Atrogin1, and oxidative damage (Padilha et al., 2019; Padilha et al., 2021). In addition, we also found an effect of exercise on cancer cachexia. Consistent with the findings of previous studies (Deuster et al., 1985; Penna et al., 2011), cancer resulted in decreased food intake and body weight, two indicators of cancer cachexia. Notably, mice subjected to high-intensity aerobic exercise showed no significant changes in food intake and body weight when compared to healthy control mice. A previous study using lung cancer-bearing mice showed the importance of changes in the thermogenic gene (*Dios2*) and skeletal muscle atrophy-related genes (*Atrogin1* and *Murf1*) in driving cancer cachexia (Kir et al., 2014). The cancer cachexia increases the resting energy expenditure by browning fat thermogenesis. The thermogenic gene *Dios2* and two muscle atrophy-related genes, *Atrogin1* and *Murf1,* were upregulated by parathyroid hormone-related protein (PTHrP), which is responsible for most of the cancer cell-derived browning adipose cell activity. This study argued that neutralizing the PTHrP could block adipose tissue browning and subsequent loss of muscle mass and strength. Interestingly, our mRNA-seq data also showed changes in the expression of *Dios2* in the lungs and *Atrogin1* and *Murf1* in the skeletal muscles of cancer-bearing mice, and the above mechanism may apply. Notably, aerobic exercise rescued the cancer-driven changes in *Astogin1* and *Murf1* expressions. Accordingly, our analysis may justify the reduced muscular strength and exercise capacity during cancer cachexia and how aerobic exercise can ameliorate cancer cachexia.

The tumor-suppressive effects of exercise also vary according to the exercise type, intensity, frequency, and type of cancer. Therefore, creative exercise interventions for individual patients should be developed. Different types of exercise, such as aerobic resistance training, elicit disputable effects, and the optimal intensity and frequency are important factors that determine the potential benefits. In this study, mice were subjected to high-intensity aerobic exercise (90% of the maximal heart rate) as a model to study the importance of preventive exercise and an exercise-centered lifestyle. However, adequate mouse models mimicking human subjects, who do not exercise regularly, should be developed to study the differences and compare the signaling pathways between the two groups to emphasize the exercise-centered lifestyle to pursue a healthy state of the body.

The mouse cancer model used in this study was established after its physical fitness was raised to imitate the effects of regular physical activity. In the future, it will be important to revalidate the effects of high-intensity aerobic exercise in human subjects. Pollán et al. (2020) indicated that the most effective behavioral interventions that may bring health improvements and changes in the lifestyles of patients vary depending on the intensity and duration of the physical activity. However, the type of workout effective for each type of cancer is unclear and remains to be investigated.

Exercise seems to prevent cancer primarily by reducing the circulating levels of hormones and growth factors, such as insulin-like growth factor 1. Preclinical studies have shown that exercise induces hyperphosphorylation of the retinoblastoma protein, which causes the phosphorylation of β-catenin and subsequent reduction in the expression of multiple microRNAs involved in a wide range of biological functions, including cellular proliferation, differentiation, regulation of inflammation, antioxidation, glucose transportation, angiogenesis, and muscle contraction (Baltgalvis et al., 2008; Ju et al., 2008; Zhu et al., 2008; Jiang et al., 2009; Horak et al., 2018). Various reports have described the potential mechanisms underlying the anticancer effects of exercise. Most of these reports have focused on the suppression of cancer with an enhanced immune system, such as the stimulation of natural killer cells. In contrast, the present study focused primarily on the effects of exercise on the skeletal muscle, suggesting a role of myokines in this tissue. Although not much is known about their role in this context, myokines are considered to have a direct anticancer effect *via* the secretion of acidic proteins (Horak et al., 2018). In our results, *IL15* and *Myostatin* were listed in the DEGs of the skeletal muscle.

The practical use of the results from the current study requires future investigation of aerobic exercise using human subjects. In particular, the expression of the anticancer candidate genes identified in this study needs to be examined using human skeletal muscle under the same condition. In addition, the human analogous of aerobic exercise that induces sufficient changes in anticancer gene expression needs to be devised. Lastly, we anticipate that there will be difficulty in making cancer patients to undergo high-intensity aerobic exercise.

Analyzing the effect of exercise on the liver in the cancer model was important because the liver tissue is closely associated with maintaining body weight, insulin sensitivity,

and chronic inflammation. Moreover, we also found that central cancer regulating pathways related to the risk of hepatocellular carcinoma (Saran et al., 2018), such as the AMPK pathways, were affected by high-intensity aerobic exercise. Another limitation of the study includes the lack of *in vivo* validation of candidate genes through CRISPR knockout mice. Particularly, *in vitro* transfection of *Six2* resulted in too high expression of the target gene. *In vivo* validation using a more physiologically relevant promoter will further support our results. These are the limitations of the current study, which warrant future analysis. In particular, the transcriptome-wide gene expression changes in the livers of cancer-bearing and high-intensity aerobic exercise mice may provide important insights into the effects of exercise on cancer prevention and cancer cachexia. Despite these limitations, our study clearly suggests that the high-intensity aerobic exercise resulted in changes in the expression of muscle-derived anticancer genes (*Fos*, *Col1a1*, *Trim63*, *and Six2*) that may account for the cancer-preventive effect of aerobic exercise.

In conclusion, our study confirmed the effects of high-intensity aerobic exercise in a mouse model of cancer and revealed aerobic exercise-derived gene expression changes in the skeletal muscle, lungs, and heart. Selected genes derived from the skeletal muscle of the high-intensity aerobic exercise group displayed an approximately 20% decrease in cancer cell viability when properly modulated. Further studies on the impact of exercise on cancer, for example, different types of physical activities and analyses conducted prior to and following an exercise regimen, are warranted.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GEO, accession number(s) GSE191281, GSE191283, GSE191284.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal use and maintenance protocols were approved by the Yeungnam University Medical Centre Institutional Animal Care and Use Committee (YUMC-AEC2020-008).

AUTHOR CONTRIBUTIONS

Conceptualization: HJ and YK; Formal analysis and interpretation of data: HJ, KH, and YK; Development of methodology: HJ and YK; Acquisition of data: HJ, EP, MK, and YK; Writing, review, and/or revision of the manuscript: HJ and YK; Supervision: HJ and YK.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2022.818470/full#supplementary-material

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