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Effects of Mongolian Medicine Modified Sugmul-7 on Hyperplasia of the Breast

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Objectives: The aim of this study was to explore the mechanism of regulating the endocrine function of hyperplasia breast in rats with Mongolian Medicine Modified Sugmul-7 (MMMS-7) by Proteomics and provide an experimental basis for its development and clinical application. **Methods:** Ninety female SD rats were randomly divided into 6 groups: the intact group – the animal group that was injected with neither estradiol, nor progesterone, nor administered Mongolian Medicine Modified Sugmul-7; the negative control group – the group injected with estradiol and progesterone; the positive control group - injected with estradiol, progesterone and administered mastodynon 0.06 g/kg; and the treatment groups – injected with estradiol and progesterone, each in their respective dose groups. After the start of the experiment, each rat except the intact group was intra peritoneally injected with estradiol 25d and progesterone 6d at a standard dose of 0.3 mg / kg for a total of 31 days. **Results:** Four differential proteins were found through proteomic analysis, namely Ppp3cb, Cacybp, Gstz1, and Nmd3 and their 5 related pathways. **Conclusions:** The above pathways are all known to be related to the treatment of breast hyperplasia caused by endocrine disorders. Treatment with Mongolian Medicine Modified Sugmul-7 is completed through regulation of the gastrointestinal and endocrine systems.

Kyewords: Mongolian Medicine, Hyperplasia of the Breast, Endocrine Function, Proteomics

Introduction

Breast hyperplasia is a common disease that generally occurs in 40% of women aged 18-50 years and accounts for 80% of all breast disorders. It has been characterized by abnormal proliferation of the epithelial cell lining of the ducts which results in formation of more than four cell layers. It has been thought that the risk factors of the breast hyperplasia are increased estrogen level, imbalance of the estrogen and progesterone, irregular lifestyle, smoking, alcohol intake, breastfeeding, as well as mental pressure. There are two types of hyperplasia depends on its pathophysiology. Physiological hyperplacia occurs during the menstruation and closely related to the level of the estrogen. On the other hand, pathological hyperplacia (atypical hyperplacia) usually requires detailed examination due to its increased risk for breast tumors [1-3]. In modern medicine, physiological hyperplacia does not require any treatment, while in some cases of atyplical hyperplacia, in order to decrease the risk of breast cancers, surgical treatment or synthetic drugs such as tamoxifen are prescribed.

Despite of the prescription of synthetic drugs, numerous studies have proved that oriental medicines have protective as well as healing effect against breast hyperplacia. Phytolaccae Radix, including Phytolacca acinosa and P. Americana, shows good therapeutic effect in the treatment of hyperplacia. It has been showed that total saponins of Phytolaccae Radix significantly reduce histological changes induced by estrogen and progesterone and mammary phosphorylation levels of ERK1/2, as well as reduced the mRNA and protein overexpression of VEGF and bFGF in mammary of rats [4]. Moreover, Liu et al. demonstrated that the traditional mongolian medicine RuXian-I also known as "Hu hun e ru le" has protective and therapeutic effects on HMG rats induced by estrogen and progestogen possibly via promoting apoptotic pathway regulated by CRYAB and is a promising agent for treating HMG. RuXian-I inhibit the upregulation level of antiapoptotic protein CRYAB of HMG rats and promote mammary gland cell apoptosis of HMG rats via increases of promoting apoptosis protein caspases-3, 8, and 9 and Bax and tumor suppressor protein p53, decreases of antiapoptosis protein Bcl-2, and release of cytochrome c [5-7]. A meta-analysis conducted by Li et al. revealed that using Ru-Pi-Xiao, chinese traditional medicine, in combiantion with tamoxifen could exhibit better therapeutic effects against MGH than that of tamoxifen alone (OR: 3.79; 95% CI: 3.09–4.65; $p < 0.000$. Further, the combined treatment resulted in a decrease in the level of progesterone, decreasing the size of breast lumps. Ru-Pi-Xiao consists of some medicinal plants such as Dandelion Herba Taraxaci, Thallus Laminariae, Snakegourd Root Radix Trichosanthis, Notoginseng Root Radix Notoginseng, Red Peony Root Radix Paeoniae Rubra, Common Aucklandia Root Radix Aucklandiae, Radix Scrophulariae as well as Safflower Flos Carthami [8]. A meta-analysis study conducted by Lai et al. also showed a potential beneficial effect from Rupi Sanjae capsules in treating breast pain. The capsule showed a significant effects in shortening duration of the breast pain (MD-6.51 days, 95%CI [-8.57, -4.45], n = 82, 1 trial), shortening the duration of breast mass (MD-5.17 days, 95%CI [-7.56, -2.78], n = 82, 1 trial), improving clinical cure rate (RR 1.55, 95% CI [1.21, 2.00], I^2 = 64%, n = 1398, 10 trials) and had a improved total effective rate (RR 1.08, 95% CI [1.03, 1.14], I^2 = 71%, n = 2170, 14 trials) compared to Tamoxifen [9]. Hu et al. also reveled that traditional chinese medicine has been widely accepted in the tretament of mammary gland hyperplasia, however, the underneath mechanisms are not yet clear [10].

According to Traditional Mongolian medicine main theory, its function is to suppress "rlung" and strengthen the body. It is mainly used for treating heart and kidney "rlung" disease, excessive leucorrhea, aching waist and legs, cold lower abdomen, irregular menstruation, weak body weight, ect [11]. Use of Mongolian Medicine Modified Sugmul-7 (MMMS-7) plus safflower has been practiced by Inner Mongolian physicians for many years and has been shown to be effective in clinical practice. Composition of Medically Modified Sugmul-7 plus safflower prescription included: the main component, 150 g of Amomum kravanh Pierre ex Gagnep., others are Polygonatum sibiricum Delar. Ex Redoute, Gymnadenia conopsea (L.) R. Br., Asparagus cochinchinensis (Lour.) Merr., Myristica fragrans Houtt., Eugenia caryophyllata Thunb., Aquilaria sinensis Lour each of them 25 g, and plus Carthamus tinctorius L. 25 g. As mentioned in numerous studies, traditional medicine is markedly different in each region of the world due to its belief, theory, culture and time. Further, as might be expected, overall efficacy and safety is exceedingly dependent on its components. The herbal components of MMMS-7 are significantly different from the above mentioned Chinese traditional medicines.

In this study, therefore, we have aimed to determine the

benefits of the MMMS-7 ingredient in the treatment of breast enlargement, and the mechanism of regulating the endocrine function of hyperplasia breast through scientifically based research and experimental methods.

Materials and Methods

Study subjects

Ninety female SD rats were selected, and after being adapted to experimental conditions for 2-4 days, they were randomly divided into intact, negative control, positive control (mastodynon 0.06 g/kg) and three treatment group of MMMS7 differing in dose: dose A (1.2 g/kg), dose B (2.4 g/kg), and dose C (3.6 g/kg) for a total of 6 groups, 15 per group. After the start of the experiment, each rat except the intact group was intraperitoneally injected with estradiol 25 d and progesterone 6d at a standard dose of 0.3 mg / kg for a total of 31 days. Each rat in the intact group was intraperitoneally injected with the same dose of 0.9% saline. Seven days after the start of the trial, each treatment groups was additionally infused with the corresponding dose of 0.5% CMC-Na suspension of MMMS-7 (plus safflower) orally, and the positive control group was administered mastodynon 0.06 mg/kg orally for a total of 25 days. The intact group and the negative control group were given the same dose of 0.5 % CMC-Na solution. After the last administration, fasting of food and water for 24 hours was imposed, then an intraperitoneal injection of 10% chloral hydrate for anesthesia, then blood was extraction from the abdominal aorta. Blood was centrifugation, separated of serum, stored at -80 ° C, and tests were performed sequentially. Tests for serum 5-HT (serotonin), cGMP (cyclic guanosine monophosphate), T3, T4 (thyroid hormones), TSH (thyroid stimulating hormone), GnRH (gonadotropin - releasing hormone), LH (luteinizing hormone) and other indicators were strictly preformed and measurements obtained in accordance with the kit instructions. The Q Exactive mass spectrometer from Thermo Scientific was used to identify and quantify proteins by secondary mass spectrometry.

Reagents and equipment

The main reagents were: 5-hydroxytryptamine 5-HT (batch number: 20181105, Shanghai Yuanye Biotechnology Co., Ltd.); cyclic guanosine cGMP (batch number: 20181105, Shanghai Yuanye Biotechnology Co., Ltd.); cyclic adenosine cAMP (Batch number: 20181105, Shanghai Yuanye Biotechnology Co., Ltd.; Triiodothyronine T3 (Lot number: 20181105, Shanghai Yuanye Biotechnology Co., Ltd.); Thyroxine T4 (Lot number: 20181105, Shanghai Yuanye Biotechnology Co., Ltd. Co., Ltd.); thyroid stimulating hormone TSH (batch number: 20181105, Shanghai Yuanye Biotechnology Co., Ltd.); gonadotropin releasing hormone GnRH (batch number: 20181105, Shanghai Yuanye Biotechnology Co., Ltd.); RP C18 column; and finally, Pierce company TMT Mass Tagging Kits and Reagents kit.

The equipment used was: a Thermo Scientific company Q Exactive mass spectrometer, the HPLC system by Diane NCS3500 systems, a YP-5002 electronic balance (YuyaoJinnuo Electronic Balance Equipment Co., Ltd.), a SUNRISE microplate reader, a TG16-WS ultracentrifuge (Xiangyi centrifuge company), a HH-W600 constant temperature water bath (Jiangsu Jinyi Equipment Technology) Co., Ltd.), a YDS-10B liquid nitrogen tank (Xi'an HuachenLotte Lab Equipment Co., Ltd.), a U410-86 ultra-freezing freezer (UK), a BCH 198K freezer (Haier Co., Ltd.), and a YB1201 electronic balance (Shanghai Haikang Equipment Co., Ltd.))

The experimental drugs were: Mongolian Medically Modified Sugmul-7 (provided by the Mongolian Medicine Preparation Room of the Affiliated Hospital of Inner Mongolia University for Nationalities), estradiol (provided by Tianjin Jinyao Amino Acid Co., Ltd., standard: 1 mg / ml, batch number: 201805211), and progestrone provided by Zhejiang Xianju Co., Ltd., standard: 1 ml / 20 mg, batch number: 201712001). Mastodynon 60 tablets (made in Germany by Bionorica SE: 07429605) were purchased from Monos drugstore in Mongolia.

The research was conducted at the Mongolian Pharmacology Laboratory of the Mongolian Medicine and Pharmaceutical School of Inner Mongolia University for Nationalities in Tongliao, the China and International School of Mongolian Traditional Medicine, and the Mongolian National University of Medical Sciences in Ulaanbaatar, Mongolia.

Statistical analysis

The mean value of continuous variables which are estrogen, progesterone, cGMP, T3 as well as T4 in the intact, negative and positive controls, and treatment dose A, B, and C groups were compared using a one-way variance (ANOVA) test. Tukey's multiple comparison test was used to determine the difference between age groups. Significant values have been adjusted by

the Bonferroni correction for multiple comparison tests. A critical p-value of < 0.05 was used. SPSS version 24 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Ethical statement

The experimental protocols were designed under ethical guidelines and the study protocol was approved by the Biomedical Research Ethical Review Board, Mongolian National University of Medical Sciences, under permission No 2019/2-2019-15.

Results

Effect of MMMS-7 on serum hormone levels of rats with hyperplasia of the breast

The effect of MMMS-7 on serum E2 (estrogen), Prog (progesterone), cGMP, T3, T4, and TSH hormones in estradiol - and progesterone-induced breast hyperplasia rats are summarized in Table 1.

The mean serum E2 level in the negative control group increased 1.1 times compared to the intact group ($p < 0.01$), while positive control and treatment groups are decreased significantly 1,94 – 1.43 times compared to the negative control group ($p < 0.01$). The treatment dose B group decreased in serum E2 level 1.05 times compared to the positive control group ($p < 0.05$).

The mean serum Prog hormone of positive control and treatment Dose A, B groups is increased significantly 1.14-1.22 times compared to the negative control group respectively. The mean serum cGMP level in the positive control group and

Table 1. Effect of Mongolian Medicine Modified Sugmul-7 on serum E2, Prog, cGMP, T3, T4 hormone levels of rats with hyperplasia of the breast.

Variables	Intact (n = 15)	Negative control (n = 15)	Positive control (n = 15)	Treatment Dose A (1.2g/kg) (n = 15)	Treatment Dose B (2.4g/kg) (n = 15)	Treatment Dose C (3.6g/kg) (n = 15)	*p-value
E2 (pmol/L) [a, b, c]	13.76 ± 1.48	15.25 ± 1.65	7.84 ± 1.52	10.65 ± 2.14	7.43 ± 0.76	7.86 ± 1.92	0.000
Prog (ng/mL) [d, e]	2.57 ± 0.17	2.26 ± 0.22	2.75 ± 0.38	2.19 ± 0.43	2.58 ± 0.18	2.77 ± 0.52	0.000
cGMP (nmol/L) [f, g]	3.53 ± 1.40	2.84 ± 0.28	3.05 ± 0.34	3.07 ± 0.23	3.26 ± 0.38	3.08 ± 0.35	0.037
T4 (ng/mL) [h]	40.89 ± 9.51	34.44 ± 4.26	35.67 ± 5.53	31.89 ± 3.49	34.49 ± 4.86	37.51 ± 4.39	0.000
T3 (ng/mL) [i]	2.07 ± 1.07	1.43 ± 0.11	1.50 ± 0.12	1.40 ± 0.13	1.35 ± 0.17	1.59 ± 0.25	0.002
TSH (mu/L)	3.72 ± 0.32	3.21 ± 0.37	3.60 ± 0.53	3.31 ± 0.50	3.15 ± 0.41	3.59 ± 0.54	0.150

*One-way ANOVA, multiple comparison: [a]intact vs. negative control, $p < 0.001$; [b]intact vs. dose A, $p < 0.052$; [c]positive control vs. dose B, $p < 0.014$; [d]intact vs. dose A $p < 0.024$; [e]positive control vs. dose C, $p < 0.053$; [f]intact vs. dose A, $p < 0.050$; [g]dose B vs. dose C, $p < 0.041$; [h]negative control vs. dose C, $p < 0.001$; [i]intact vs. dose A $p < 0.014$.

Table 2. Effect of Mongolian Medicine Modified Sugmul-7 on serum FSH, LH, T, 5-HT, GnRH, PRL hormone levels of rats with hyperplasia of the breast.

Variables	Intact (n = 15)	Negative control (n = 15)	Positive control (n = 15)	Treatment Dose A (1.2g/kg) (n = 15)	Treatment Dose B (2.4g/kg) (n = 15)	Treatment Dose C (3.6g/kg) (n = 15)	*p-value
FSH (IU/L) [a, b, c]	2.42 ± 0.27	2.23 ± 0.12	2.45 ± 0.28	2.24 ± 0.18	2.38 ± 0.16	2.66 ± 0.29	0.000
LH (mIu/mL)	10.42 ± 3.84	8.08 ± 0.99	10.25 ± 0.78	8.51 ± 0.57	8.93 ± 1.07	10.22 ± 1.36	0.071
T (pg/mL) [d, e]	1.85 ± 0.47	1.51 ± 0.07	1.82 ± 0.16	1.57 ± 0.14	1.59 ± 0.12	1.84 ± 0.20	0.002
5-HT (ng/mL) [f, g]	22.92 ± 2.42	25.92 ± 2.06	23.47 ± 1.57	23.66 ± 1.76	24.96 ± 1.44	30.22 ± 2.28	0.000
GnRH (mIu/mL) [h]	17.76 ± 1.81	16.28 ± 1.70	17.32 ± 1.56	16.33 ± 1.78	17.57 ± 1.32	17.45 ± 1.43	0.002
PRL (ng/mL) [i, j, k]	6.91 ± 0.51[j]	7.60 ± 0.92	6.34 ± 0.67	6.11 ± 0.48	6.46 ± 0.75	6.80 ± 0.95	0.000

*One-way ANOVA, multiple comparison: [a]intact vs. dose A, $p < 0.000$; [b]positive control vs. dose B, $p < 0.005$; [c]intact vs. dose C, $p < 0.010$; [d]intact vs. dose B $p < 0.031$; [e]intact vs. dose A, $p < 0.031$; [f]negative control vs. dose A, $p < 0.000$; [g]dose B vs. dose C, $p < 0.000$; [h]intact vs. dose A, $p < 0.005$; [i]intact vs. positive control, $p < 0.000$; [j]intact vs. dose A, $p < 0.000$; [k]dose A vs. dose C, $p < 0.000$.

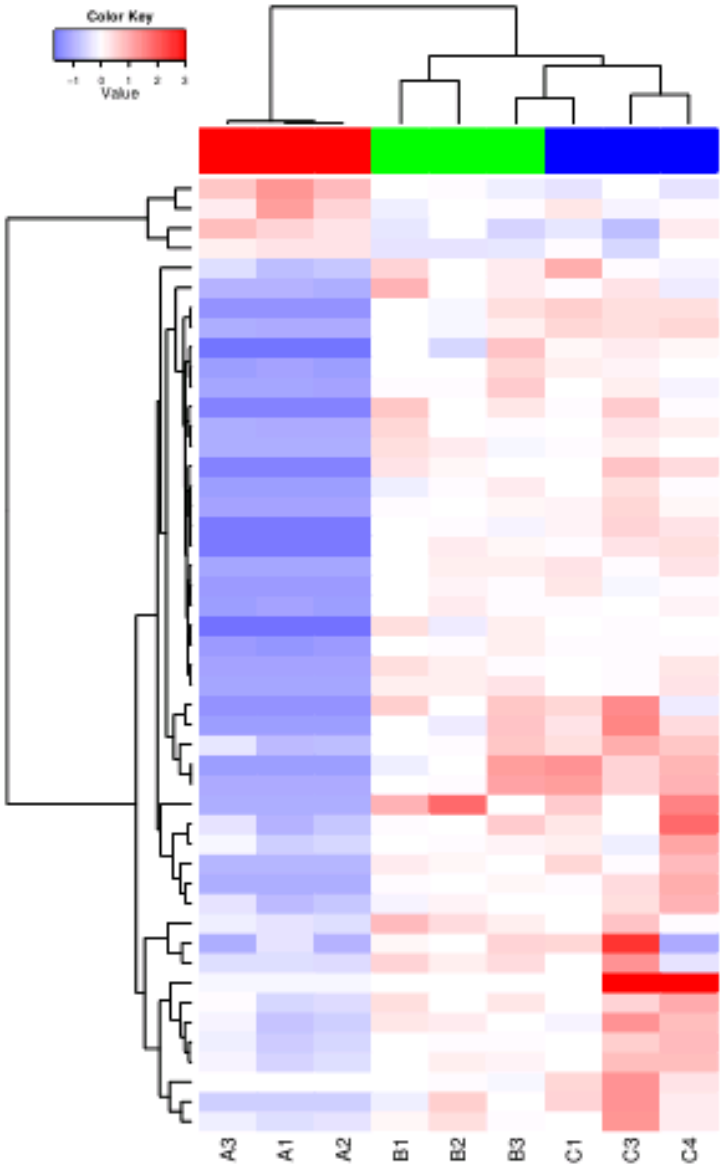

Figure 1. Differential protein clustering.

Table 3. LC / MS determination and stability of protein changes in breast hyperplasia rats.

Accession	Gene_ID	Gene_Symbol	A-vs-B.FC	B-vs-C.FC
NP_058738.1	24675	Ppp3cb	0.85	1.13
NP_001004208.1	289144	Cacybp	0.91	1.10
XP_006240461.1	681913	Gstz1	1.14	1.07
NP_001101152.1	310512	Nmd3	0.90	1.20

The mean serum T3 level in the positive control group increased 1.04 times and treatment dose C groups increased 1.11 times compared to the negative control group ($p < 0.05$) respectively. The mean serum TSH level in the positive control group increased 1.12 times and treatment dose C groups increased 1.11 times compared to the negative control group ($p < 0.05$) respectively. According to the experimental data in Table 1, MMMS-7 also increased TSH, Prog, T4, T3, and cGMP which in the secondary signal of cells ($p<0.05$, or $p<0.01$).

treatment dose A, B, C groups is increased 1.07-1.15 times compared to the negative control group ($p < 0.05$) respectively. This was especially true for the treatment dose B group which increased in the serum cGMP level 1.06 times compared to the positive control group ($p < 0.05$). The mean serum T4 level in the positive control group increased 1.03 times and treatment dose C groups increased 1.09 times compared to the negative control group ($p < 0.05$) respectively. This was especially true for the treatment dose C group which increased in the serum T4 level 1.05 times compared to the positive control group ($p < 0.05$).

The effect of MMMS-7 on serum FSH (follicle - stimulating hormone), LH, T, 5-HT, GnRH, PRL (prolactin) hormones in estradiol and progesterone-induced breast hyperplasia rats is summarized in Tables 2. The mean serum FSH level in the negative control group decreased 1.08 times compared to intact ($p < 0.01$), while positive control and treatment dose B, C groups increased 1,07 – 1.19 times compared to the negative control group ($p < 0.05$, $p < 0.01$). Treatment dose C group increased in serum FSH level 1.08 times compared to the positive control group ($p < 0.05$). The mean serum LH level in the negative control group decreased 1.29 times compared to the intact group ($p < 0.05$), but positive control and treatment dose B, C groups increased 1.10-1.26 times compared to the negative control group ($p < 0.05$, $p < 0.01$). The mean serum T level in the negative control group decreased 1.21 times compared to the intact group ($p < 0.05$), but positive control and treatment dose B, C groups increased 1.05-1.21 times compared to the negative control group ($p < 0.05$, $p < 0.01$). The mean serum 5-HT level in the negative control group increased 1.13 times compared to the intact group ($p < 0.05$), but positive control and treatment dose A groups decreased 1.10-1.09 times compared to the negative control group ($p < 0.05$, $p < 0.01$). The mean serum GnRH level in the negative control group decreased 1.09 times compared to the intact group ($p < 0.05$), but positive control and treatment dose B, C groups increased 1.06-1.08 times compared to the negative control group ($p < 0.05$). The mean serum PRL level in the negative control group increased 1.09 times compared to the intact group ($p < 0.05$), but positive control and treatment dose groups decreased 1.11-1.14 times compared to the negative control group ($p < 0.05$, $p < 0.01$). Treatment dose

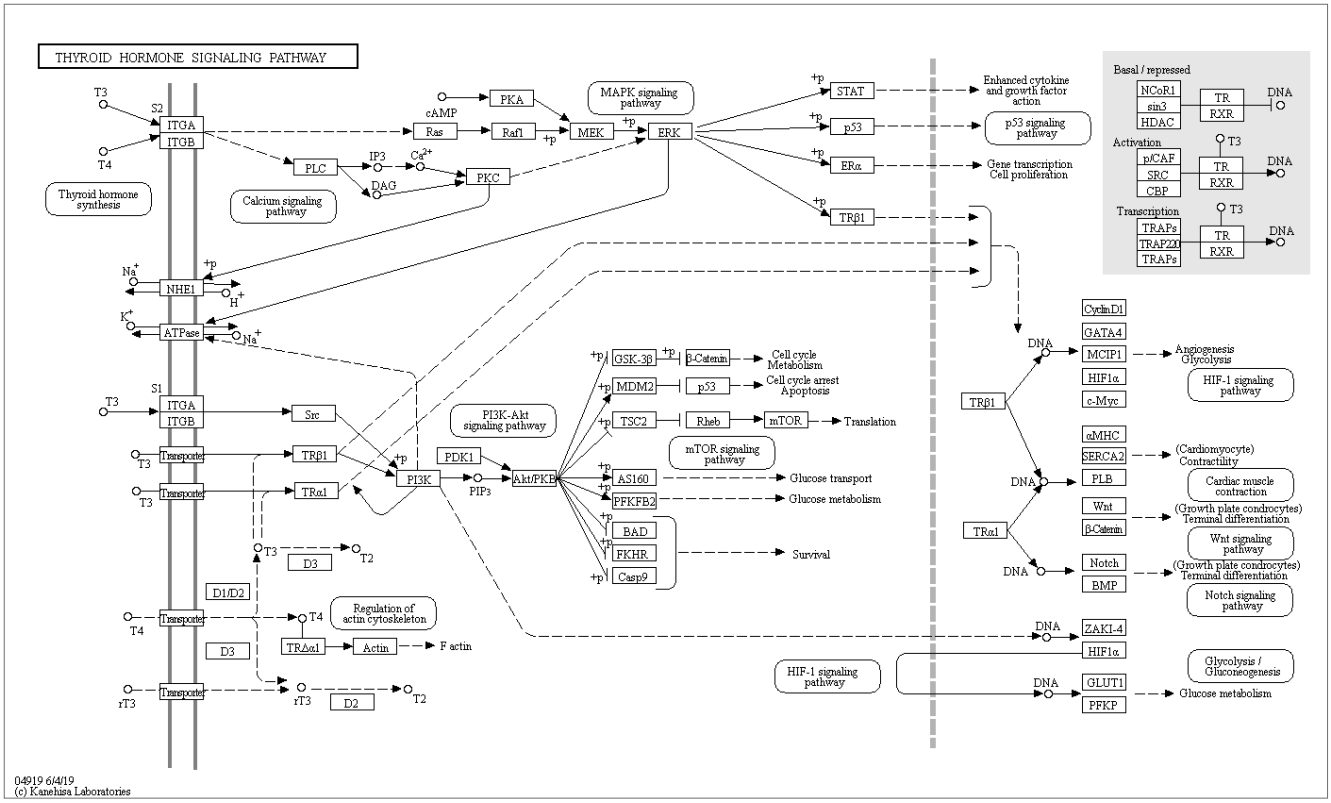

Figure2. Map04919 thyroid hormone signaling pathway.

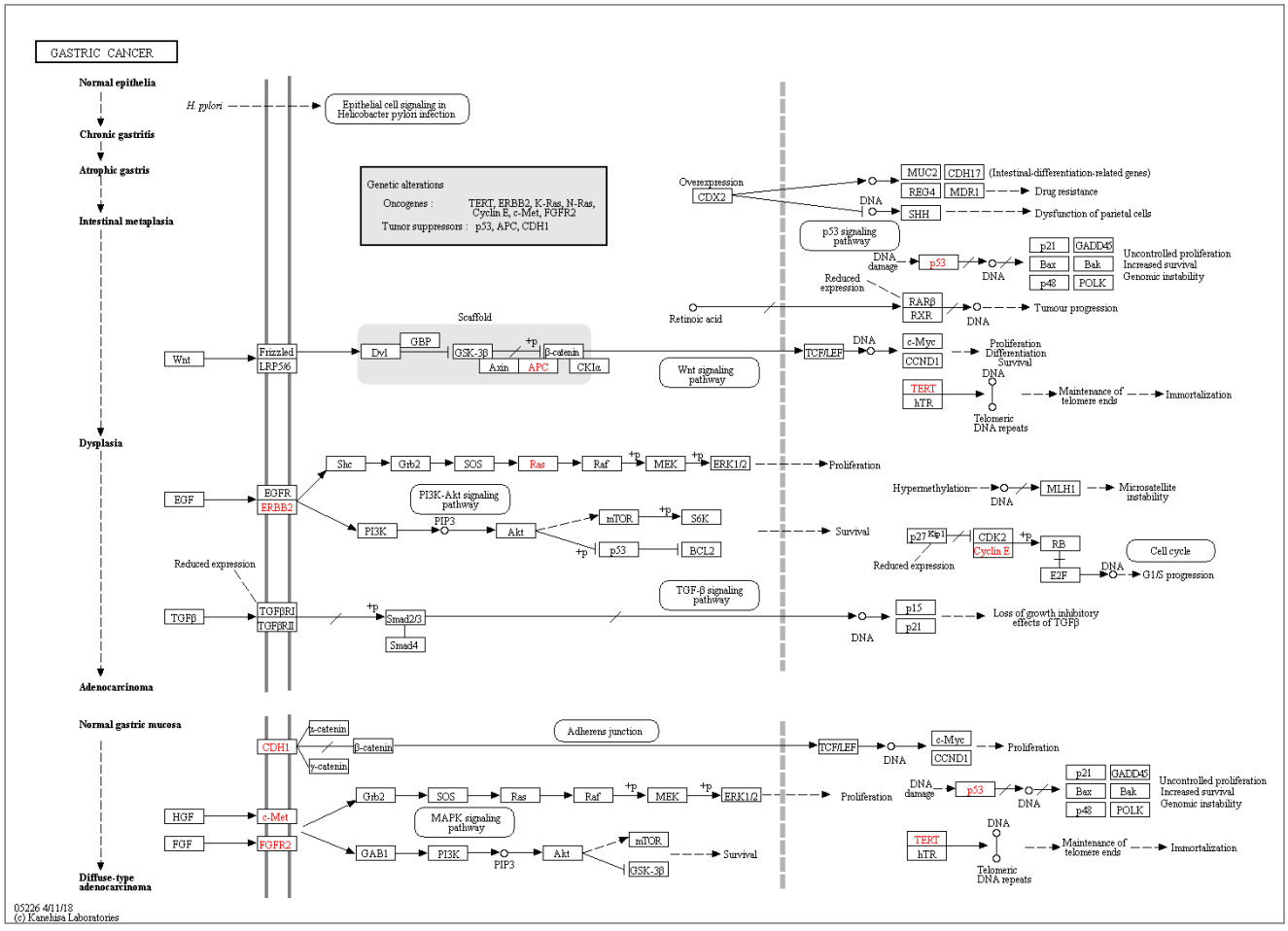

Figure 3. Map05226 Path to the stomach cancer.

A group decreased in serum PRL level 1.04 times compared to the positive control group ($p < 0.05$). MMMS-7 increased the serum female sex hormone (E2), FSH, LH, testosterone, GnRH and 5-HT ($p < 0.05$) while PRL was decreased ($p < 0.05$). The above hormone parameters have been shown to be effective in the treatment groups dose A, B, C, and in the positive control group. It was special true that the treatment dose C group increased the amount of FSH 1.08 times ($p < 0.05$), and the treatment dose A group decreased the amount of PRL hormone 1.04 times compared with the positive control group ($p < 0.05$) which confirmed the statistical validity of the therapeutic effect respectively.

MMMS-7 on Mass Spectral Analysis of breast Tissue Protein in Rats with Hyperplasia of the breast

Differential protein clustering results

In this test, the number of proteins identified in the negative

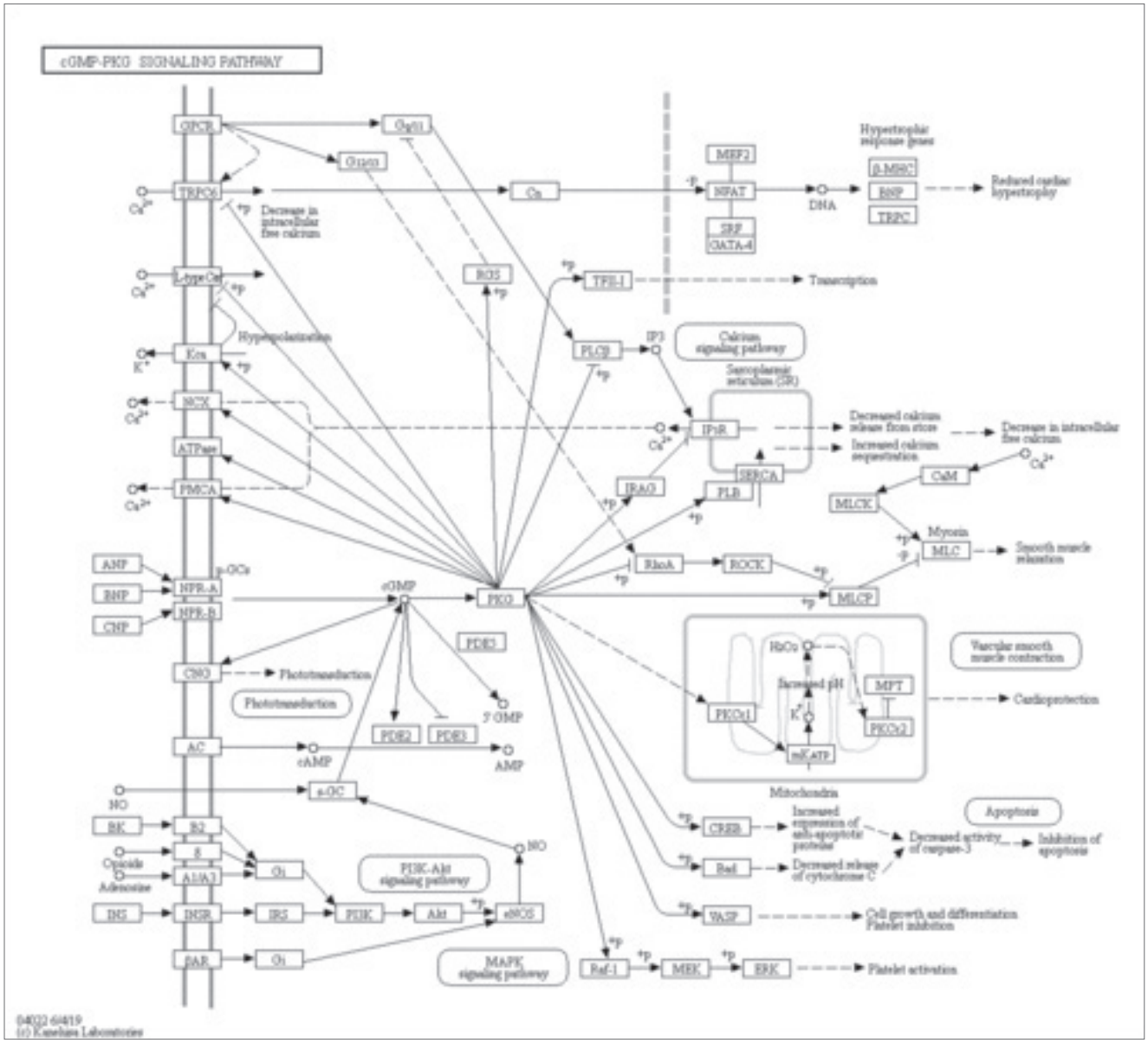

Figure 4. Map04022 Pathway cGMP-PKG signaling.

control group and the intact group was 6129. The standard is 1.2 times 0.8333 times and the protein that satisfies $p < 0.05$. The intact group and the negative control group have 132 proteins of which there were 50 up-regulated and 82 down-regulated; compared with the negative control group, there were 27 different proteins in the treatment group, of which 12 were up-regulated proteins and 15 were down-regulated proteins.

Enrichment results-1.2-fold change and $p < 0.05$ differential protein

Based on the traditional Mongolian medicine theory and the pathogenesis of breast hyperplasia, 1.2 times to 0.8333 times as the standard, and the difference proteins and related websites with $p < 0.05$ are shown in Table 3 and the difference protein interaction network diagrams 1-10.

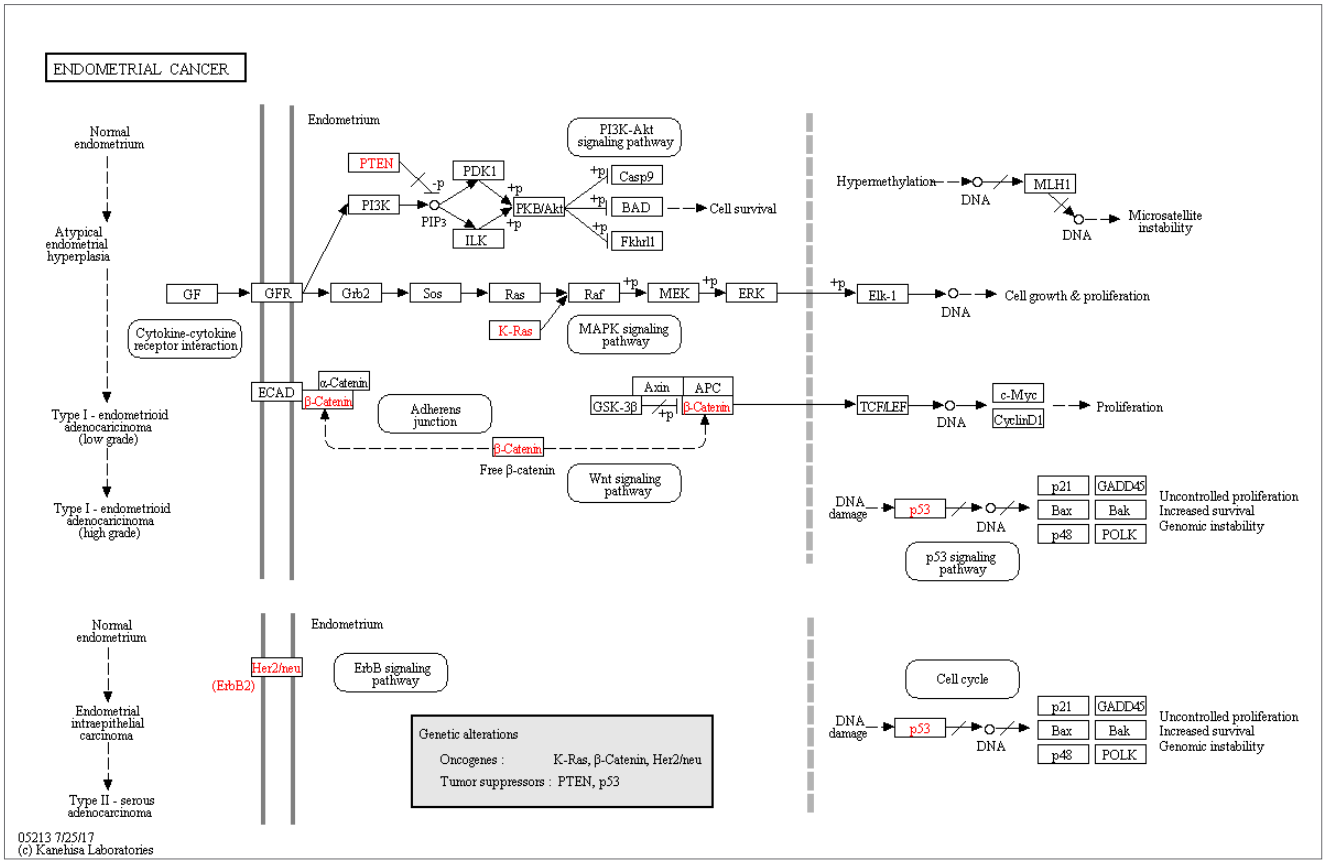

Figure 5. Map05213Route endometrial cancer.

Discussion

MMMS-7 prescription is a Mongolian medicine with long clinical experience based on the etiology and pathogenesis of the "breast mass" of Mongolian medicine. It has been used in the clinic for a long time and has achieved good therapeutic effects [11]. No research report on the modern pharmacological action mechanism of MMMS-7 prescription has been found. Aspects of Traditional Mongolian Medicine (TMM) are explained by the "Rlung, Mkhris, Badgan" theory [12]. Some Mongolian scientists revealed that the functional and structural unit of living cells such as the membrane – redox potentials three state line systems (first compartment) have close relationships with the second (blood serum), third (body mass) and fourth (membrane structures) compartments of the body [13].

According to TMM main theory, the function of MMS-7 is to suppress "Rlung" and strengthen the body [14]. The modern chemical studies on this prescription`s components are reported such as, Amomum kravanh which contains volatile oil, 5-hydroxy-3, 7, 4'-trimethoxyflavone [14-17] and trace elements such as Ca, Mn, and K. It has the effects of anti-gastric ulcer, enhances gastric motility, and protects gastric mucosa [18]. Further, Polygonatum, Gymnadenia conopsea, and Asparagus mainly contain polysaccharides and has the function of improving the immune system and regulating endocrine disorders [18, 19]. Asparagus cochinchinensis contains steroidal saponin, furostanol glycoside, and aspacochinoside [18]. It has been demonstrated that water-soluble polysaccharides possess immunological activity and the immunostimulatory mechanism may be attributed to the NF-kB and p38 MAPK pathways similar to the mechanism of lipopolysaccharides [20]. Safflower contains safflower yellow pigment, which can protect the liver and increases the "heat and strength of the bodily constituents" in Traditional Mongolian medicine. It supplies to the humor, bodily constituents and excretions, ensures good health, energy, radiance of complexion, and long/ life [18]. Flos Carthami major

constituents are pigments, flavonoids and phenolic acid which has an antioxidant activity [21]. Myristica contains volatile and nonvolatile components oily ingredients and has a sedative effect in MTT. Moreover, modern pharmacological studies have shown that Myristica fragrans Houtt has strong antibacterial, anti-inflammatory, analgesic, anti-tumor and other effects [15]. Eugenia is rich in the clove oil component and has obvious sedative and hypnotic [20], antimicrobial, antioxidant and anti-Inflammatory effects as well as protecting the gastric mucosa and preventing gastric ulcers; Aquilaria sinensis contains volatile oils and has analgesic and antipyretic effects [16-20].

According to the above analysis, from the perspective of the composition of MMMS-7, it consists of drugs that protect liver function, protect gastric mucosa, and drugs that regulate immune function and regulate endocrine function. Therefore, the formula's mechanism for clinically treating hyperplasia of mammary glands is likely to be to treat hyperplasia of the breast by regulating "endocrine function" disorders. In this study, the proteomics method was used to find the differential protein of MMMS-7 for the treatment of breast hyperplasia, and to explain the endocrine regulation mechanism of the prescription for the treatment of breast hyperplasia.

Our experiment was based on the traditional theory of Mongolian medicine and the pathogenesis of breast hyperplasia, 1.2 times to 0.8333 times are used as the standard. As a result, four differential proteins Ppp3cb, Cacybp, Gstz1Gstz1, Nmd3 and a total of 10 related pathways have been found [22]. As for the cGMP-PKG signaling pathway, the cGMP, the second messenger in the cell, mediates the effects of nitric oxide and natriuretic peptide, and regulates a wide range of physiological processes. Increased intracellular cGMP levels exert their physiological roles through two forms of cGMP-dependent protein kinase (PKG), cGMP-regulated phosphodiesterase (PDE2, PDE3), and cGMP-gated cation channels, of which PKGs may be the main medium. The PKG1 subtype-specific activation of established substrates leads to a decrease in cytosolic calcium concentration and / or a decrease in the sensitivity of myofilaments to Ca2 + (Ca2 + -desensitization), leading to smooth muscle relaxation [22-24]. In the present study, cGMP was significantly reduced in the negative control group compared with the intact group. This may be the mechanism of calcium loss caused by clinically induced endocrine disorders in females caused by decreased calcium levels in the body's myofilaments and bone significantly increased. MMMS-7 is one of the mechanisms of regulating calcium loss caused by endocrine disorders [23].

Further, as for the Thyroid hormone signaling pathway, the effect of thyroid hormone is mainly mediated by T3 (3, 5, 3'-triiodo-1-thyrosine). Thyroid hormones, levothyroxine (T4), and T3 enter cells through transporters. Although the main form of thyroid hormone in the blood is T4, it is converted intracellularly to the more active hormone T3. T3 binds to nuclear thyroid hormone receptors (TRs), which act as ligand-dependent transcription factors and control the expression of target genes (genomic effects). Non-genetic mechanisms begin at the integrin receptor. The plasma membrane α (v) β (3) -integrin has different binding sites for T3 and T4 [24]. One binding site only binds T3 and activates the phosphatidylinositol 3-kinase (PI3K) pathway. The other binding site binds T3 and T4 and activates the ERK1 / 2 MAP kinase pathway [23]. Our experiment shows that T3 and T4 are decreased in the negative control group compared with the intact group; and T3 and T4 are significantly increased in the treatment dose C group compared with the negative and positive control groups.

Next, as for the Estrogen signaling pathway, estrogen regulates its cellular effects through two signaling pathways, which are divided into "nuclear-induced steroid signaling" and "membrane-induced steroid signaling." In the "nuclear" pathway, estrogen binds to ER alpha or ER beta, which in turn is translocated to the nucleus, binds to DNA on the ERE element, and activates ERE-dependent gene expression. In the "membrane" pathway, estrogen can function through a subpopulation of estrogen receptors on the plasma membrane or new G protein-coupled E2 receptors (GPER). After activating these receptors, various signaling pathways (i.e., Ca2 +, cAMP, and protein kinase cascades) are rapidly activated and eventually affect downstream transcription factors. Studies have shown that this pathway causes abnormalities in breast cancer [24]. We showed in this study that the negative contol group is significantly higher than the intact group, and each dose treatment groups and positive control group are significantly lower than the negative control group.

As for Gastrointestinal signal-related pathways, intestinal metaplasia is characterized by mutations in the p53 gene, reduced expression of retinoic acid receptor beta (RARβ), and reduced expression of telomerase reverse transcriptase. Gastric adenomas further showed APC gene mutations, decreased

p27 expression, and cyclin E amplification. In addition, in more advanced gas chromatography, amplification and over expression of c-ErbB2, reduced expression of TGF-β receptor i-type, and complete loss of p27 expression are usually observed. The main molecular changes observed in diffuse GCs include CDH1 mutations and loss of E-cadherin function due to MET and FGFR2F amplification [25]. In according to TMM, all "internal" disease begins with a decrease in stomach "heat" – it is called a "not absorbed or cold disease". It's related to the female "rlung" disease [25].

There is also another pathway of reproductive system-related pathways. Here, endometrial cancer is the most common gynecological malignancy and the fourth largest malignancy after breast cancer, colorectal cancer, and lung cancer among women in developed countries. The two endometrial cancers are different biologically and clinically. Type uterine cancer is associated with endometrial hyperplasia, frequent expression of estrogen and progesterone receptors, and younger age, while type uterine cancer has nothing to do with estrogen, and is frequently associated with atrophic endometrium, estrogen, and progesterone receptors. Lack is related to older age. The morphological differences of these cancers are reflected in their molecular genetic maps. The I type shows DNA mismatch repair and mutation defects in PTEN, K-ras and β-catenin, and the II type shows aneuploidy, p53 mutations, her2 / neu amplification [25].

In summary, the above pathways are related to the treatment of hyperplasia of the breast caused by endocrine disorders with MMMS-7, which is completed by the gastrointestinal and endocrine regulating functions. However, our study has a limitation. MMMS-7 is composed of several plants, and each of the plant may have a modifying effect. Therefore, we cannot explain still which plant has the most modifying effects. Therefore, future studies could expand the applicability of present results by investigating each of the plant extracts in females and determining the effective compounds of MMMS-7.

Conclusions

In conclusion, the above pathways are all related to breast hyperplasia caused by endocrine disorders and benefit from treatment with MMMS-7, which is completed through the regulation of the gastrointestinal tract and endocrine tract.

Conflict of Interest

The authors state no conflict of interest.

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References

1. Leung AY. Encyclopedia of Chinese and Mongolian medicine. Shanghai, China: Shanghai Science and Technology Press; 1992. p 274-86.
2. Robert B. Biomembranes molecular structure and function. New York, USA: Springer Press; 1989: p 86-90.
3. Ulzii O. Herbs and prescriptions used in Traditional Mongolian medicine. Hohhot, China: Inner Mongolian Scientific Technology Printing House; 2013. p 287-9.
4. Li X, Wang Z, Wang Y, Zhang Y, Lei X, Xin P, et al. Anti-hyperplasia effects of total saponins from phytolaccae radix in rats with mammary gland hyperplasia via Inhibition of proliferation and induction of apoptosis. Front Pharmacol 2018; 9: 467-71.
5. Liu J, Zhang JF, Gong GH, Zhang B, Wei CH. Therapeutic effect of Mongolian medicine RuXian-I on hyperplasia of mammary gland induced by estrogen/progesterone through CRYAB-promoted apoptosis. eCAM 2020; 10: 15-21.
6. Zhang JF, Liu J, Gong GH, Zhang B, Wei CH. Mongolian medicine RuXian-I treatment of estrogen-induced mammary gland hyperplasia in rats related to TCTP regulating apoptosis. eCAM 2019; 9: 37-40.
7. Jiang M, Liang Y, Pei Z, Wang X, Zhou F, Wei C, et al. Diagnosis of breast hyperplasia and evaluation of RuXian-I based on metabolomics deep belief networks. Int J Mol Sci 2019; 20: 2620-7.
8. Li HT, Liu HH, Yang YX, Wang T, Zhou XL, Yu Y, et al. Therapeutic effects of a traditional Chinese medicine formula plus Tamoxifen vs. Tamoxifen for the treatment of

mammary gland hyperplasia: a meta-analysis of randomized trials. Front Pharmacol 2018; 9: 45-9.

9. Lai BY, Jia AY, Yu BW, Liang SB, Chu AJ, Cao HJ, et al. Chinese herbal medicine (Rupi Sanjie capsule) for the treatment of breast pain: a systematic review and meta-analysis of randomized clinical trials. Integr Med Res 2021; 10: 88-93.
10. Li X, Xin P, Wang C, Wang Z, Wang Q, Kuang H. Mechanisms of traditional Chinese medicine in the treatment of mammary gland hyperplasia. Am J Chin Med 2017; 45: 443-58.
11. Jin M, Li JR, Wu W. Study on the antioxidative effect of safflower yellow. J Tradit Chin Med 2004; 29: 447-9.
12. Politeo O, Jukic M, Milos M. Comparison of chemical composition and antioxidant activity of glycosidically bound and free volatiles from clove. J Food Biochem 2010; 34: 129-41.
13. Fazly B, Bibi SK, Bahman J. Solid lipid nanoparticles (SLN) carrying\r Eugenia caryophyllata\r essential oil: the novel nanoparticulate systems with broad-spectrum antimicrobial activity. Lett Appl Microbiol 2018; 66: 506-13.
14. Leem HH, Seo M, Kim E, Cho SW. Antioxidant and anti-inflammatory activities of eugenol and its derivatives from Clove. J Korean Soc Food Sci Nutr 2011; 40: 10-7.
15. Chen H, Yang Y, Jian X, Wei, J, Zhen Z, Chen H. Comparison of compositions and antimicrobial activities of essential oils from chemically stimulated agarwood, wild agarwood and healthy aquilaria sinensis. Molecules 2011; 16: 4884-96.
16. Chen D, Song YL, Nie CX, Ma X, Tu PF. Chemical constituents from aquilaria sinensis. J Chin Pharm Sci 2012; 21: 173-9.
17. Murthy KS. Signaling for contraction and relaxation in smooth muscle of the gut. Annu Rev Physiol 2006; 68: 345-74.
18. Andreas F, Peter S, Achim S. cGMP: a unique 2nd messenger molecule – recent developments in cGMP research and development. Naunyn Schmiedebergs Arch Pharmacol 2020; 393: 287–302.
19. Davis PL, Davis FB, Luidens MK, Lin HY. Membrane receptor for thyroid hormone: physiologic and pharmacologic implications. Annu Rev Pharmacol 2011; 51: 99-115.
20. Kelly MJ, Qiu J, Ronnekleiv OK. Estrogen signaling in the hypothalamus. Vitam Horm 2005; 71: 123-45.
21. Wessler S, Krisch LM, Dominik PE, Aberger F. From inflammation to gastric cancer - the importance of Hedgehog/GLI signaling in Helicobacter pylori-induced chronic inflammatory and neoplastic diseases. J Cell Commun Signal 2017; 15: 26-31.
22. Lax SF. Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Arch 2004; 444: 213-23.
23. Liau PR, Wu MS, Lee CK. Inhibitory effects of scutellaria baicalensis root extract on linoleic acid hydroperoxide-induced lung mitochondrial lipid peroxidation and antioxidant. Molecules 2019; 24: 2143.
24. Jiao D, Jiang Q, Liu Y, Ji L. Nephroprotective effect of wogonin against cadmium-induced nephrotoxicity via inhibition of oxidative stress-induced mapk and nf-kb pathway in sprague dawley rats. Hum Exp Toxicol 2019; 38: 1082-91.
25. Cheng CS, Chen J, Tan HY, Wang N, Chen Z, Feng Y. Scutellaria baicalensis and cancer treatment: recent progress and perspectives in biomedical and clinical studies. Am J Chin Med 2018; 46: 25-54.