Taurine Attenuates Dimethylbenz[a]anthracene-induced Breast Tumorigenesis in Rats: A Plasma Metabolomic Study

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Abstract. *Breast cancer is the most common malignancy and the leading cause of cancer-related mortality in women worldwide. Taurine, the most abundant free amino acid, plays a role in several biological processes in humans and has been shown to have activity against breast cancer and other tumors. To investigate the role and mechanism of taurine action in breast cancer, we used dimethylbenz[a]anthracene (DMBA)-induced breast carcinogenesis in rats as a model of breast cancer. The administration of taurine significantly reduced the DMBA-induced breast cancer rate from 80% to 40% in rats ($p<0.05$). Metabolomic studies using time-of-flight gas chromatography-mass spectrometry identified 23 differential metabolites in the plasma of taurine-administered rats. Bioinformatic analysis further revealed that these metabolites are involved in multiple metabolic pathways, including energy, glucose, amino acid, and nucleic acid metabolism, suggesting that the antitumor activity of taurine in rats is mediated through altered metabolism of breast cancer cells. We propose that these differential metabolites may be potential biomarkers for monitoring cancer therapy and prognosis in the clinic. This study provides a scientific basis for further investigations of the antitumor mechanism of taurine and the development of novel therapeutic strategies to treat breast cancer.*

Breast cancer is the most common cancer and the leading cause of cancer-related death in women worldwide, accounting for nearly one in eight cancer deaths (1). Despite intensive investigation, the pathogenesis and development of breast cancer are still largely unknown. In the late 1920s, Otto Warburg and colleagues first described altered metabolism in cancer cells, demonstrating altered aerobic glycolysis in tumors (2, 3). Since then, more metabolic changes have been identified in cancer cells, including metabolism of amino acids and nucleic acids. It is now widely accepted that cancer is a range of metabolic diseases; therefore, understanding the interactions between altered metabolism and cancer biology is important not only for therapeutic interventions of cancer but also for prevention of malignant development (4).

Accumulating evidence has demonstrated that dietary patterns and specific dietary factors correlate with higher incidence of breast cancer. Consumption of fruits, vegetables, low-fat dairy products, fish, polyunsaturated fatty acids, vitamin D, calcium, and phytoestrogens appear to reduce the risk for breast cancer, whereas a high intake of meat, poultry, total energy, total fat, and saturated fatty acids may be causative factors for this disease (5). Although the mechanisms underlying these phenomena are still elusive, recent research reports that dietary compounds such as folate, genistein, tea polyphenols, and resveratrol affect breast cancer tumorigenesis and progression by modulating epigenetic mechanisms, including DNA methylation and histone modifications (6). This is consistent with the notion that a change in the ratio between histone acetylation and histone deacetylation may be a driving force in causing normal cells to become malignant.

Taurine, or 2-aminoethanesulfonic acid, is the most abundant free amino acid in the body and plays important roles in several essential biological processes, including bile acid conjugation, maintenance of calcium homeostasis, osmoregulation, and membrane stabilization. In addition, taurine serves a wide variety of functions in the central nervous system and is associated with cardiomyopathy, renal dysfunction, developmental abnormalities, and severe damage to retinal neurons (7). Furthermore, studies have demonstrated that taurine has an inhibitory effect on different types of tumors, including the suppression of breast cancer cell growth *in vitro* and *in vivo* (8-10). The mechanism accounting for the antitumor effects of taurine is not clear,

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but recent evidence suggests that taurine induces tumor cell apoptosis, enhances immune function, and repairs DNA damage in various human and animal tumors (11-14).

Metabolomics is a powerful technology for detecting thousands of metabolites in cells, tissues, and biological fluids; it is currently being applied to identify biomarkers and altered metabolic pathways in cancer. Metabolomics can also be used for selecting individualized therapeutics and for monitoring drug response in the clinical laboratory (15-17).

In the current study, we investigated the effect and mechanism of taurine in a dimethylbenz[a]anthracene (DMBA)-induced breast cancer model in rats. Using a metabolomic approach, we analyzed the metabolite patterns in the plasma of this animal model providing a basis for an understanding of the pathogenesis of breast cancer as well as the antitumor mechanism of taurine.

Materials and Methods

Establishment of the rat breast cancer model and animal breeding. A total of 30 healthy female SD rats were purchased from Guangxi Animal Center (Nanning, P. R. China). The rats had never given birth and weighed 169-205 g each. They were raised at the Experimental Animal Center of Guangxi Medical University under specific pathogen-free conditions and were cared for in an environmentally controlled room with a 12-h light/12-h dark cycle and a constant temperature of 22˚C. The rats were provided with a standard diet and water in accordance with the guidelines for the treatment of experimental animals published by the Ministry of Science and Technology of the People's Republic of China in 2006. The 30 rats were randomly divided into three groups: control group (n=10), breast cancer group (n=10), and taurine intervention group (n=10). Before starting the experiments, all of the rats were fed for 7 days to allow them to adapt to the environment. The breast cancer and taurine intervention groups were administered DMBA (Sigma-Aldrich Corp., St. Louis, MO, USA) at 15 mg/100 g by gavage. The taurine intervention group was freely given water containing 3% taurine at the start of the experiments. The rats were checked for tumor growth, and breast tumors were counted and measured for each animal every week. The rats were killed humanely 16 weeks later, and breast tumors were resected for histopathological examination. All measurements were performed in a coded, blinded fashion. This study was carried out in strict accordance with the recommendations set forth in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Eighth Edition, 2010) (18). The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Guangxi Medical University (approval no. KY-015; Nanning, P. R. China).

Sample collection and preparation. At the end of 16 weeks, blood samples were collected from the rats under anesthesia (3% barbiturates), after a 12-h food fasting period. Venous blood was collected from the primary abdominal veins and stored in heparin-coated vacuum blood collection tubes. The plasma was separated by centrifugation at 1,000 × *g*, and 500 μl of each plasma sample were cryopreserved in liquid nitrogen for 30 min, followed by storage at −80˚C. Ten rat plasma samples from the control group, 10 samples from the breast cancer group, and eight samples from the taurine-intervention group were collected. Immediately after blood sample collection, breast cancer tissues of each rat were excised and stored in 10% formalin for histological analysis.

Analysis of samples by high-throughput gas chromatography with time-of-flight mass spectrometry (HT GC-TOFMS). To prepare samples for HT GC-TOFMS, 100 μl of a plasma sample, 0.35 ml of methanol, and 40 μl of internal standard (L-2-chlorophenylalanine; 0.1 mg/ml stock in dH_2O) were added to a 1.5-ml Eppendorf tube and vortex-mixed for 10 sec. After centrifugation at 13,000 × *g* for 10 min at 4˚C, 0.4 ml of supernatant was transferred to a fresh 2-ml gas chromatography-mass spectrometry (GC-MS) glass vial and dried in a vacuum concentrator without heat, followed by the addition of 60 μl of methoxyamination reagent (20 mg/ml in pyridine) and shaking for 2 h at 37˚C. For silylation, 0.08 ml of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) plus 1% trimethylsilyl chloride (v/v) were added to the sample, followed by shaking for 1 h at 70˚C. After the sampler cooled to room temperature, 10 μl of a standard mixture of fatty acid methyl esters (FAME: C8-C16, 1 mg/ml; C18-C30, 0.5 mg/ml in chloroform) were mixed with the sample, and GC-TOFMS analysis was performed using an Agilent 7890 gas chromatography system coupled to a Pegasus high-throughput time-of-flight mass spectrometer (LECO UK; Hazel Grove, Stockport, UK). The system uses a DB-5MS capillary column coated with 5% diphenyl cross-linked with 95% dimethylpolysiloxane (30 m × 250 μm inner diameter, 0.25 μm film thickness; J & W Scientific, Folsom, CA, USA). The analyte (1 μl) was injected in splitless mode with helium as the carrier gas. The front inlet purge flow rate was 3 ml/min, and the gas flow rate through the column was 20 ml/min. The temperature was held at 50˚C for 1 min, raised to 330˚C at a rate of 10˚C/min, and held at 330˚C for 5 min. The injector, transfer line, and ion source temperatures were 280˚C, 280˚C and 220˚C, respectively. The energy was −70 eV in electron impact mode. Mass spectrometry data were acquired in full-scan mode with an m/z range of 85-600 and at a rate of 20 spectra/sec after a solvent delay of 366 sec.

Analysis of GC-TOFMS data. The Chroma TOF4.3X software program (LECO Corp., St. Joseph, MI, USA) and the LECO-Fiehn Rtx5 database were used for raw peak extraction, data baseline filtering, baseline calibration, peak alignment, deconvolution analysis, peak identification, and peak are integration (19). The retention time index (RI) method was used for peak identification, with an RI tolerance of 5,000. After missing values in the raw data were assigned half of the minimum value, 673 peaks were detected; 403 metabolites remained after interquartile range de-noising. Internal standard normalization was applied for the data analysis.

Statistical data analysis. Using the SIMCA-P 13.0 software package (Umetrics, Umea, Sweden), the three-dimensional data, including peak number, sample name, and normalized peak area, were analyzed by principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA). PCA showed the distribution of the original data. Based on the OPLS-DA, a loading plot was constructed, revealing the contributions of variables to differences between the two groups. The loading plot also showed important variables situated far from the origin, but the plot was complex owing to the large number of variables. To refine the analysis, the first principal component of the Variable Importance in Projection (VIP) was obtained. VIP values exceeding 1.0 were initially selected to

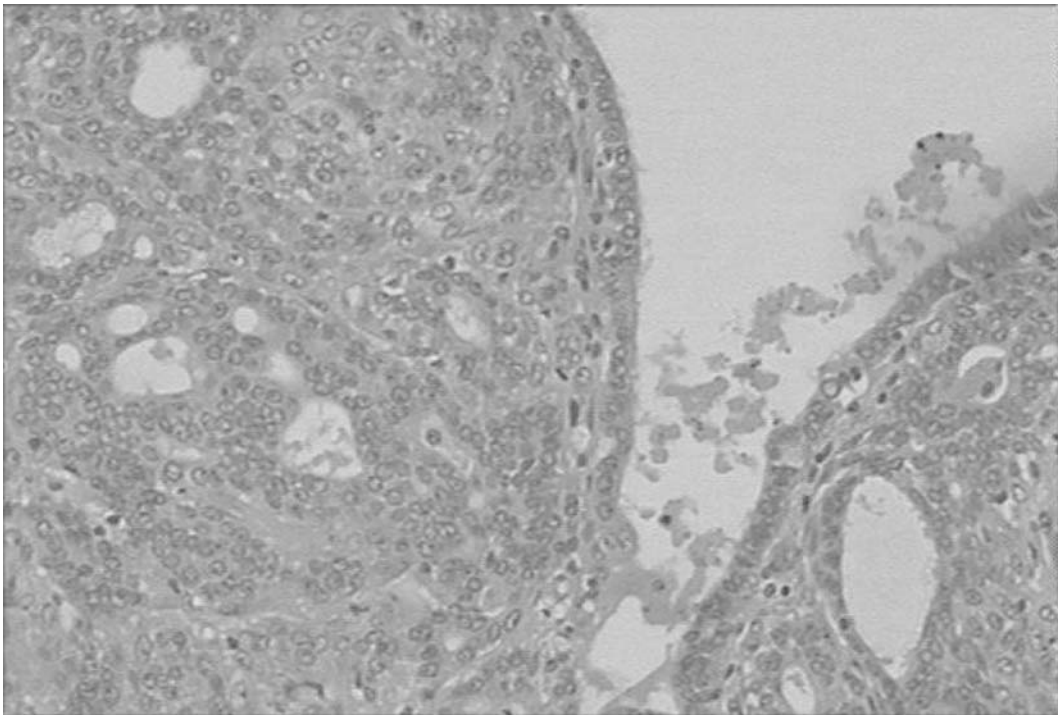

Figure 1. *Hematoxylin and eosin staining of tumor tissue from the dimethylbenz[a]anthracene-induced breast cancer group. The tumor was diagnosed as infiltrative ductal breast carcinoma based on the standard of the World Health Organization for pathological diagnosis of tumors of the breast (20) (original magnification, ×10).*

signify changed metabolites. The remaining variables were assessed by Student's *t*-test, and insignificant variables between two comparison groups were discarded ($p>0.05$). The relative average normalized quantities of the identified differential metabolites were plotted in a heat map using the MeV software package. Commercial databases, including the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) and the National Institute of Standards and Technology (NIST) database (http://www.nist.gov/index.html), were used to search for the pathways of the identified differential metabolites. We also used MetaboAnalyst 2.0 software (http://www.metaboanalyst.ca) for metabolite set enrichment analysis.

Results

Taurine significantly attenuates DMBA-induced breast carcinogenesis in rats. During the course of the experiments, tumor growth in rats was examined for all experimental groups. The average incubation period of tumor growth was 43 days for the breast cancer group and 58 days for the taurine intervention group ($p<0.05$). Breast cancer incidence in the taurine intervention group was 40%, that was significantly lower than that (80%) in the breast cancer group ($p<0.05$). These results clearly showed that taurine inhibited tumorigenesis in the rat model of breast cancer. Histopathological examination of the tumor tissues from the model rats revealed infiltrating ductal breast carcinoma (Figure 1), showing the successful establishment of the experimental breast cancer model. The diagnosis of breast carcinoma in the rat model was based on the pathological diagnostic standard for mammary gland of the World Health Organization classification of tumors (20). Two rats in the taurine intervention group unexpectedly died during the experiments. One rat died on day 3 after rat gavage with DMBA, possibly due to acute DMBA toxicity. The other rat died on the day the experiments were terminated and had no detectable tumor; the cause of death in this rat was unclear. Hence, the plasma samples were not collected from these two rats, and only eight plasma samples from the taurine intervention group were available for the metabolomics study.

Identification of 23 differential metabolites in the plasma of taurine-administered rats by GC-MS-based metabolomic analysis. We analyzed a total of 28 plasma samples from the three rat groups, and the GC-MS spectra of representative plasma samples from the control, breast cancer, and taurine intervention groups are shown in Figure 2. The ion flow charts of the samples from the three groups were similar; however, the peaks at certain retention times differed among the three groups, suggesting differences in plasma metabolites among the three groups of rats.

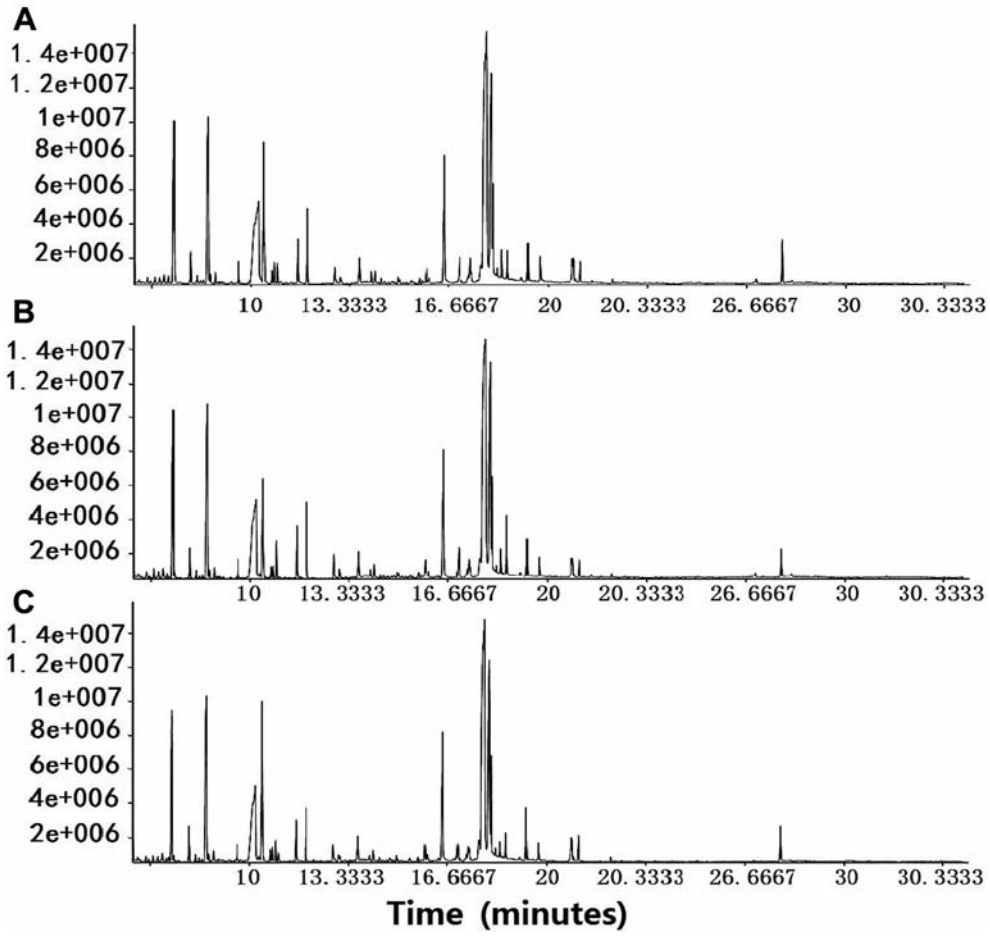

Figure 2. *Total ion chromatogram of plasma from the three groups of rats. Gas chromatography–mass spectrometric spectra of typical metabolites in the healthy control group (A), breast cancer group (B), and taurine intervention group (C). The horizontal axis is the retention time, and the ordinate scale represents peak size.*

Next, we examined the metabolomic data by multivariate statistical analysis. We first performed unmonitored PCA. SIMCA-P+ software (V13.0, Umetrics AB, Umea, Sweden) was used for multivariate analysis after data normalization and pattern recognition, and PCA was performed after unit variance scaling. For automatic modeling analysis of the plasma metabolic profiles, we used unmonitored PCA, comparing the control and breast cancer groups or the breast cancer and taurine intervention groups, respectively. The control group, breast cancer group, and taurine intervention group were not well separated in the PCA models (Figure 3), indicating the limited discriminant ability of PCA analysis as a method for unmonitored pattern recognition, especially for analyzing large numbers of samples and variables. In the present study, there were multiple samples with different levels of classification in the dataset. As unmonitored PCA was unable to identify differential metabolites in the plasma samples, further discriminant analysis was necessary.

We next performed OPLS-DA, that is a monitored analysis method. Monitored pattern recognition uses a multiple parameter model to identify and classify samples based on known classification information in order to estimate factors leading to classifications between samples. Monitored pattern recognition can maximize the separation of samples with different levels of classification as well as find metabolic differences at each level. OPLS-DA using orthogonal signal correction partial least squares discriminant analysis was used for the first and second principal component modeling analysis between the control and breast cancer groups or between the breast cancer and taurine intervention groups. As shown in Figure 4, OPLS-DA produced good separation between the control and breast cancer groups and between the breast cancer and taurine intervention groups.

We also identified differential metabolites by OPLS-DA. To improve the reliability of the results, we filtered-out and

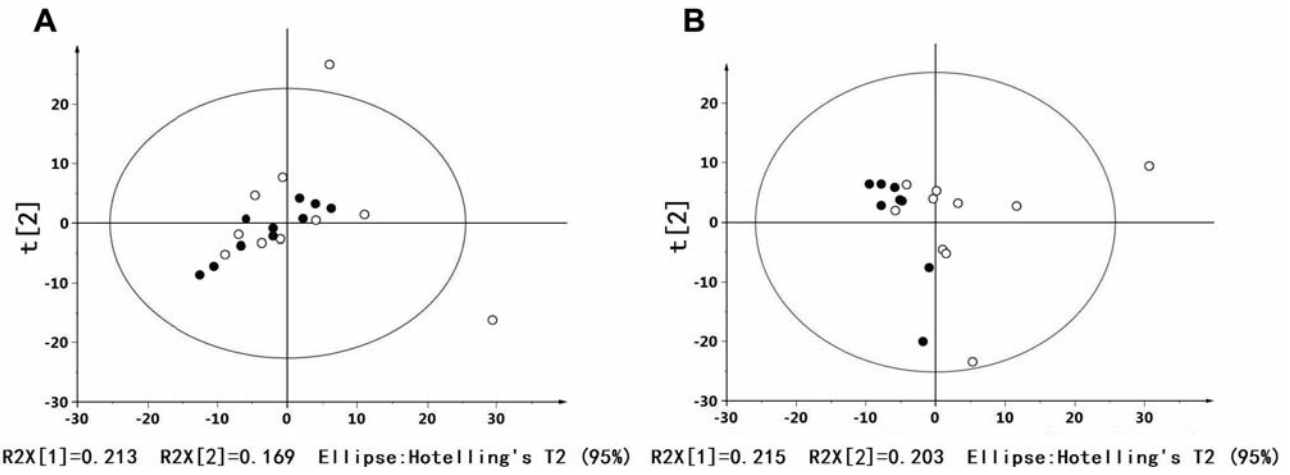

Figure 3. *General distribution of the original data samples in principal component analysis (PCA) scoring charts. PCA scoring between the control (solid dots) and breast cancer (circle dots) groups (A) and between the breast cancer (circle dots) and taurine intervention (solid dots) groups (B). The metabolites in the plasma samples of different groups are not clearly separated. The samples lie within the 95% confidence interval.*

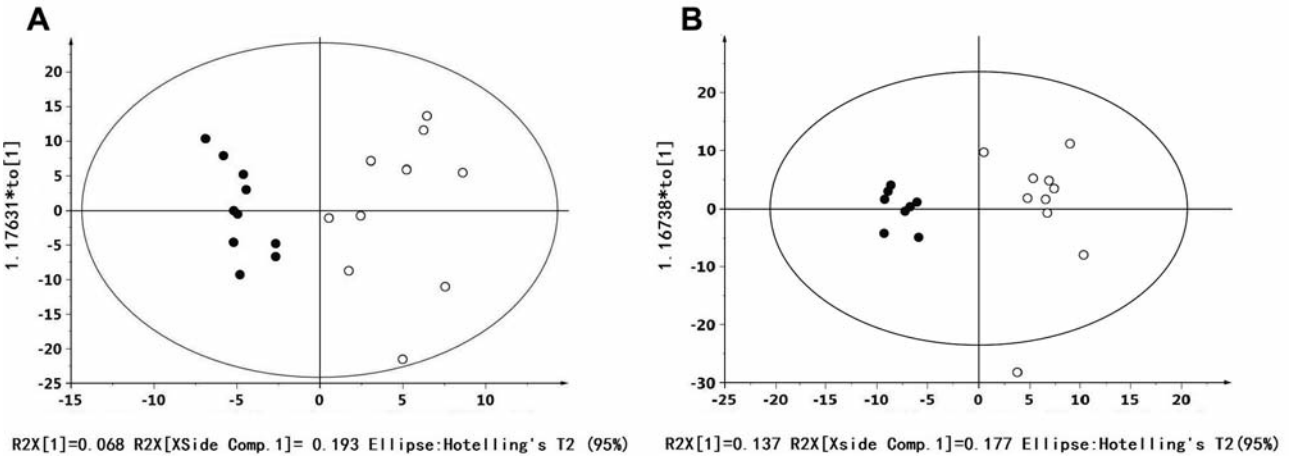

Figure 4. *General distribution of the data samples in orthogonal projections to latent structures discriminant analysis (OPLS-DA) scoring charts. OPLS-DA scoring between the control (solid dots) and breast cancer (circle dots) groups (A) and between the breast cancer (circle dots) and taurine intervention (solid dots) groups (B). Metabolites are well separated between the control and breast cancer groups (A) and between the breast cancer and taurine intervention groups (B).*

removed unrelated orthogonal signals with OPLS-DA. To identify differential metabolites, we used first principal component VIP value >1 in the OPLS-DA model and used t-test p-values of less than 0.05. Differential metabolites were identified by comparison with metabolites in the KEGG and the NIST database. Five identified metabolites were differentially expressed in the plasma samples of the breast cancer group compared to the control group (Table I), and 23 differential metabolites were identified in the plasma samples of the taurine intervention group compared to the breast cancer group (Table II).

The calculated average of the relative quantities of the five differential metabolites in the plasma samples of the breast cancer group after normalization to the control group values were plotted on a heat map using MeV software (Figure 5A). Similarly, the calculated average of the relative quantities of the 23 differential metabolites in the plasma samples of the taurine intervention group after normalization to the breast cancer group values were plotted on a heat map (Figure 5B). The metabolites were clustered according to their Pearson correlation.

Finally, using MetaboAnalyst 2.0 software, we analyzed the metabolic pathways involving the identified metabolites

Table I. *Identification of differential metabolites in the plasma of rats in the breast cancer group by gas chromatography with time-of-flight mass spectrometric analysis.*

Metabolite	VIP	*p*-Value	Fold change	Up- or down-regulation*
Proline	2.18539	0.030482267	1.234665182	↓
α-Ketoisocaproic acid 1	2.32108	0.015591737	1.704390145	↓
2-Keto-isovaleric acid 1	2.26931	0.018491959	1.432773877	↓
D-Arabitol	3.07321	0.000638255	0.783737324	↑
D-Galacturonic acid 2	2.68631	0.005695505	0.587107887	↑

**Versus* plasma of rats in the healthy control group; arrows indicate a decrease or increase in metabolite level. VIP: Variable importance in projection.

Table II. *Identification of differential metabolites in the plasma of rats in the taurine intervention group by gas chromatography with time-of-flight mass spectrometric analysis.*

Metabolite	VIP	*p*-Value	Fold change	Up- or down-regulation*
2-Hydroxybutanoic acid	1.22477	0.047764616	1.420353265	↓
L-Malic acid	1.64406	0.012435328	2.255580607	↓
Pyruvic acid	1.85065	0.002535449	1.783417908	↓
Threonine 1	1.79237	0.003872813	1.507968804	↓
Myo-inositol	1.62907	0.009311225	1.352179681	↓
Valine	1.16913	0.029881469	1.141611552	↓
Cystine	1.80624	0.003947669	2.128801069	↓
Isoleucine	1.42938	0.006907471	1.126311586	↓
Citric acid	1.88521	0.00567549	1.547768256	↓
Uric acid	1.67918	0.006613147	1.630199684	↓
Uracil	1.43585	0.036613226	1.388161736	↓
3-Hydroxybutyric acid	1.91082	0.002822329	1.73259583	↓
Fumaric acid	1.72597	0.007871309	1.957966184	↓
Cystine	1.2671	0.044998084	1.490521624	↓
4-Hydroxypyridine	1.65226	0.029437549	0.847537605	↑
Hydroxyurea	2.03414	0.001290708	0.707232575	↑
α-Ketoglutaric acid	1.39772	0.031716172	1.538140324	↓
Ibose	1.66325	0.010417499	2.074583769	↑
Dodecanol	2.62289	8.82524E-05	0.381689907	↑
Lactic acid	1.5837	0.012194176	1.492274774	↓
D-Arabitol	1.73317	0.013533302	0.701939284	↑
Malonic acid 1	1.73017	0.014893653	1.269326369	↓
Aspartic acid 2	1.96366	0.00180171	1.590560886	↓

**Versus* plasma of rats in the breast cancer group without taurine administration; arrows indicate a decrease or increase in metabolite level. VIP: Variable importance in projection.

and considered the pathways with a raw $p<0.05$ as differential metabolic pathways. As shown in Figure 6A, the five metabolites that differed between the control and breast cancer groups were involved in amino acid metabolism, including branched-chain amino acid, aspartic acid, arginine, and proline metabolism, and protein synthesis. The 23 metabolites that differed between the breast cancer and taurine intervention groups participated in metabolic pathways of the urea cycle, Krebs cycle, protein synthesis, aspartic acid metabolism, alanine metabolism, ammonia circulation, and the malic acid-aspartic acid shuttle (Figure 6B). KEGG metabolic pathway analysis showed significantly lower levels of the Krebs cycle metabolites pyruvic acid, fumaric acid, malic acid, α-ketoglutaric acid, and citric acid in the taurine intervention group compared with the breast cancer group (Figure 7A). The levels of threonine, valine, isoleucine, and pyruvic acid in biosynthesis pathways were also reduced in the taurine intervention group (Figure 7B), indicating that taurine diminished amino acid and energy metabolism in the rat model for breast cancer.

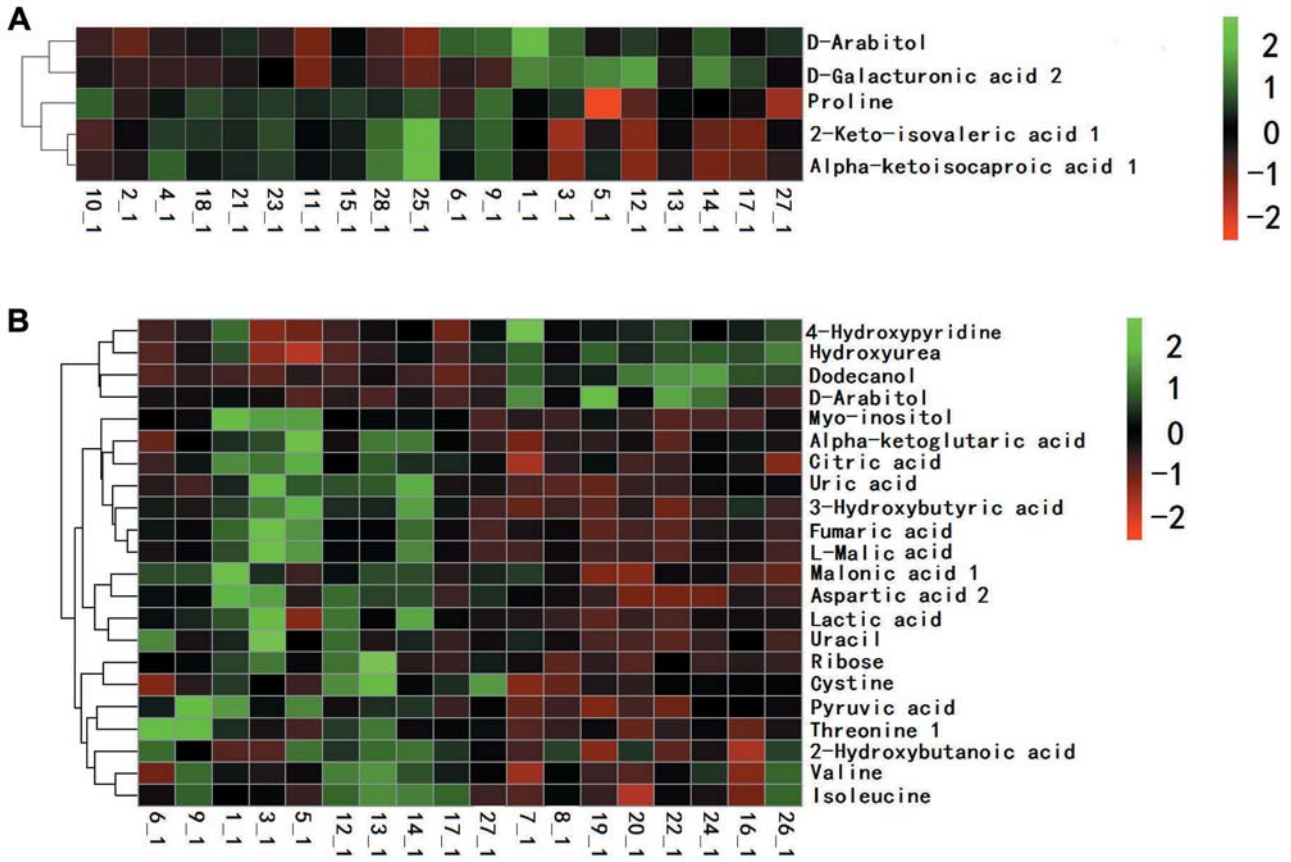

Figure 5. *Profiles of differential metabolite levels in plasma of the rats. Heat maps showing clustering analysis of the metabolites with different levels in the breast cancer group compared to the control group (A) and in the taurine intervention group compared to the breast cancer group (B). Metabolites were annotated based on the Kyoto Encyclopedia of Genes and Genomes and the National Institute of Standards and Technology database. The color scale from green to red indicates the relative metabolite levels from high to low.*

Discussion

In the present study, taurine had antitumor activity in a rat animal model of breast cancer when used as a nutritional additive in drinking water. Taurine not only delayed the onset and development of breast cancer but also reduced the incidence and average number of tumors in rats. These data are consistent with previous studies from other groups showing that taurine in drinking water or food inhibits breast cancer in rats (8, 9). Our investigation together with the studies from others suggests that taurine may have potential as an antitumor agent for breast cancer therapy. Although evidence shows that taurine induces apoptosis in human breast cancer cell lines and inhibits tumor growth in nude mice (10), the antitumor mechanism of taurine remains elusive.

Cancers are known to be metabolic diseases (15-17, 19, 21), and cancer cells consume more nutrients and energy in support of the rapid growth of tumors, resulting in metabolic changes in the body. Salient features of cancer cells are their high rates of glycolysis and glutamine metabolism, which allow malignant cells to meet their high energy and anabolic requirements. The production of lactic acid from glucose, even under non-hypoxic conditions, is commonly exhibited by tumor tissues; this process, known as the Warburg effect, has been recognized as a feature of cancer cells for more than 70 years. The Warburg effect offers several advantages to cancer cells, including a high rate of ATP production and the production of Krebs cycle intermediates such as citrate, which feed biosynthetic pathways and support the production of proteins and nucleic acids for cell proliferation (22, 23). Profiles of plasma amino acids differ significantly between patients with cancer and matched controls (24-32), suggesting that changes in plasma amino acids may represent tumor-induced protein metabolism in patients with cancer (33), and may be useful for cancer diagnosis (27).

In the present study, we used a metabolomics approach to assess metabolic alterations in DMBA-induced breast carcinogenesis in rats. Our experiments identified five metabolites involved in amino acid and glucose metabolism

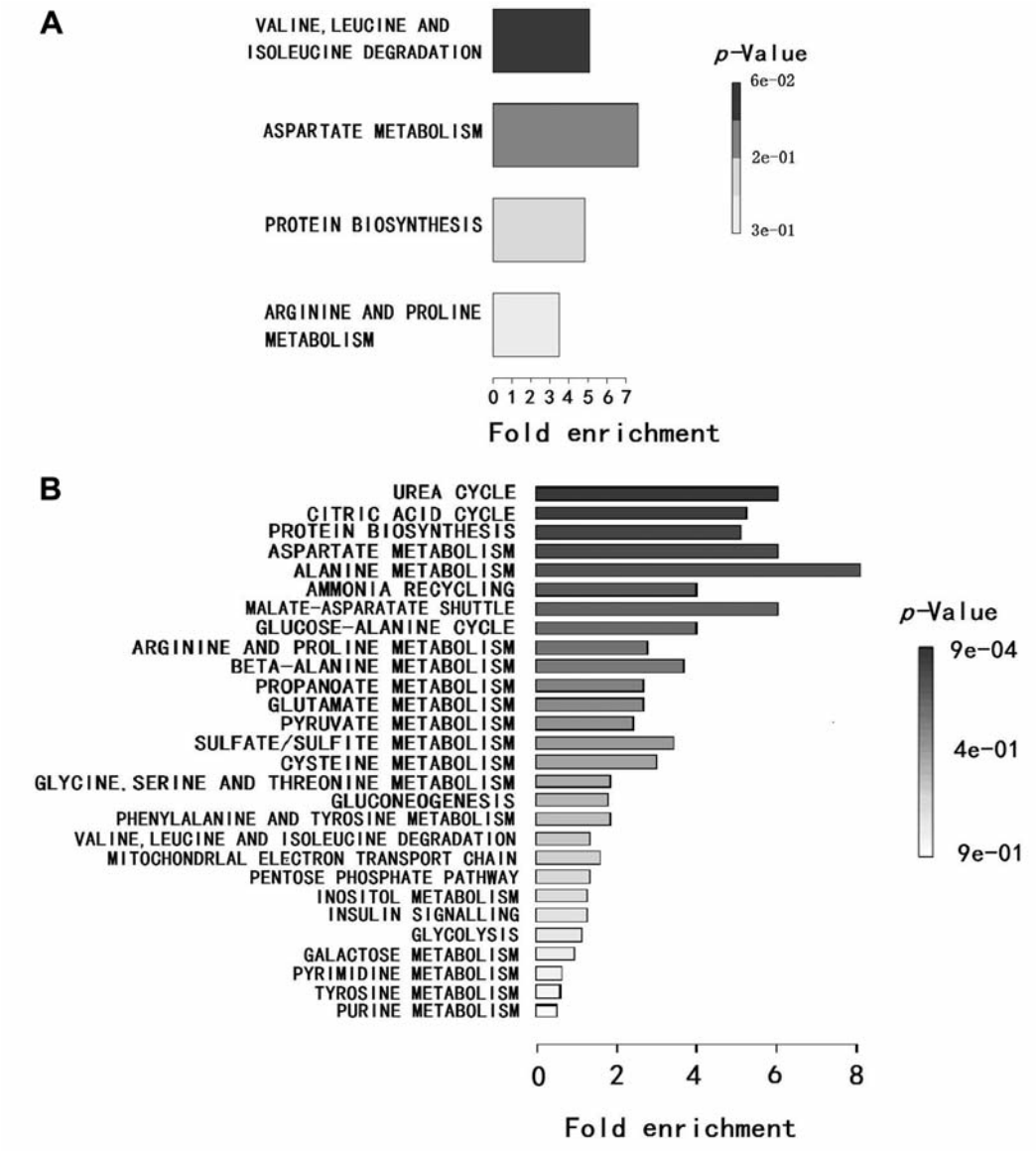

Figure 6. *Identification of the metabolic pathways for the differential metabolites using MetaboAnalyst 2.0 software. A: The metabolic pathways for the five metabolites that differed between the control and breast cancer groups. B: The metabolic pathways for the 23 metabolites that differed between the breast cancer and taurine intervention groups. The Krebs cycle is also known as the citric acid cycle.*

that were differentially expressed between the diseased and healthy samples. These differential metabolites represent potential diagnostic biomarkers for breast cancer. Of the five metabolites, proline, α-ketoisocaproic acid (involved in leucine metabolism), and 1,2-keto-isovaleric acid 1 (involved in valine metabolism) were present at higher levels in the breast cancer rats. Two metabolites related to pentose and glucuronate interconversions (D-arabitol and D-galacturonic acid 2) occurred at lower levels in the rats with breast cancer. According to the metabolite set enrichment analysis, the differential metabolites are involved in several major metabolic pathways, including branched-chain amino acid metabolism, aspartate metabolism, protein biosynthesis, and arginine and proline metabolism. In a previous breast cancer study, serum amino acid levels varied with intrinsic tumor subtype and were influenced by the pro-inflammatory state (34). In particular, alanine, arginine, aspartate, and cysteine have been shown to have proliferative effects in breast cancer cells (35). However, the role and mechanism of amino acid metabolism in tumor proliferation and progression in our rat breast cancer model remains to be determined.

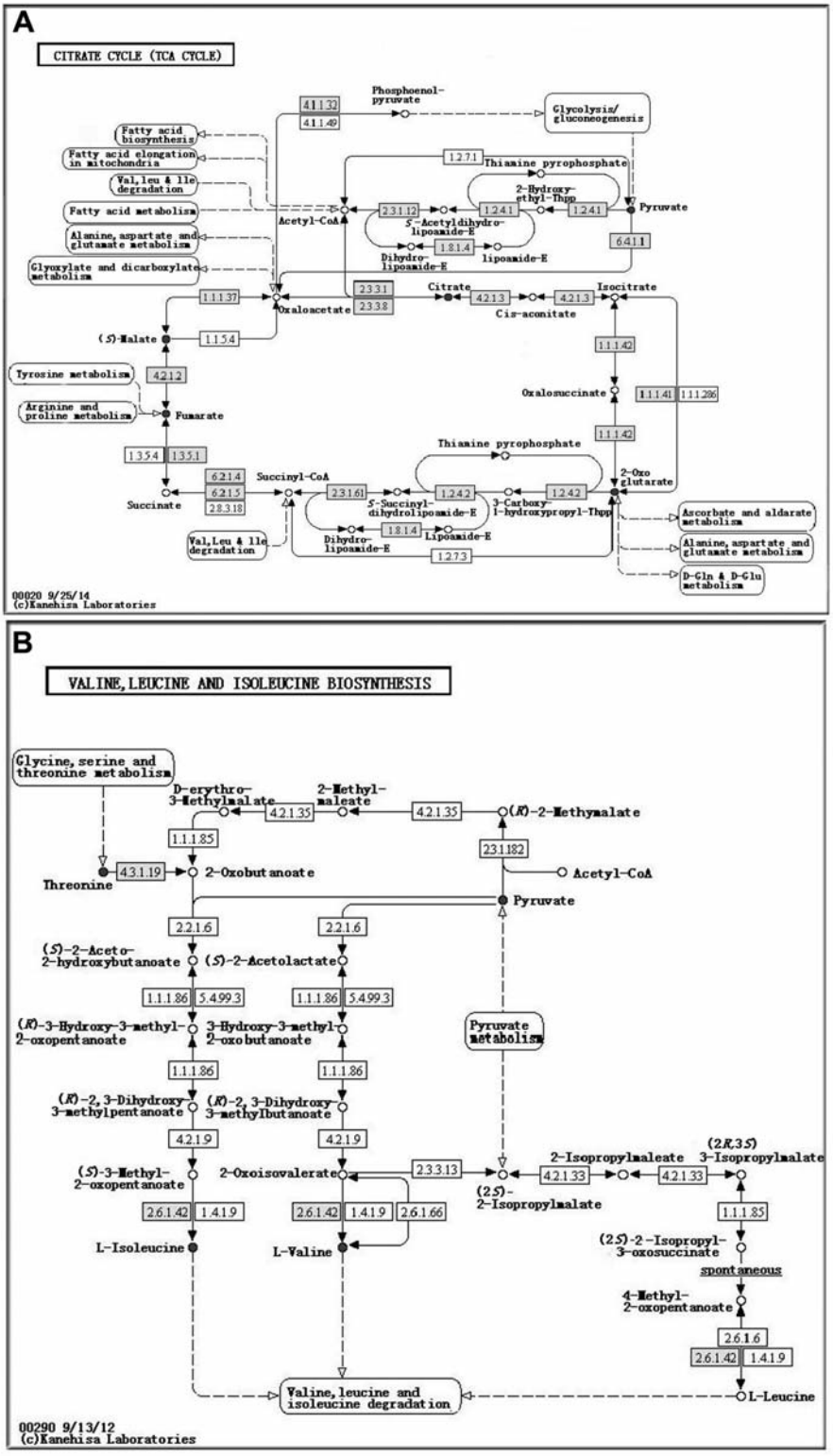

Figure 7. *Kyoto Encyclopedia of Genes and Genomes metabolic pathway analyses for the breast cancer and taurine intervention groups. A: The Krebs cycle is compared between the two groups of rats; the levels of the Krebs cycle metabolites pyruvic acid, fumaric acid, malic acid, α-ketoglutaric acid, and citric acid were significantly lower in the taurine intervention group than in the breast cancer group. The Krebs cycle is also known as the citric acid cycle or the tricarboxylic acid (TCA) cycle. B: The valine, leucine, and isoleucine biosynthesis pathways compared between the two groups of rats; the levels of threonine, valine, isoleucine, and pyruvic acid were reduced in the taurine intervention group compared with the breast cancer group.*

Taurine, the most abundant free amino acid in the body, plays an important role in nutrient metabolism. It is a substrate for the formation of bile salts and facilitates an increase in low-density lipoprotein receptors, thus reducing blood plasma cholesterol levels. Other studies have indicated that taurine attenuates insulin resistance and helps regulate glucose metabolism in animal models of diabetes (36-39). In the current study, taurine inhibited breast tumor growth by altering metabolic processes in the rat model. Taurine caused several metabolic changes, including reduced glycolysis and diminished energy, amino acid, and nucleic acid metabolism. Enrichment analysis revealed that the main metabolic pathways involved were protein biosynthesis, the urea cycle, the Krebs cycle, ammonia recycling, and alanine metabolism. The plasma concentrations of fumarate, malate, citrate, α-ketoglutarate, and pyruvate were significantly lower in the taurine-intervention group than in the breast cancer group. These metabolites participate in energy generation through the Krebs cycle, suggesting that the inhibitory effect of taurine on breast tumor growth may be mediated *via* the down-regulation of energy metabolism. Recent studies have observed that several other compounds also inhibit tumor cells through reducing energy metabolism, which suggests that blocking or reducing energy metabolism may be a novel strategy for therapeutic intervention in cancer (40, 41).

The role of metabolic alterations and adaptations in tumorigenicity is increasingly recognized. Some metabolic alterations are now considered a hallmark of cancer (4). Our metabolomic analysis revealed changes in four major metabolisms in the taurine intervention group. Valine, isoleucine, and aspartic acid are converted into propionyl-coenzyme A and oxaloacetic acid, which are Krebs cycle intermediates. In this study, the plasma levels of valine, isoleucine, and aspartic acid were lower in the taurine intervention group, implying that taurine suppressed amino acid metabolism as well as the Krebs cycle *in vivo*. The levels of some metabolites associated with nucleic acid metabolism were also decreased in the taurine-administered rats, suggesting that taurine suppresses nucleic acid metabolism and proliferation of rat breast cancer cells. This is consistent with the lower plasma levels of uric acid, ribose, and uracil in taurine-administered rats compared to matched controls. Lastly, it is well known that cancer cells have unusually high rates of glucose uptake and lactate production compared with rates in normal tissues of origin, even in the presence of oxygen (2). The present results demonstrated that the levels of pyruvate and lactate, which are intermediates in glycolysis, were significantly diminished in the taurine intervention group, indicating improved aerobic glycolysis following taurine administration. This observation suggests that the antitumor activity of taurine in rat breast cancer is attributable, at least in part, to its regulation and inhibition of glycolysis.

Conclusion

In this study, we found changes in amino acid and glucose metabolism in DMBA-induced rat breast cancer. Taurine had an inhibitory effect on DMBA-induced breast carcinogenesis in rats. Metabolomic data analysis further revealed alterations in multiple metabolic activities, including amino acid, glucose, and nucleic acid metabolism, in taurine-administered rats. These results suggest that the antitumor effect of taurine in rat breast cancer is mediated through modulation of these metabolic activities as well as aerobic glycolysis, leading to decreased energy metabolism. Our findings may be helpful for developing new therapeutic approaches targeted at modulating relevant metabolic pathways or at inhibiting glycolysis and amino acid biosynthesis in tumors. Further studies are needed to investigate the anticancer activity of taurine *via* the modulation of signaling pathways (*e.g.* phosphatidylinositol 3'-kinase (PI3K)-AKT-mTOR pathway) or the inhibition of enzymes in metabolic pathways influencing carcinogenicity.

Conflicts of Interest

The Authors declare that they have no conflict of interest.

Ethical Approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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