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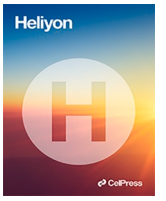

Research article

Transcriptomics and metabonomics study on the effect of exercise combined with curcumin supplementation on breast cancer in mice

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ABSTRACT

Curcumin and exercise have been reported to show good anti-tumour effects. However, relevant research on the combined effects of physical exercise and curcumin supplementation on cancer and the underlying mechanisms is still lacking. The current study aimed to construct an anti-breast tumour mouse model using the combined effects of curcumin treatment and swimming exercise. Transcriptomic and metabolomic techniques were used to screen for differentially expressed genes and metabolites, evaluate the anticancer effects, and analyse the molecular regulatory mechanisms related to metabolism. Observation of the mouse phenotypes, including tumour appearance, *in-vivo* tumour imaging, and HE staining results of pathological sections, suggested a more obvious inhibitory effect of the combination of curcumin administration and exercise intervention on breast cancer than that of a single treatment. The combination treatment group had a total of 445 differentially expressed (154 upregulated and 291 downregulated) genes. Functional enrichment analysis showed the calcium signalling pathway, Wnt signalling pathway, PI3K Akt signalling pathway, and IL-17 signalling pathway to significantly participate in the anti-breast cancer process of curcumin-exercise combination treatment. Results of the intergroup differential metabolite analysis showed that the combined effect of curcumin and exercise involves two unique pathways, namely the amino sugar and nucleotide sugar metabolism, which includes chitosan, D-glucosamine 6-phosphate, L-fucose, and N-acetyl beta-mannosamine, and the amino acid biosynthesis, which includes DL-isoleucine, DL-tyrosine, and homocysteine. Collectively, the top-ranked genes and metabolites with the highest degree of associations were further revealed by O2PLS analysis. Overall, the study helped reveal the mechanism of action of curcumin-exercise combination treatment on breast cancer at multi-omics level.

Abbreviations: NGS, next-generation sequencing; PE, paired-end; GSEA, gene set enrichment analysis; UHPLC, ultra-high-performance liquid chromatography; O2PLS, two-way orthogonal partial least squares.

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1. Introduction

Curcumin is a plant polyphenol with good antitumour effects, especially in breast cancer. The anti-tumour processes by curcumin may involve the inhibition of tumour angiogenesis, retardation of the activity of metalloproteinases and NF-κB related proteins, inhibition of cell adhesion and movement to induce cell cycle arrest and apoptosis, and stoppage of tumour cell invasion and metastasis [1–10]. The International Agency for Cancer Research has reported that approximately 25% of cancer cases worldwide are caused by obesity and sedentary behaviour. A large body of evidence has shown that higher levels of physical activity can reduce the risk of some cancers; in particular, the relationship between breast cancer and exercise has been widely studied [11–23]. The risk of breast cancer in women who are active in sports is reduced by 20%, which may be related to the effects of oestrogen [24–29]. A series of studies have shown that physical activity can reduce the risk of breast cancer by reducing fat, and is accompanied by a significant reduction in oestrogen and a significant increase in sex hormone-binding globulin [30–33]. The mechanism underlying the effect of exercise on cancer is complex, and activation of the immune system forms an important part of this process. Previous studies had shown that moderate physical activity can enhance immune function by increasing the number and activity of immune cells, such as neutrophils, eosinophils, monocytes, and lymphocytes, and by stimulating natural killer cells [34–46]. Recent research has found that lactic acid produced by exercise can effectively activate CD8+T cells and enhance antitumour immunity, adding new evidence to the theory of exercise against cancer [47].

With the development and widespread application of omics technology, systemic treatment scenarios for breast cancer are constantly evolving [48–51]. However, relevant research on the combined effects of curcumin and exercise on cancer and the underlying mechanisms is still lacking. In this study, we constructed an anti-breast tumour mouse model using the combined effects of curcumin treatment and swimming exercise. Transcriptomic and metabolomic techniques were used to screen for differentially expressed genes and metabolites, evaluate the anticancer effects of the combination treatment, and analyse the molecular regulatory mechanisms related to metabolism.

2. Materials and methods

2.1. Cell culture and mouse model establishment

The experimental animals used in this study were female BALB/c mice aged 4–6 weeks and weighing approximately 18–20 g. The experimental cell line was a 4T1-luc breast cancer cell line (sourced from BALB/c mice). The 4T1 breast cancer cells of mice were placed in a complete medium containing 90% DMEM, 10% foetal bovine serum (high-quality), and 1% penicillin streptomycin, and cultured and expanded in a 5% CO_2 incubator at 37 °C.

Thirty of the healthy BALB/c mice were randomly divided into five groups with six mice each. The treatment groups were named the tumour group (Tnm group), tumour + swimming group (Tnm_swim group), tumour + curcumin group (Tnm_cur group), and tumour + combination group (Tnm_union group). Mice in the CK group were injected with equal amounts of physiological saline, whereas those in the other four groups were subcutaneously inoculated with 0.2 ml of the corresponding concentrations of cell suspensions (with a cell count of approximately 2×10^6) in the chest wall at the fifth or sixth intercostal and lateral abdominal walls. Every afternoon, mice in the Tnm and Tnm_swim groups were gavaged with 200 μL of solution while those in the Tnm_cur and Tnm_union groups were gavaged with 200 μL of curcumin solution dissolved in corn oil (100 mg/kg). Half an hour after gavage, the mice in the Tnm_swim and Tnm_union groups underwent a 30-min swimming exercise, and their body weight and tumour volume were measured every two days.

2.2. Sample collection

After injecting 4T1-luc cells, we observed the daily diet of the mice and the growth of tumour masses at the inoculation site, including the time of tumour appearance, tumour size, and tumour formation rate. We used a Vernier calliper to measure the diameter of the tumour every 2 days and calculated the tumour volume to draw a growth curve. On the last day of sampling, mice were subjected to gavage and exercise interventions before fasting for 6 h. Anaesthetic agents and D-fluorescein potassium salt were injected into the mice, and in-vivo imaging was performed using a Tanon 5200 fully automated chemiluminescence image analysis system. After the mice were euthanised, serum from each was frozen and stored at −80 °C. The tumour body was photographed and divided into three parts. One part was sent for transcriptomics testing, another part was fixed with 4% paraformaldehyde and paraffin-sectioned for pathological HE staining, and the other part was frozen at −80 °C for subsequent molecular validation. The fetch intestinal contents were prepared for metabolomics analysis.

2.3. RNA sequencing and data analysis

Solid tissue samples from three mouse breast tumours were selected from each tumour group. Tumour tissue cells were ground and broken, and RNA was extracted therefrom. After RNA extraction, purification, and library construction, the samples were subjected to next-generation sequencing (NGS) using the Illumina HiSeq sequencing platform to perform paired-end (PE) sequencing of these libraries. We used HISAT2 software to compare the filtered reads against the reference genome (*Mus musculus*, GRCm39). We used DESeq2 for the analysis of differential gene expression and screened the genes under the following conditions: multiple expression

differences | log 2 FoldChange | > 1, and P-value for significance <0.05. The R language ggplot 2 software package was used to draw volcano maps and MA maps of the differentially expressed genes. The R language circle package was used to label the differentially expressed RNAs in the genome based on the genomic information and RNA differential expression analysis results. We used the R language pheatmap software package to perform bidirectional clustering analysis of the union of differentially expressed genes and samples of all comparative groups. We used topGO for GO enrichment analysis and gene set enrichment analysis (GSEA) for KEGG pathway enrichment analysis of the differentially expressed genes.

2.4. Metabonomics analysis

After slowly thawing the sample of intestinal contents at 4 °C, we added an appropriate amount of sample to a pre-cooled methanol/acetonitrile/water solution (2:2:1, v/v), vortex mixed, treated with low-temperature ultrasound for 30 min, allowed it to stand at −20 °C for 10 min, centrifuged at 14000×*g* at 4 °C for 20 min, vacuum dried the supernatant, and added 100 μL acetonitrile aqueous solution (acetonitrile: water = 1:1, v/v); it was subsequently re-dissolved, swirled, centrifuged at 4 °C for 15 min at 14000×*g*, and then the supernatant taken for analysis. UHPLC-Q-TOF MS technology was used for chromatography-mass spectrometry analysis. The samples were separated using an Agilent 1290 Infinity LC ultra-high-performance liquid chromatography (UHPLC) HILIC column, and the primary and secondary spectra of the samples were collected using an AB Triple TOF 6600 mass spectrometer. Based on univariate analysis, differential analysis was conducted on all metabolites detected in positive- and negative-ion modes (including unidentified metabolites), and the threshold criteria for screening differential metabolites were FC > 1.5 or FC < 0.67 with a P-value <0.05. Using the Fisher's exact test, the significance levels of metabolite enrichment in each pathway were analysed and calculated to determine the metabolic and signal transduction pathways that were significantly affected.

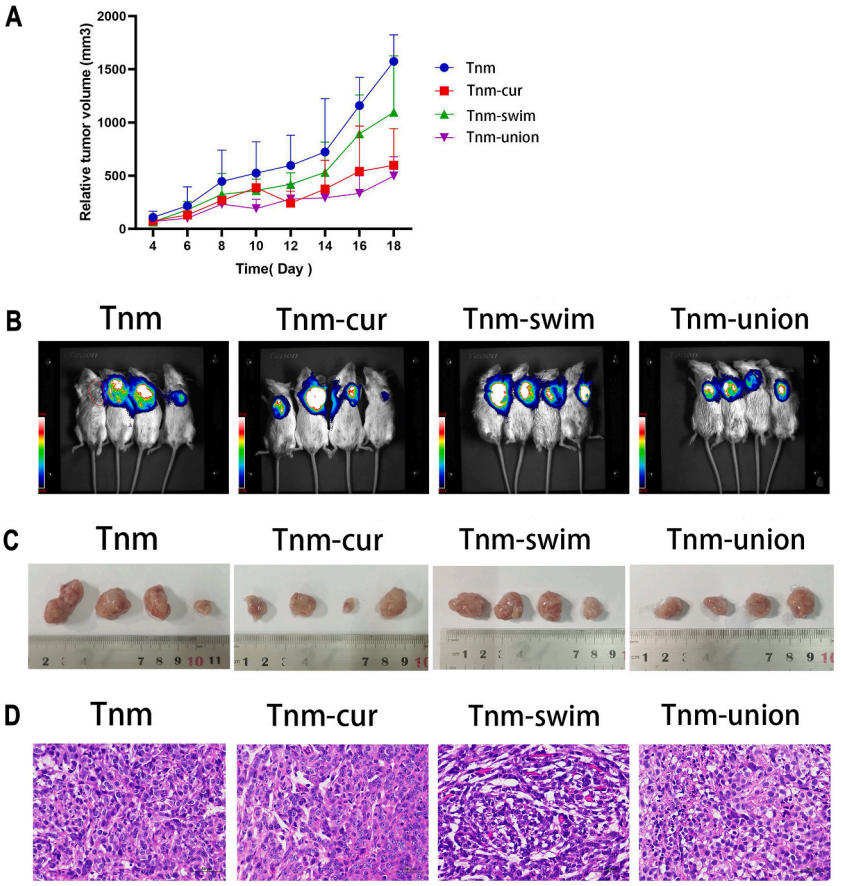


Fig. 1. Phenotypic observation of the effect of curcumin and exercise in combination on anti-breast tumour mice A. **Growth curve of tumour volume in mice after combined treatment with curcumin and exercise**. The horizontal axis represents the different days, whereas the vertical axis represents the relative tumour volume (mm3). Curves of the four groups are depicted separately as follows: tumour group (Tnm group), tumour + swimming group (Tnm_swim group), tumour + curcumin group (Tnm_cur group), and tumour + union group (Tnm_union group). B. *In-vivo* imaging of tumours under the condition of curcumin combined with exercise. C. Detection of stripped mouse tumour entities. D. H&E staining of solid tissue sections of mouse tumours in each group (400 ×).

2.5. Integrative analysis of multi-omics data

According to the quantitative detection data from metabolomics and transcriptomics mentioned above, we conducted correlation and integrative analyses based on the two-way orthogonal partial least squares (O2PLS) model to identify genes and metabolites with the highest degree of association.

2.6. Statistical analysis

Data obtained from the experimental measurements were analysed using GraphPad Prism 8 statistical software with one-way ANOVA, and the difference was considered statistically significant at $p < 0.05$.

3. Results

3.1. Curcumin combined with exercise enhanced the inhibitory effect on breast cancer

According to the phenotypic observations of mice in each group, including tumour appearance and in-vivo tumour imaging, compared to that in the Tnm group, the growth curve of tumours in the Tnm_swim, Tnm_cur, and Tnm_union groups clearly showed the

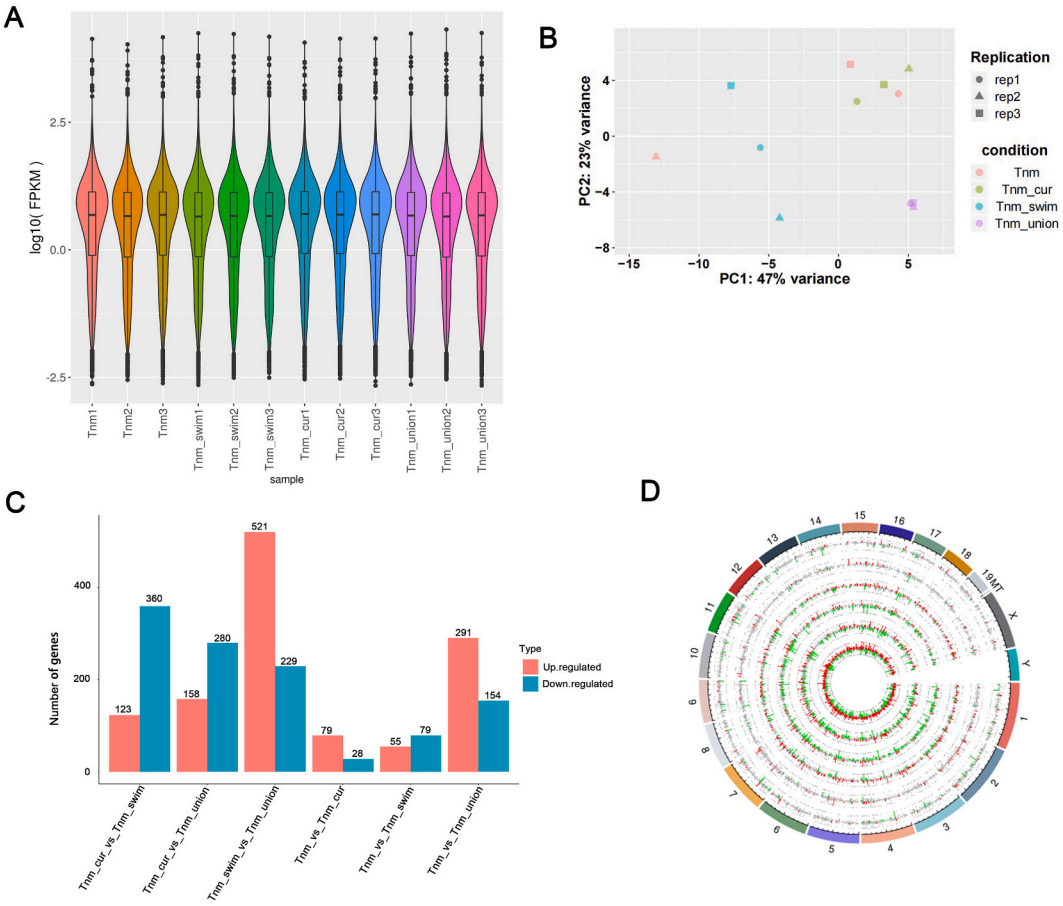


Fig. 2. RNA-seq data quality and differentially expressed gene anal Violin plot of the FPKM density distribution.
The horizontal axis represents the sequencing samples for each group, and the vertical axis represents the log 10 (FPKM) value distribution of all genes. The median is the horizontal line in the middle of the violin box shape.
B. Principal component analysis of RNA-seq samples.
Different colours represent different groups, and different shapes represent different duplicate samples within a group.
C statistical results of differentially expressed genes across different groups.
The horizontal axis represents the comparison of different groups, the vertical axis represents the number of differentially expressed genes, the red bar chart represents upregulated genes, and the green bar chart represents downregulated genes.
D. Distribution of differentially expressed genes in the genome. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

intervention by curcumin administration and swimming to inhibit the growth of tumours to varying degrees, and the combined treatment of curcumin administration and exercise intervention to have a more obvious inhibitory effect on breast cancer than the single treatment (Fig. 1A and B). We further observed stripped tumour entities in mice in each group, and the results showed the combined treatment of curcumin administration and exercise intervention to have a more significant inhibitory effect on breast cancer than the single treatments, though difference within the group was small, as shown in Fig. 1C. The HE staining results of the pathological sections suggested that the three treatment groups had a decrease in nuclear deviation, an increase in tumour cell interstitium, and relatively consistent cells. Moreover, the phenomenon of nuclear division was significantly reduced in the Tnm_union group, which showed striped staining and reduced cytoplasm (Fig. 1D). Overall, the combined treatment with curcumin and exercise could enhance the inhibitory effects on breast cancer.

3.2. Transcriptomic analysis of curcumin-exercise combination treatment effects on breast tumour in mice

The RNA sequencing results of 12 mouse tumour samples in the Tnm, Tnm_swim, Tnm_cur, and Tnm_union groups showed that Q20 (%) value in the RNA-seq data of each sample was controlled at almost 98%, and Q30 (%) value was controlled between 94% and 95% (Fig. 2A and Table 1). This indicated that the RNA-seq data quality of this experiment was superior and could be used for subsequent transcriptome analysis.

Principal component analysis results showed that except for one sample in the Tnm group that underwent separation, the other sequencing samples could be well clustered together through gene expression values. The best clustering effect of the PCA results was obtained from three samples in the union group, i.e. three samples of mouse tumours under the combined effect of curcumin and swimming exercise (Fig. 2B). It further showed that the experimental samples from mice with breast cancer under the combined effect of curcumin and swimming had good repeatability, which is more conducive to the subsequent analysis and screening of differentially expressed genes.

Next, we conducted a statistical analysis of the DEGs between different groups. The results showed that compared to the tumour group (Tnm group), the Tnm_cur group had a total of 107 differentially expressed genes, including 28 upregulated and 79 downregulated genes, the Tnm_swim group had a total of 134 differentially expressed genes, including 79 upregulated and 55 downregulated genes, and the Tnm_union group had a total of 445 differentially expressed genes, including 154 upregulated and 291 downregulated genes (Fig. 2C). From a quantitative perspective, there was a greater change in gene expression at the transcriptome level due to the combination treatment of curcumin and aerobic exercise; the distribution of DEGs in the genome is shown in Fig. 2D.

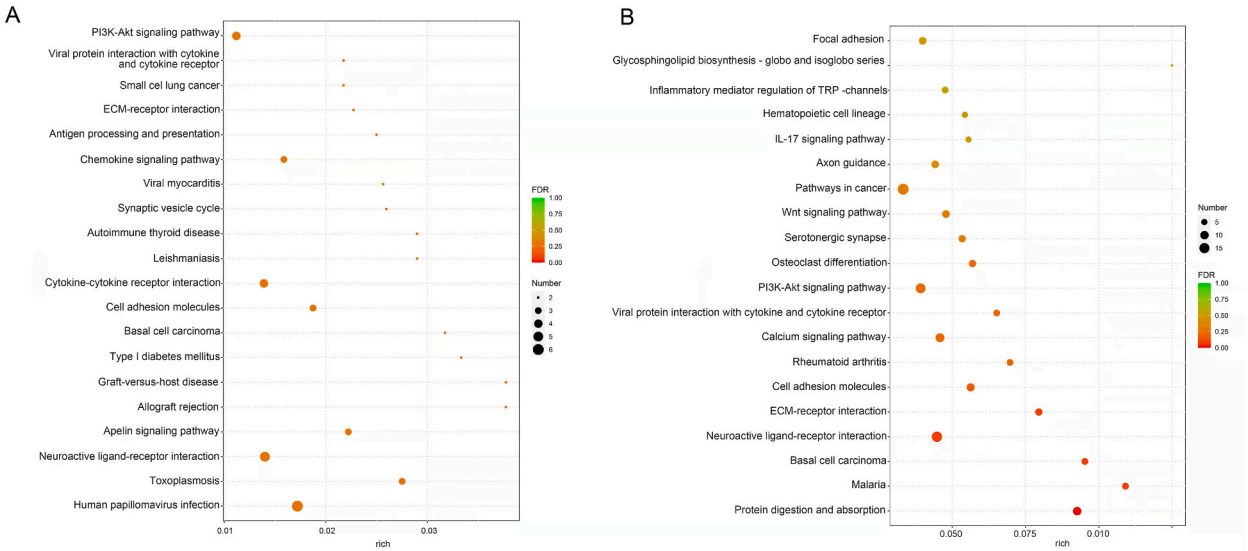
Further, we conducted GO and KEGG pathway enrichment analyses on the differentially expressed genes, selecting the top 10 GO terms and top 20 KEGG pathways with the most significant enrichment for display; the results are shown in Figs. S1 and S2. Using the Tnm group as a control, we compared the enriched pathways of the Tnm_cur group (Fig. 3A) and the Tnm_union group (Fig. 3B). Under the condition of curcumin combined with exercise, three related signalling pathways were significantly enriched, namely the mmu 04020: calcium signalling pathway, mmu 04310: Wnt signalling pathway, and mmu 04657: IL-17 signalling pathway. The genes involved in each pathway, including CAMK1G, WNT5A, and IL6, are shown in Fig. 3C. Wnt signalling and insulin sensitivity are known to be significantly altered due to exercise intervention in breast cancer survivors (BCS) [52]. WNT5A has been identified as an essential regulator and therapeutic target in breast cancer [53–56]. Crosstalk between multiple inflammatory cytokines, including IL6 and IL-17, has been found to occur in the breast tumour microenvironment [57].

3.3. Differential analysis of metabolites in the intestinal contents of mice under the combined action of curcumin and exercise

A total of 2618 metabolites were identified using the metabolomic data of the collected samples, including 1597 positive-ion-mode (Pos) and 1021 negative-ion-mode (Neg) metabolites. According to chemical taxonomy classification information, the top-ranked chemical classifications with over a hundred metabolites include lipids and lipid-like molecules, organic acids and derivatives, organic heterocyclic compounds, benzene ring compounds, organic oxygen compounds, phenylpropanoids, and polyketides. Based on univariate analysis, inter-group differential metabolite screening was performed. Significant differences in the number of metabolites

Table 1
Statistical analysis of RNA seq data quality.

Sample	Raw Data Reads	Clean Data Reads	Q30 (bp)	Q20 (%)	Q30 (%)	Total_ Map
Tnm1	47569352	44847410	6730532409	98.09	94.32	43290816 (96.53%)
Tnm2	48976506	46233180	6922789747	98.07	94.23	44790580 (96.88%)
Tnm3	47454488	44773140	6707582218	98.06	94.23	43270873 (96.64%)
Tnm_ swim 1	44531500	41974344	6346947967	98.38	95.01	40737035 (97.05%)
Tnm_ swim 2	45490758	42930084	6431446089	98.08	94.25	41466519 (96.59%)
Tnm_ swim 3	47329652	44645702	6719853918	98.23	94.65	43106586 (96.55%)
Tnm_ cur 1	46359298	43710888	6557727331	98.07	94.30	42197266 (96.54%)
Tnm_ cur 2	43140566	40696682	6105277146	98.11	94.34	39270009 (96.49%)
Tnm_ cur 3	43843880	41339936	6199796116	98.05	94.27	39922737 (96.57%)
Tnm_ union 1	47541952	44784882	6727201154	98.10	94.33	43328930 (96.75%)
Tnm_ union 2	48820126	45965398	6892611396	97.96	94.12	44511545 (96.84%)
Tnm_ union 3	46111450	43406552	6523720329	98.03	94.31	41919193 (96.57%)



C

Signaling pathways for union	Genes
mmu04020:Calcium signaling pathway	EDNRB, FGF9, NTRK3, PTAFR, ADCY1, HTR2A, MST1R, AVPR1A, ADRA1B, HTR5B, CAMK1G
mmu04310:Wnt signaling pathway	FZD1, WNT10A, WIF1, FRZB, SFRP5, WNT5A, ROR2, AXIN2, PRICKLE1
mmu04657:IL-17 signaling pathway	IL6, CCL12, CCL2, FOSB, CCL17

Fig. 3. Enriched KEGG pathways and associated genes A. Enriched pathways of the curcumin single-treatment. B. Enriched pathways of the combination treatment. C. Associated genes in three related signalling pathways under the condition of curcumin-exercise combination.

and KEGG pathway information between the different groups are shown in Table 2.

Compared to the Tnm group, the Tnm_cur group showed significant differences in two metabolism-related KEGG pathways, including ABC transporters (mmu02010) and metabolic pathways (mmu01100). Four KEGG pathways were associated with significantly different metabolites in the Tnm_union group, including ABC transporters (mmu02010), amino sugar and nucleotide sugar metabolism (mmu00520), biosynthesis of amino acids (mmu01230), and metabolic pathways (mmu01100). Significant differences in the metabolites involved in each pathway and their clustering analysis results are shown in Fig. 4A and B, respectively for the single curcumin treatment and the synergistic effect. In addition to the common ABC transporters and metabolic pathways, the combined effect of curcumin and exercise involved the metabolism of amino sugars and nucleotides, as well as the biosynthesis of amino acids. The amino sugar and nucleotide sugar metabolism pathways included chitobiose, D-glucosamine 6-phosphate, L-fucose, and N-acetyl-beta mannosamine, whereas the biosynthetic pathways of amino acids included DL-isoleucine, DL-tyrosine, and homocysteine. For the ABC transporter pathway, the significantly different metabolites in the Tnm_cur group were chitobiose, D-ribose, DL-isoleucine, inosine, and myo-inositol. The significantly different metabolites in the Tnm_union group were 2′- deoxycytidine, 2′-deoxynosine, cytidine, chitobiose, DL-isoleucine, and myo-inositol. Among them, 2′-deoxycytidine, 2′-deoxynosine, and cytidine were unique in their combined effects due to curcumin and exercise. Significant metabolic perturbations in amino acids, nucleotides, and sugars were revealed by the hepatic metabolic profiles of lifelong exercise-training rats [58].

3.4. Integrative analysis of metabolomics and transcriptomics data

As shown in Fig. 5A, the results of O2PLS analysis revealed that the top 20 metabolites related to metabolomics and transcriptomics in the positive-ion mode were (4r)-4-(5s, 7r, 9s, 10s, 13r, 14s, 17r)-7-hydroxy-10,13-dimethyl-3-oxohexadecahydro-1h-cyclopenta [a] phenanthren-17-yl) pentanoic acid, P-chloroaline, Borelidin, Flutamide, Tetrahydroprocumin, Desferrioxamine, Arctigen. In: trifloxystrobin, iodiglycol 4-methyllumbelliferyl. beta.-*d*-glucuronide, 4,2′- dihydroxy-3-methoxychalcone, Luvangetin, 1-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, N-[(2s)-2-[(1z) -1-methyl-3-oxo-3-[4-(trifluoromethyl) phenyl] -1-propen-1-yl] amino]-3-[4-[2-(5-methyl-2-phenyl-4 oxazolyl) ethoxy] phenyl] Propanamide, Histamine, 2- (4-amino-1-piperidinyl) ethanol, Uroscolic acid, gamma. - uric acid Arg Ser and (−) atropine. The top 20 genes are Cfd, Stfa2l1, Retnlg, S100a9, Gzmg, S100a8, C1qtnf3, Grem1, Clec4a1, Child3, Actn3, Saa3, Acta 1, Myl1, Atp2a1, Mybpc2, Wfdc17, Ckm, Ccl8, and Myh4.

As shown in Fig. 5B, the top 20 metabolites in O2PLS analysis results under negative-ion mode were Maltotriose, [1,1 ′- biphenyl] -2-carboxylic acid, 4’ - [5- [[(1s) -1- (4-bromophenyl) ethyl] amino] carbonyl] −2,3-dimethyl-1h indol-1-yl] methyl] -, Xanthosine, Met−Tyr, (3s, 4s)-5-[(3s, 4s) −4,10-dihydroxy-7,9-dimethyl-3-methyl-3,4-dihydro-1h-benzo [g] isochromen-5-yl] −7,9-dimethyl-3-

Table 2
Summary of differential metabolites and significantly related pathways in each comparison.

Comparison	No. of differential metabolites	Significantly related pathways
Tnm-cur_vs_Tnm	77 (Pos 52/Neg 25)	mmu02010 ABC transporters; mmu01100 Metabolic pathways
Tnm-swim_vs_Tnm	109 (Pos 69/Neg 40)	mmu 05230 Central carbon metabolism in cancer; mmu 00260 Glycine, serine and threonine metabolism; mmu02010 ABC transporters; mmu01230 Biosynthesis of amino acids; mmu04976 Bile secretion; mmu00140 Steroid hormone biosynthesis; mmu01100 Metabolic pathways
Tnm-union_vs_Tnm	126 (Pos 94/Neg 32)	mmu02010 ABC transporters; mmu00520 Amino sugar and nucleotide sugar metabolism; mmu01230 Biosynthesis of amino acids; mmu01100 Metabolic pathways
Tnm-swim_vs_Tnm-cur	143 (Pos 94/Neg 49)	mmu00240 Pyrimidine metabolism; mmu 00410 beta-Alanine metabolism; mmu 00232 Caffeine metabolism; mmu 00250 Alanine, aspartate and glutamate metabolism; mmu 00770 Pantothenate and CoA biosynthesis; mmu 04742 Taste transduction; mmu02010 ABC transporters; mmu 04080 Neuroactive ligand-receptor interaction; mmu 00230 Purine metabolism; mmu00140 Steroid hormone biosynthesis; mmu01100 Metabolic pathways
Tnm-union_vs_Tnm-cur	46 (Pos 32/Neg 14)	mmu00240 Pyrimidine metabolism; mmu02010 ABC transporters; mmu04976 Bile secretion; mmu00140 Steroid hormone biosynthesis; mmu01100 Metabolic pathways
Tnm-union_vs_Tnm-swim	134 (Pos 88/Neg 46)	mmu 04024 cAMP signaling pathway; mmu 00120 Primary bile acid biosynthesis; mmu04976 Bile secretion; mmu01100 Metabolic pathways

methyl-3,4-dihydro-1h-benzo [g] isochromene-4,10-diol, Dl sulforaphane n-acetyl-l system), Np-00000 4 (11), Monensin, 1,3,5 (10) - estratrien-3,17 beta. - diol diglucosiduronate, Ciprofloxacin piperazinyl-n4-sulfonate, S − adenosyl − l − homocysteine, Cylanilide, Bensulfuron metal Marein, D-ribose 1-phosphate, 6-phosphogluconic acid, Doxycycline, Silybin, D-*erythro*-imidazolylglycerol phosphate, and trehalose 6-phosphate. The top 20 genes are Ccl8, Prl2c3, Child3, Cd163, Saa3, Prl2c2, Wfdc17, Il24, Child 1, Mcpt1, Clec4a1, Sln, Myl1, Myh4, Ckm, S100a8, S100a9, Adamdec1, Cxcl2, and Lcn2. These genes and metabolites could serve as key factors in the combination of exercise and curcumin supplementation therapies for breast cancer.

4. Discussion

Overall, the combination of exercise and curcumin can achieve significant effects in the treatment of cancer, since both can regulate immune functions and enhance defence mechanisms. Curcumin and exercise have been shown to have significant benefits in the treatment of complex diseases. Exercise rapidly increases the demand for oxygen in the human body, accompanied by an increase in respiratory intensity and speed, thereby increasing the demand for the respiratory system. Once the pressure on the lungs is too high, it can easily lead to oxidative stress, inflammation, and other lung health issues. Curcumin has attracted considerable attention owing to its strong antioxidant properties. In addition, the heart and cardiovascular system maintain energy and material supply during exercise, and a good state of exercise requires good energy and material supply to support it. Curcumin can promote healthy cardiovascular function by improving endothelial function and balancing blood pressure. Previous studies on the post-exercise recovery effects of curcumin indicated that it can alleviate muscle damage, such as pain and inflammation, after exercise [59]. The application of curcumin in sports nutrition has been attracting increasing attention recently. With the increasing demand for natural products, plant-derived curcumin may become the backbone of future sports and health products.

In this study, we integrated and utilized transcriptomics and metabolomics data for the first time, and systematically analysed the therapeutic effects and molecular mechanisms of exercise combined with curcumin on breast cancer in mice. Based on the results of this study and the reference pathway map in KEGG database, we have preliminarily constructed a molecular regulatory mechanism of exercise combined with curcumin in the treatment of breast tumours. The combination of exercise and curcumin can inhibit the growth of breast tumours in mice by regulating such targets that act upstream of or within several processes (Fig. 6). In the Calcium signaling pathway, EDNRB, PTAFR, AVPR1A, and ADRA1B are the members of the G protein coupled receptors (GPCR), which can affect cell apoptosis by regulating ADCY1. There are relevant reports indicating that homocysteine can regulate cell life processes by affecting GPCR [60]. Both NTRK3 and MST1R are the members of the receptor tyrosine kinases (RTK), and previous studies have shown a certain association between RTK and the metabolic processes of L-Fucose and homocysteine in the regulation of cancers [61–63]. In the

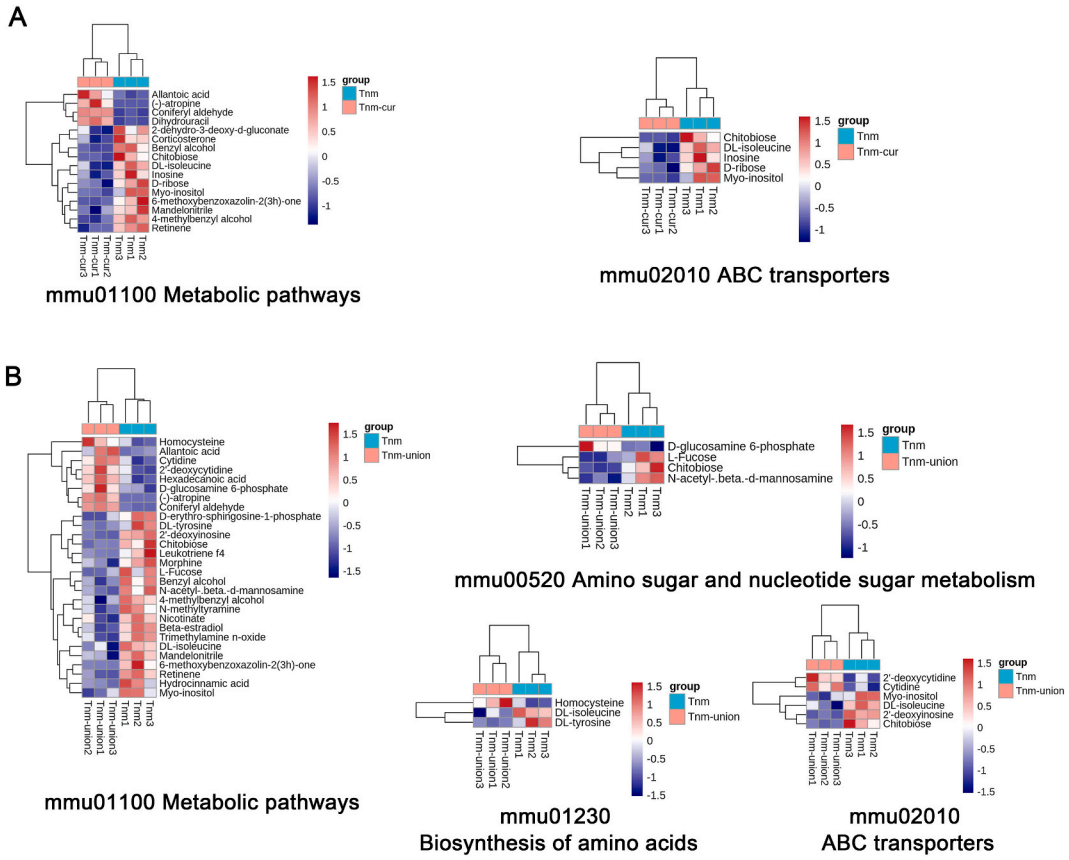


Fig. 4. Pathways associated with the significantly different metabolites A. Pathways associated with significantly different metabolites of the curcumin single-treatment. Pathways associated with significantly different metabolites in the curcumin-treated group (Tnm_cur group) compared to those in the tumour group (Tnm group).
B. Pathways associated with significantly different metabolites of the combined-effect treatment.
Pathways associated with significantly different metabolites in the combined-effect treatment group (Tnm_union group) compared to those in the tumour group (Tnm group).

Wnt signaling pathway, frizzled class receptor 1 (FZD1) enables Wnt-protein binding activity, the FRZB, SFRP5, and WIF1 genes can inhibit the Wnt protein and regulate the downstream of AXIN2 to affect the cell cycle [64–66]. In the IL-17 signaling pathway, AP-1 transcription factor subunit FOSB regulates CCL17, IL6 and CCL2 genes, thereby stimulating cell mediated immunity [67–69]. Through our integrated analysis, we identified the key genes and metabolites that function in mouse breast tumours under the condition of both exercise and curcumin intake, providing a new direction and data support for the treatment of breast cancer. Our study, however, has certain limitations. First, the number of experimental samples was relatively small, the sample size should be increased to improve the accuracy of experimental results; second, the mechanism of interaction between key metabolites and DEGs is still not clear, more molecular validation experiments should be conducted. In future, based on the results of this study, we will explore how the screened differentially expressed genes affect the production and metabolic pathways of metabolites, as well as how metabolites regulate gene expression and function.

Ethical approval

All animal experiments were approved by the Academic Ethics Committee of the Harbin Sport University (HSU-2022004).

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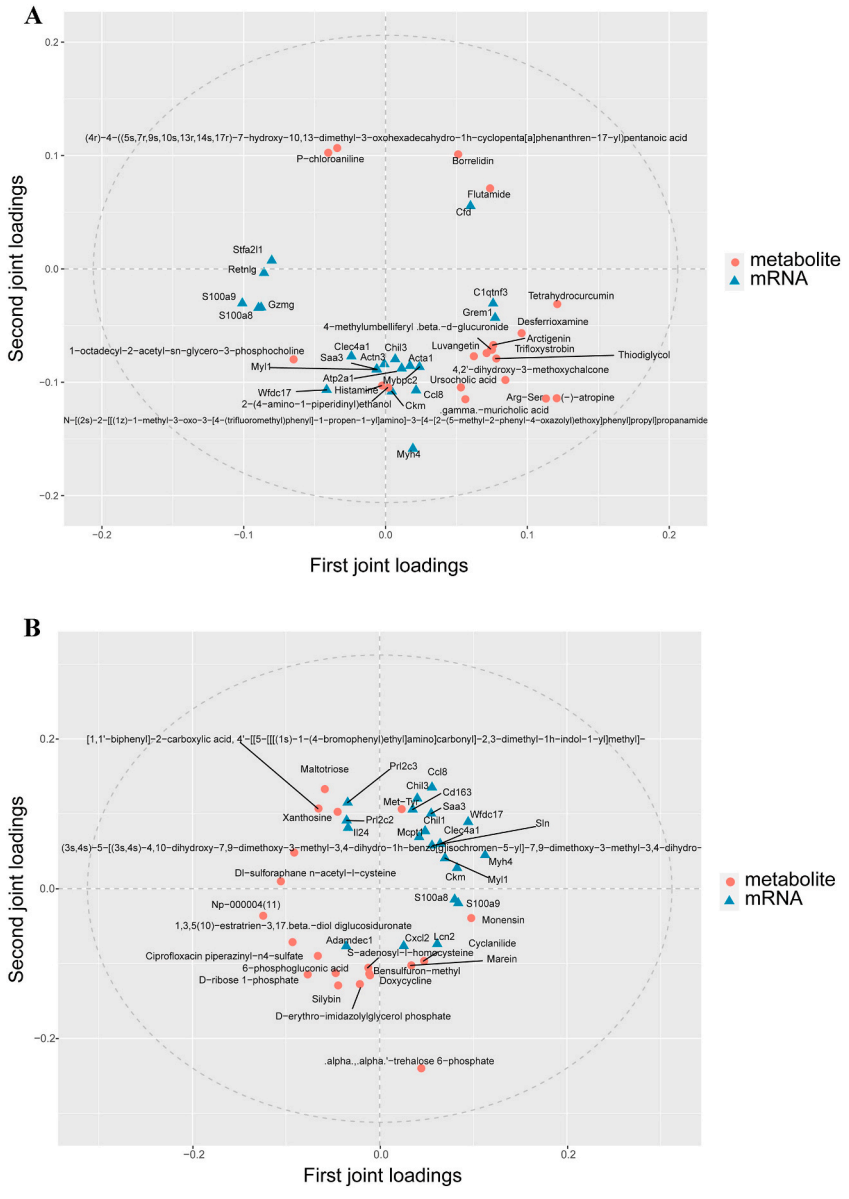

Fig. 5. Top 20 loading elements of metabolites and genes by integrative analysis based on O2PLS model A. **Top 20 loading elements of metabolites in positive-ion mode and associated genes.** Red dots represent metabolites and blue triangles represent genes. B. **Top 20 loading elements of metabolites in negative-ion mode and associated genes.** Red dots represent metabolites and blue triangles represent genes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Data availability statement

The RNA-seq data generated in this study were submitted to the Gene Expression Omnibus (GEO) repository with the accession number GSE256152. The metabonomics data that support the findings of this study are available in the database of MetaboLights under the reference number MTBLS9577.

CRediT authorship contribution statement

Yong Guo: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Jinxing Su:** Writing – original draft, Visualization, Formal analysis, Validation. **Shangquan Jiang:** Writing – review & editing, Visualization, Validation, Formal analysis. **Yan Xu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis. **Binbin Dou:** Validation, Formal analysis, Visualization, Writing – original draft. **Ting Li:** Formal analysis,

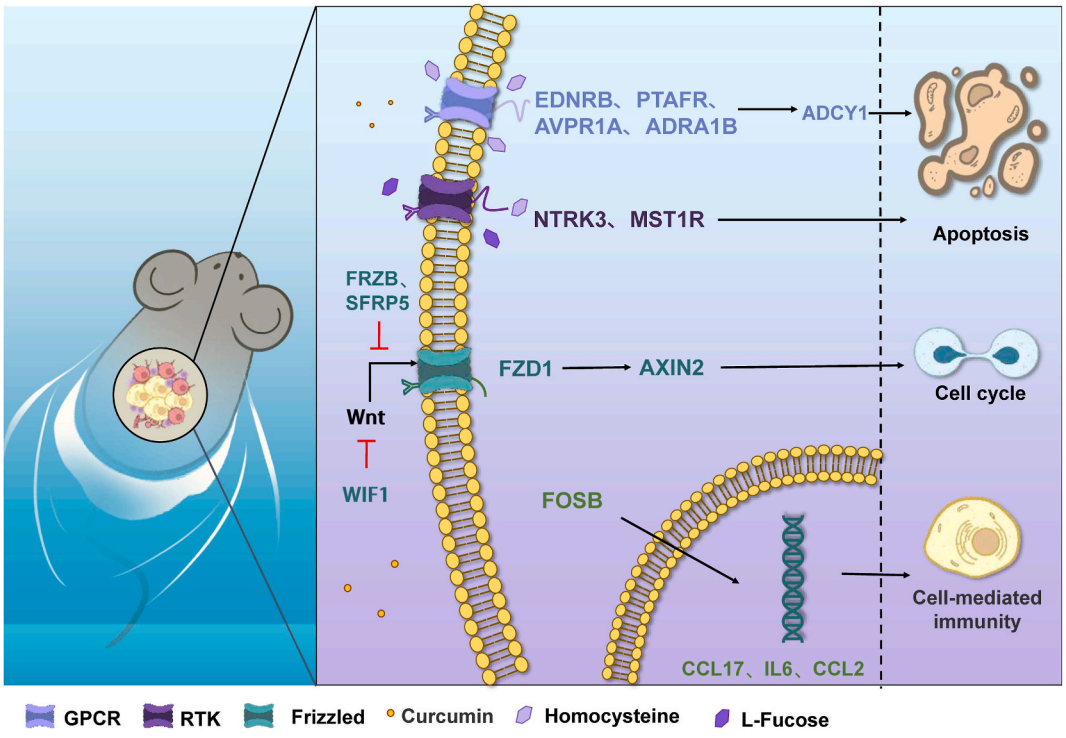

Fig. 6. Preliminarily constructed molecular regulatory mechanism of exercise combined with curcumin in the treatment of breast tumours.

Validation, Visualization, Writing – original draft. **Jiabin Zhu:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Conceptualization, Funding acquisition. **Kan He:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28807.

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