

Metabolic differentiation and classification of abnormal Savda Munziq's pharmacodynamic role on rat models with different diseases by nuclear magnetic resonance-based metabonomics

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ABSTRACT

Background: Abnormal Savda Munziq (ASMq) is a traditional Uyghur herbal preparation used as a therapy for abnormal Savda-related diseases. In this study, we investigate ASMq's dynamic effects on abnormal Savda rat models under different disease conditions. **Materials and Methods:** Abnormal Savda rat models with hepatocellular carcinoma (HCC), type 2 diabetes mellitus (T2DM), and asthma dosed of ASMq. Serum samples of each animal tested by nuclear magnetic resonance spectroscopy and analyzed by orthogonal projection to latent structure with discriminant analysis. **Results:** Compared with healthy controls, HCC rats had higher concentrations of amino acids, fat-related metabolites, lactate, myoinositol, and citrate, but lower concentrations of α -glucose, β -glucose, and glutamine. Following ASMq treatment, the serum acetone very low-density lipoprotein (VLDL), LDL, unsaturated lipids, acetylcysteine, and pyruvate concentration decreased, but α -glucose, β -glucose, and glutamine concentration increased ($P < 0.05$). T2DM rats had higher concentrations of α - and β -glucose, but lower concentrations of isoleucine, leucine, valine, glutamine, glycoprotein, lactate, tyrosine, creatine, alanine, carnitine, and phenylalanine. After ASMq treated T2DM groups showed reduced α - and β -glucose and increased creatine levels ($P < 0.05$). Asthma rats had higher acetate, carnitine, formate, and phenylalanine levels, but lower concentrations of glutamine, glycoprotein, lactate, VLDL, LDL, and unsaturated lipids. ASMq treatment showed increased glutamine and reduced carnitine, glycoprotein, formate, and phenylalanine levels ($P < 0.05$). **Conclusion:** Low immune function, decreased oxidative defense, liver function abnormalities, amino acid deficiencies, and energy metabolism disorders are common characteristics of abnormal Savda-related diseases. ASMq may improve the abnormal metabolism and immune function of rat models with different diseases combined abnormal Savda.

Key words: Abnormal Savda, abnormal Savda Munziq, metabonomics, rat model, traditional Uyghur medicine

INTRODUCTION

Influenced by the Humoral Theory of Hippocrates (c. 460-370 B.C.E.) and Galen (c. 129-216 C.E.), traditional Uyghur medicine (TUM) has distinctive theoretical foundations and cultural background. With a history spanning over 2500 years, TUM has developed a unique system for the diagnosis and treatment of illnesses.

Body fluid (Hilit) theory is a basic TUM principle. It is built around the idea that the human body is filled with four kinds of body fluids, Savda (black bile), Belghem (phlegm), Sapra (yellow bile), and Kan (blood), that are associated with the fundamental elements earth, fire, water, and air, respectively.^[1,2] Hilit is the basic matter that drives physiological activity. It is produced in the liver using various nourishment materials and circulates continuously in the body. There is a balance, inter-restriction, and complementary system between the different types of body fluids. When a body suffers from an imbalance of one fluid, then their personality and physical health is affected and the imbalance manifests as "abnormal body fluid" syndromes. Depending on the degree of imbalance and

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the disease symptoms “abnormal body fluid” is divided into abnormal Savda, abnormal Belghem, abnormal Sapra, and abnormal Kan. Abnormal Savda syndrome is a special syndrome associated with complex and intractable diseases such as a tumor, type 2 diabetes mellitus (T2DM), and asthma.^[1-3] Clinical data have shown that 72% of cancer patients, 54.2% of T2DM, and 46.5% of asthma patients show signs of abnormal Savda.^[4]

According to the “different diseases homology” pathological development theory and the “different diseases in the same treatment” therapeutic principle of Uyghur medicine, abnormal Savda Munziq (ASMq) can be used to treat various diseases such as cancer, T2DM, and asthma caused by abnormal Savda.^[5,6] ASMq has long been used by Uyghur for treatment and protection from abnormal Savda. It is composed of ten medicinal herbs, *Adiantum capillus-veneris* L., *Albati pseudalbagi* (Bieb.) Desv., *Anchusa italica* Retz., *Cordia dichotoma* G. Forst., *Euphorbia maculata* L., *Foeniculum vulgare* Mill., *Glycyrrhiza glabra* L., *Lavandula angustifolia* Mill., *Melissa officinalis* L., and *Ziziphus jujuba* Mill.^[7] ASMq therapy of abnormal Savda-related diseases results in temperament recovery and body fluid equilibrium. For years, ASMq’s pharmacological and clinical effects have been investigated, and these studies have demonstrated that it scavenges free radicals and protects DNA,^[8] decreases oxidative stress,^[9] protects against hydroxide radical-induced oxidative damage,^[10] inhibits cancer cell proliferation,^[11,12] and affects the immune system.^[13]

Blood contains significant information about physiological and/or pathological changes in metabolite content. These metabolite pattern differences can provide insight into underlying molecular mechanisms. We hypothesized that the occurrence and development of abnormal Savda results from metabolic abnormality; therefore, metabolomics was used to explain “different body fluid” theory concepts. Metabolomics is a method used to study dynamic endogenous metabolite changes in bio-systems. It combines chemical instrumental analysis techniques such as nuclear magnetic resonance (NMR), high-performance liquid chromatography/mass spectrometry, and gas chromatography with chemometric methods to detect pathophysiological stimuli or genetic modification.^[14,15] NMR is an efficient, reproducible, noninvasive, and nondestructive technique that can provide complete structural analysis of a wide range of organic molecules in complex mixtures.^[16] It is widely used in metabolomic studies to acquire metabolic profiles of biological specimens and disease biomarker profiles for diagnostic and prognostic purposes without extensive sample preparation.^[17,18] NMR-based metabolomics is also used to identify possible biomarkers of different diseases including

cancer,^[19-22] hypertension,^[23] schistosomiasis,^[24] stress,^[25] and aging.^[26]

In the present study, we applied ¹H NMR spectroscopy with multivariate data analysis to investigate metabolomic changes in abnormal Savda rat models with hepatocellular carcinoma (HCC), diabetes (T2DM), and asthma following oral ASMq treatment. The primary goal was to determine the dynamic metabolomic responses following ASMq treatment in these models and to identify the metabolic signatures that may indicate ASMq’s drug mechanisms. These results may provide valuable information for identifying common characteristics shared by different abnormal Savda disease conditions and insight into the abnormal Savda pathophysiology described in TUM.

MATERIALS AND METHODS

Reagents

Streptozotocin (STZ), ovalbumin (OVA), diethylnitrosamine (DEN), and deuterium oxide (D₂O) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Aluminum hydroxide (Al(OH)₃) was purchased from Tianjin Yongsheng Fine Chemical Co., Ltd. K₂HPO₄·2H₂O and NaH₂PO₄·12H₂O were purchased from Guoyao Chemical Co., Ltd. (Shanghai, China). ASMq was prepared according to the patented preparation method (Patent No. C082130082.8).

Abnormal Savda syndrome rat model

Healthy, specific pathogen free, adult male Sprague–Dawley rats (weight 150 ± 30 g) were provided by the experimental animal center of Xinjiang Medical University, China. All animal procedures were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals as well as the guidelines of the Animal Welfare Act. The animals were housed in metabolic cages with free access to food and water at 25°C. After 1-week acclimatization, the animals were randomly divided into a normal control group and the abnormal Savda syndrome groups. Abnormal Savda was induced as described previously.^[27,28] Briefly, abnormal Savda syndrome groups were exposed to a cool, dry environment (temperature 6 ± 1°C, relative humidity 25–32.8%, 6 h/day in the first week, 8 h/day in the second week, and 10 h/day in the third week), stimulated with chronic, and intermittent electric foot shocks (30 min/day, with 20 V in the first week, 35 min/day with 25 V in the second week, and 40 min/day, with 30 V in the third week), and fed a dry, cold diet (1:1 ratio of barley and coriander combined with normal rat food in a 3:7 ratio and granulated). The abnormal Savda syndrome rat model was further divided into three groups to establish HCC, T2DM, and asthma along with the abnormal Savda syndrome.

Hepatocellular carcinoma with abnormal Savda syndrome model procedure and drug administration

Beginning in the fourth week of abnormal Savda induction, the rats' water was supplemented with 0.1 mg/ml DEN in addition to abnormal Savda stimulation for 11 weeks. The rats were further subdivided into a control HCC with abnormal Savda group, and ASMq low dose, medium dose, and high dose treatment groups ($n = 8/\text{group}$). The HCC with abnormal Savda control group was orally administered physiological saline 2 ml/day. The ASMq low-dose treatment group was orally administered 1.5 g ASMq/kg body weight; the medium dose treatment group, 3 g ASMq/kg; and the high dose treatment group, 6 g ASMq/kg for an additional 3 weeks. Following treatment, the blood sample was collected. The serum was separated by centrifugation at 3500 rpm/min for 5 min and immediately stored at -80°C until used for NMR spectroscopy.

Type 2 diabetes mellitus with abnormal Savda syndrome model procedure and drug administration

Beginning in the fourth week of abnormal Savda induction, the rats were orally administered a 1 ml/100 g fat emulsion every day in addition to abnormal Savda stimulation for 8 weeks. In the thirteenth week 25 mg STZ/kg body weight was intraperitoneally injected 3 times to establish T2DM with Abnormal Savda syndrome. The rats were further subdivided into a T2DM with Abnormal Savda control group and the ASMq low-, medium-, and high- dose treatment groups ($n = 8/\text{group}$). The T2DM with abnormal Savda control group was orally administered physiological saline 2 ml/day. The ASMq low-dose treatment group was orally administered 1.5 g ASMq/kg body weight; medium dose treatment group, 3 g ASMq/kg; and high dose treatment group, 6 g ASMq/kg for an additional 3 weeks. In the 16th week, blood samples were collected and the serum samples were prepared and stored as described above.

Asthma with abnormal Savda syndrome model procedure and drug administration

Beginning in the fourth week of abnormal Savda induction, the rats were immunized with a 1 ml suspension containing 10 mg OVA and 100 mg $\text{Al}(\text{OH})_3$ injected intraperitoneally in addition to abnormal Savda stimulation. Two weeks following immunization, rats were challenged using 10% OVA in a phosphate-buffered saline aerosol, 20 min/day, for 1-week to establish the asthma with abnormal Savda syndrome rat model. The rats were subdivided into the asthma with abnormal Savda control group and the ASMq low-, medium-, and high-dose treatment groups ($n = 8/\text{group}$). The asthma with abnormal Savda control group was orally administered physiological saline 2 ml/day. ASMq low-dose treatment were orally administered 1.5 g ASMq/kg body weight; medium-dose

treatment group, 3 g ASMq/kg; and high-dose treatment group, 6 g ASMq/kg for an additional 3 weeks. Following treatment blood samples were collected, prepared, and stored as described above.

^1H nuclear magnetic resonance spectroscopy of serum

For NMR analysis, the samples were thawed at room temperature. Serum samples were prepared by mixing 200 μL serum with 400 μL saline buffer solution (45 mM NaH_2PO_4 , 45 mM K_2HPO_4 , 0.9% saline in a 20% v/v D_2O , 80% v/v H_2O solvent, pH 7.4). The serum-buffer mixture was kept at room temperature for 10 min then centrifuged at 10,000 rpm/min for 10 min. The clarified supernatant (550 μL) was transferred into a 5 mm NMR tube for spectroscopic analysis. The serum ^1H NMR spectrum of each sample was recorded using the Carr-Purcell-Meiboom-Gill pulse sequence ($\text{RD} - 90^{\circ} - [\tau - 180^{\circ} - \tau]_n - \text{AQ}$) on a Varian Unity Inova600 NMR spectrometer at a proton frequency of 599.95 MHz. For each sample, 128 scans were collected with 32,768 data points over a 10,000 Hz spectral width, resulting in a 1.64 s acquisition time and a 2 s relaxation delay at 298 K. The water signal was suppressed using a presaturation sequence on the water-signal frequency during the relaxation delay. For assignment purposes, selected samples were subjected to two-dimensional NMR, including ^1H - ^1H homonuclear correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), and J-resolved spectroscopy (J-Res).

Data analysis

Prior to Fourier transformation, 0.3 Hz line broadening was applied to the free induction decays. The Fourier-transformed ^1H NMR spectra were manually phased and baseline corrected. Chemical shifts were referenced to the α -glucose anomeric proton signal at a chemical shift (δ) of 5.233 ppm. NMR spectra over the δ 9.0–0.5 ppm range were segmented into 0.003 ppm integral regions, and all regions were normalized by the total integrated area of each spectrum. Due to the high variability in water intensity, the δ_{H} 5.22–4.68 ppm region was excluded from analysis. Pattern recognition analysis was carried out on the normalized NMR data sets using the SIMCA-P⁺ software (Version 11.0, Umetrics Inc., Umea, Sweden). We used a supervised multivariate data analysis tool called the orthogonal projection to latent structure for discriminant analysis (OPLS-DA) with unit variance scaling.^[29,30] Coefficient plots were back calculated from the coefficients, incorporating the weight of the variables contributing to the sample model classification.

The OPLS-DA model is an extension of the partial least-square regression method featuring an integrated orthogonal signal correction filter. It is constructed with

the NMR data as the X matrix and the class information identifier for the different groups as the Y variable using one partial-least-square and one orthogonal component. In this study, the OPLS-DA model quality was described using the parameters R^2X and Q^2 . R^2X represented the total explained variation for the X matrix, and Q^2 indicated the model predictability related to its statistical validity.

The OPLS-DA coefficient plot was generated with MATLAB (version 7.0) scripts. The coefficient plot showed that the variables contributed to classification and indicated the significance of this contribution. The coefficient plots were color-coded by the absolute value of the correlation coefficients. The direction of the signals in the coefficient plot indicated the changes in relative metabolite concentration in the class of interest with respect to the other model classes. The signal color in the coefficient plot represents the metabolite significance in the two groups; red indicates a more significant contribution to the class separation than blue.

RESULTS AND DISCUSSION

A representative serum sample 1H NMR spectrum is shown in Figure 1. Fourier-transformed 1H NMR spectra were analyzed using OPLS-DA and the results are shown in the score plots and coefficient plots in Figure 2. We identified a total of 23 distinguishable metabolites that were modified in the different abnormal Savda-coupled disease conditions and showed dynamic metabonomic responses to ASMq treatment. The metabolites are summarized in Table 1.

Metabolic changes in different disease conditions with abnormal Savda

Several metabolite differences were frequently observed in the serum spectra from HCC, T2DM, and asthma with abnormal Savda groups compared with healthy controls. The score plots from the abnormal Savda groups and the healthy controls are completely distinct, demonstrating

that serum metabolite concentrations between the two groups have significant differences. Figure 2 shows the OPLS-DA score and coefficient plots for the serum of HCC, T2DM, and asthma with abnormal Savda compared to healthy controls.

The R^2X and Q^2 values for the OPLS-DA models are 0.53 and 0.91 for HCC, 0.53 and 0.89 for T2DM, and 0.47 and 0.89 for asthma, respectively, indicating high model reliability. Based on the number of samples in each group ($n = 8$), we used a Pearson's product-moment correlation coefficient (r) of 0.632 as the cut-off value with a statistical significance of $P < 0.05$ and obtained a list of metabolites that clearly differed between the HCC, T2DM, and asthma with abnormal Savda groups and healthy controls. The OPLS-DA model provides correlation coefficients with either positive or negative value that is, representative of a relatively lower or higher serum metabolite concentration. A negative value indicates a relatively increased metabolite concentration in the HCC, T2DM, and asthma with abnormal Savda serum and a positive value indicates a relatively decreased metabolite concentration. Based on their correlation coefficients and the COSY, TOCSY, and J-Res spectra results, we determined the identities of the various differential metabolites [Table 1].

Our preliminary data indicated that compared with healthy controls, HCC with abnormal Savda had higher concentrations of amino acids including isoleucine, leucine, valine, acetylcysteine, tyrosine, alanine, and phenylalanine, the fat-related metabolites very low density lipoprotein (VLDL), LDL, unsaturated lipids, acetone, pyruvate, formate, and acetate, as well as lactate, myoinositol, and citrate. However, HCC rats had lower concentrations of α -glucose, β -glucose, and glutamine. T2DM with abnormal Savda had higher concentrations of α - and β -glucose, but had lower concentrations of isoleucine, leucine, valine, glutamine, glycoprotein, lactate, tyrosine, creatine, alanine, carnitine, and phenylalanine. Asthma with abnormal Savda had increased acetate, carnitine, formate, and phenylalanine concentrations, but decreased glutamine, glycoprotein, lactate, VLDL, LDL, and unsaturated lipids.

Metabolic changes in different disease conditions with abnormal Savda following oral abnormal Savda Munziq treatment

Data analysis showed that, compared to the untreated HCC with abnormal Savda syndrome group, the ASMq low dose, medium dose, and high dose treatment groups showed reduced acetylcysteine and pyruvate concentrations and increased α - and β -glucose concentrations ($P < 0.05$). The ASMq medium and high dose treatment groups showed

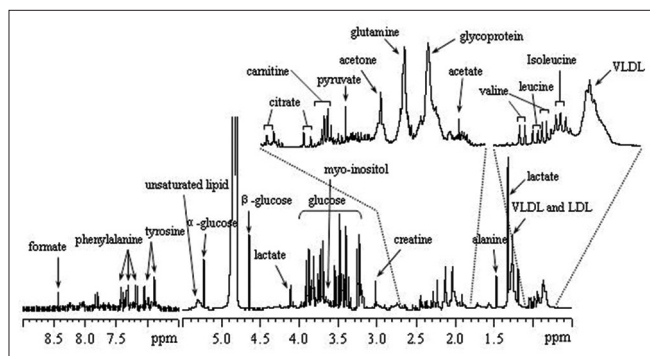


Figure 1: A typical rat serum sample 1H -nuclear magnetic resonance spectrum. The δ 9.0–6.5 ppm region was expanded 16 times and compared with the δ 5.5–0.5 ppm region

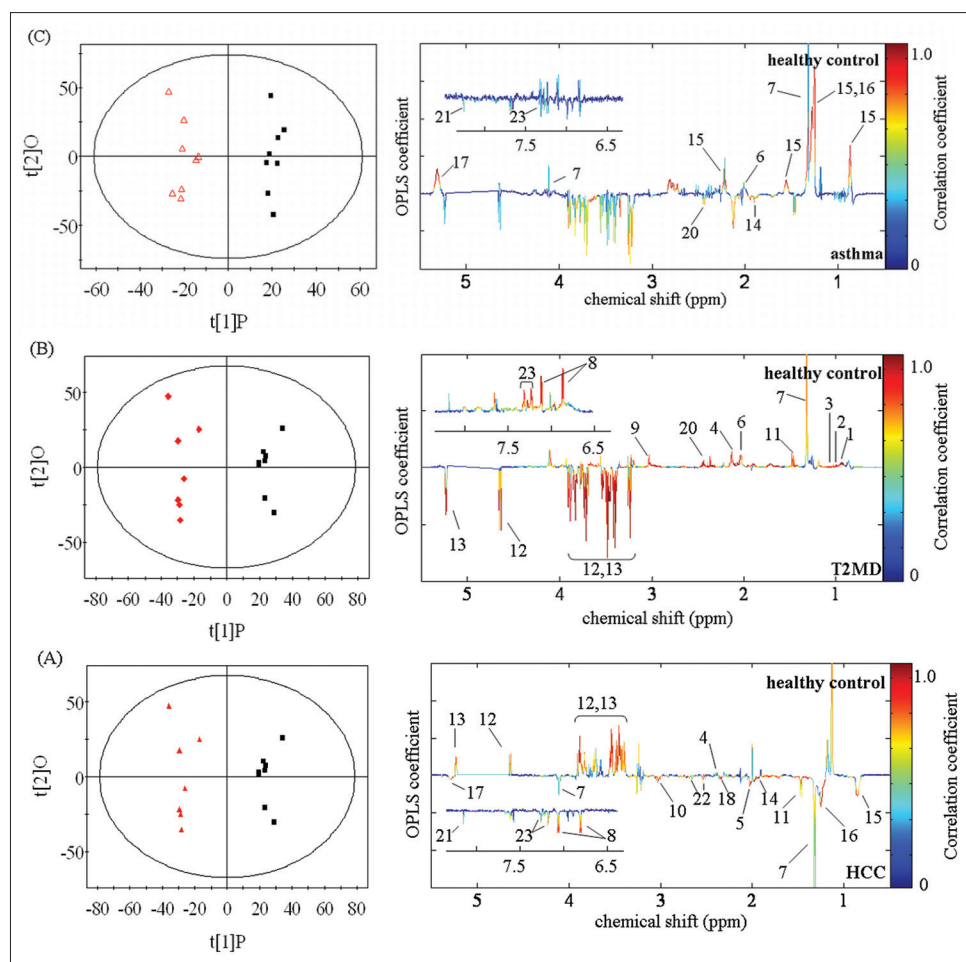


Figure 2: Orthogonal projection to latent structure for discriminant analysis score plots (left) and coefficient plots (right) of serum metabolites. (a) Hepatocellular carcinoma (HCC), (b) type 2 diabetes mellitus (T2DM), and (c) asthma with abnormal Savda compared with healthy controls. The color scale shows the significance of metabolite variation between the two classes: ■: Healthy controls, ▲: HCC with abnormal Savda rat model, ◆: T2DM with abnormal Savda rat model, and △: Asthma with abnormal Savda rat model. Peak labels are as follows: (1) Isoleucine, (2) leucine, (3) valine, (4) glutamine, (5) acetylcysteine, (6) glycoprotein, (7) lactate, (8) tyrosine, (9) creatine, (10) myoinositol, (11) alanine, (12) β-glucose, (13) α-glucose, (14) acetate, (15) very low density lipoprotein (LDL) lipid, (16) LDL lipid, (17) unsaturated lipid, (18) pyruvate, (19) acetone, (20) carnitine, (21) formate, (22) citrate, (23) phenylalanine

reduced acetone and lipid concentrations, including VLDL, LDL, and unsaturated lipids, and increased glutamine levels ($P < 0.05$). Compared to the untreated T2DM with abnormal Savda control group, the ASMq low dose, medium dose, and high dose treatment groups showed reduced α- and β-glucose concentrations ($P < 0.05$). The medium and high dose treatment groups also showed an increased creatine concentration ($P < 0.05$). Compared to the untreated asthma with abnormal Savda control group, the ASMq low dose, medium dose, and high dose treatment groups showed increased glutamine and decreased carnitine ($P < 0.05$). The ASMq medium and high dose treatment groups showed reduced formate and phenylalanine concentrations and increased glycoprotein ($P < 0.05$). The results are listed in Table 1.

In TUM theory, Savda is described as a black-colored and sour-tasting liquid secreted from the liver and stored in

the spleen. It plays several important roles in the human body, such as maintaining organ characteristics as well as storing and transporting various nutrients. It is related to memory, thought, sensibility, and also has a certain effect on digestive function.^[4,31] Abnormal Savda change is a hotbed for diseases. Our previous studies indicated that amino acid deficiency and energy metabolism disorders might be related to the generation of abnormal Savda.^[32] In this work, NMR profiling suggested that several metabolite concentrations changed in the serum of different diseases with abnormal Savda rat models. The metabolite variations allowed us to obtain important metabolic information about the mechanisms involved in abnormal Savda evolution.

ASMq had significant effects on the different disease condition rat models. These findings are consistent with existing research on ASMq. Typically diseases are

Table 1: Potential ASMq drug mechanism biomarkers from rat models of different disease conditions with abnormal Savda

Metabolites	Healthy/ HCC	Healthy/ T2DM	Healthy/ asthma	ASMq high dose			ASMq medium dose			ASMq low dose		
				HCC	T2DM	Asthma	HCC	T2DM	Asthma	HCC	T2DM	Asthma
Isoleucine	-0.85	0.98										
Leucine	-0.78	0.98										
Valine	-0.74	0.92										
Glutamine	0.68	0.98	0.90	0.77		0.86	0.82		0.77			0.74
Acetylcysteine	-0.71			-0.68			-0.76			-0.83		
Glycoprotein		0.91	0.65			0.72			0.71			
Lactate	-0.88	0.9	0.66									
Tyrosine	-0.92	0.94										
Creatine		0.77			0.72			0.83				
Myoinositol	-0.90											
Alanine	-0.88	0.89										
β-glucose	0.89	-0.97		0.78	-0.77		0.84	-0.81		0.80	-0.71	
α-glucose	0.88	-0.96		0.81	-0.74		0.82	-0.71		0.81	-0.86	
Acetate	-0.91		-0.84									
Lipid (VLDL)	-0.92		0.95	-0.71			-0.92					
Lipid (LDL)	-0.72		0.93	-0.70			-0.74					
Unsaturated lipid	-0.88		0.97	-0.70			-0.88					
Pyruvate	-0.77			-0.68			-0.70			-0.81		
Acetone	-0.76			-0.70			-0.71					
Carnitine		0.93	-0.94			-0.75			-0.66			-0.77
Formate	-0.71		-0.84			-0.68			-0.72			
Citrate	-0.96											
Phenylalanine	-0.84	0.88	-0.73			-0.75			-0.77			

ASMq: Abnormal Savda Munziq; HCC: Hepatocellular carcinoma; T2DM: Type 2 diabetes mellitus

considered to be restricted to a certain organ; however, abnormal Savda is believed to be the state of the patient and an indication that the whole body has shifted to a pathological state. Therefore, the blood of abnormal Savda patients contains significant information concerning pathogenic changes based on the metabolite content, which reflects abnormalities in multiple organ and tissue functions.

We observed that the serum glutamine concentration decreased significantly in the HCC, T2DM, and asthma with abnormal Savda rat models. In TUM disease homology theory, similar metabolic changes between different diseases indicate a similar pathogenic process. Glutamine is related to cell membrane protection and immune function. The immune system is the body's main anti-disease defense system and glutamine maintains immune system function and along with glycoproteins, increases the total lymphocyte count.^[33] Glutamine also protects cells, tissues, and organs from free radical damage through glutathione stores.^[34] Decreased serum glutamine levels indicate decreased cell antioxidant activity that may lead to intensified oxidative damage. Previous studies have demonstrated that oxidase enzymatic activity decreased and lipid peroxidation damage was exacerbated with abnormal Savda.^[35] This was a common characteristic in the HCC,

T2DM, and asthma with abnormal Savda rat models. In this study, ASMq treatment was evaluated in rat models of different disease conditions with abnormal Savda. Results showed that the serum glutamine concentration increased in the medium and high dose ASMq-treated HCC rat model and all three ASMq treatment groups of the asthma rat model, but did not change in the T2DM rat model. One of ASMq's therapeutic mechanisms may relate to immunomodulatory effects that could have the potential clinical significance. This may be the result of the antioxidant effects of ASMq, leading to an enhanced defense against stress, free radical scavenging, and antioxidant enzyme activity.

The T2DM with abnormal Savda rat model had relatively low serum glycoprotein and creatine levels. Similarly, the asthma rat model had decreased serum glycoprotein. However, the HCC rat model had no significant change in glycoprotein or creatine concentration. Creatine is an important energy storage and utilization compound. Low creatine levels are related to dysfunctional energy metabolism.^[36] Following medium and high dose ASMq treatment, the serum creatine level increased in the T2DM with abnormal Savda rat model. These results indicate that ASMq can improve energy storage in the T2DM model. High and medium dose ASMq treatment increased the

serum glycoprotein in the asthma with abnormal Savda model. Glycoprotein primarily protects the epithelial cell integrity and enhances immune function.^[37] Increased glycoprotein levels may relate to immune modulation in the asthma model.

Serum α - and β -glucose concentrations decreased in the HCC group, increased in T2DM group, and showed no change in the asthma model. The energy needed to survive requires adenosine triphosphate, which primarily originates from glucose oxidation through glycolysis. The T2DM rat model lacks or cannot effectively use insulin, leading to impaired glucose uptake and utilization.^[38] The observed serum glucose decrease in the HCC model may indicate perturbation of liver glucose metabolism. Compared with the various untreated abnormal Savda rat model groups, the ASMq treatment had significant effects on glucose metabolism. Following oral ASMq treatment, glucose concentration increased in the HCC model and decreased in T2DM model. Similarly, the serum citrate and pyruvate levels in the HCC model increased. Citrate is a three acid cycle (TCA) cycle intermediate and is essential for energy metabolism. Pyruvate is an important glycolysis product that can be converted into acetyl-CoA prior to entering the TCA cycle. The alterations in these two metabolites may be the product of muscle proteolysis or liver parenchyma necrosis.^[39]

Lactate is a glycolysis product and its increase indicates that glycolysis is directed toward glycolytic intermediate production. Serum lactate increased in the HCC rat model, but decreased in the T2DM and asthma models. While ASMq treatment did affect glucose levels, it had no effect on serum lactate concentration. These alterations may reflect an altered or deregulated energy metabolism to compensate for the disease's energy consumption. Previous studies found that lactate and alanine, the precursors of gluconeogenesis, were found at lower concentrations in patients with T2DM, breast cancer, and cervical carcinoma, indicating altered energy metabolism in these diseases.^[32,40] This also confirmed that tumors rely on glycolysis as their main energy source even in the presence of oxygen.^[41,42] Our findings are consistent with these previous studies. Our results suggest that the HCC with abnormal Savda may suppress the TCA cycle, leading to an energy metabolism disorder. When rats were subjected to repetitive, acute anaerobic conditions, serum lactate first increased, and then decreased.^[43] During the early stages of anaerobic exposure, glucose aerobic oxidation was inhibited. To compensate, glycolysis was enhanced, producing large amounts of lactate. However, hypoxia enhances the pentose phosphate pathway, reducing serum lactate levels.^[43] Thus, internal lactate concentration decreased in later stages of anaerobic conditioning. Therefore, the low serum lactate levels in

the T2DM model may also be associated with glucose metabolism disorders.

In addition to glycolysis, approximately 50% of the energy consumed by a respiratory muscle comes from lipid metabolism.^[44] The serum concentration of lipid-related metabolites including VLDL, LDL, unsaturated lipid, pyruvate, acetone, acetate, and formate were significantly different between the different diseases. Following ASMq treatment, the serum lipid-related metabolite levels decreased in the medium and high dose treatment groups of the HCC rat model, but showed no effect on the other diseases. Lipid concentration increases indicated reinforced fat mobilization in the HCC rat model. Lipid peroxidation is a process that leads to cell damage. Fat oxidation produces massive quantities of free radicals, causing a toxic accumulation in the cell. Previous studies indicate that ASMq can scavenge free radicals, prevent hydroxyl radical-induced oxidative DNA damage, and mitochondrial oxidative damage by increasing superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) levels.^[5] Both SOD and GSH-PX can be used to evaluate the oxidation resistance levels.^[45] GSH-PX is an important anti-oxidase that can convert lipid peroxide back to its original fatty acid alcohol state, eliminating the free radical. Coupled with SOD, these enzymes work together to form an oxidation defense system.^[46] Our results indicate that ASMq might reduce lipid peroxidation in the HCC model.

Many blood amino acids including isoleucine, leucine, valine, tyrosine, alanine, and phenylalanine were found at higher concentrations in the HCC with abnormal Savda group when compared with the healthy control group, but at lower concentrations in the T2DM group with the exception of acetylcysteine. In asthma with abnormal Savda group, only phenylalanine was up-regulated. The branched-chain amino acids (BCAAs) leucine, valine, and isoleucine are critical for maintaining skeletal muscle protein metabolism. Symptoms of abnormal Savda patients frequently include weight loss^[3,4] and energy metabolism disorders.^[47,48] Overall, these changes may reflect changes in skeletal and lung muscles. BCAAs are also thought to affect glucose metabolism^[49] and weight-loss conditions have been shown to increase gluconeogenesis rates.^[50] Another amino acid, tyrosine is synthesized from the essential amino acid phenylalanine, a critical metabolic substrate for tumor cells. Phenylalanine is important for assessing liver metabolism and liver dysfunction severity.^[51] High serum phenylalanine concentrations in the HCC and asthma with abnormal Savda groups may be related to liver dysfunction. According to the TUM body fluid theory, body fluids are produced in the liver.^[1,2] Liver is the primary organ involved in amino acid metabolism. Disrupting liver function can affect protein metabolism

by forming plasma proteins, interconverting amino acids, deaminating amino acids, and up-regulating urea synthesis. Both liver and muscle utilize amino acid metabolism, so these results may indicate increased protein degradation in skeletal muscle and/or liver function abnormality in the HCC with abnormal Savda rat model. In addition to metabolic functions, acetylcysteine is converted to cysteine during DNA and RNA methylation reactions. Therefore, perturbations in acetylcysteine levels may affect gene methylation levels. Following ASMq treatment, the acetylcysteine levels in the HCC groups and the phenylalanine levels in the asthma groups decreased, but no effect was seen on the other amino acids levels.

The present study combined NMR metabolite profiling of rat serum with chemometrics to explore metabolic pathway perturbations in different primary diseases coupled with abnormal Savda. The NMR results show a wide range of metabolite changes, indicating different pathological states. However, some abnormal Savda-coupled diseases showed similar clinical manifestations or pathological processes consistent with the “different diseases with the same syndrome” principles of TUM. The use of ASMq to treat different diseases with abnormal Savda based on the principle of “disease homology theory” may have some biological basis.

CONCLUSION

These findings suggest that the HCC, T2DM, and asthma with abnormal Savda rat models have common characteristics along with significant differences. Our data indicate decreased immune function, decreased oxidative defense, liver function abnormalities, amino acid deficiencies, and energy metabolism disorders that are common characteristics of abnormal Savda-associated diseases and may be related to the formation of abnormal Savda. The altered metabolites in plasma may reflect the occurrence and development of complicated diseases, and may be helpful in developing a metabolic signature of the Savda described in TUM. Compare to T2DM with abnormal Savda, ASMq have a more significant effect for HCC and asthma with abnormal Savda rat models, which improve the abnormal metabolism and immune function. For T2DM with abnormal Savda rat models, ASMq significantly effect on blood glucose.

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