Research on the correlation between colorectal adenoma and sucrase-isomaltase based on constitution theory

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Abstract. Intestinal cancer is one of the common malignant tumors in the digestive system. Colorectal adenoma is closely related to colorectal cancer, which seriously threatens people's health. This study selected colorectal adenoma tissue samples from patients with different traditional Chinese medicine constitutions as experimental objects, and used Sucrase-isomaltase (SI) as a marker to perform the research: the expression of SI in different samples; SI separation and purification; SI biochemical characteristic detection; the difference in the expression and characteristic of SI, supplemented by database analysis. The constitution susceptible to colorectal adenoma is mainly Yang deficiency type, followed by blood stasis type. It is expected to establish the relationship between SI and colorectal adenoma and traditional Chinese medicine constitutions.

Keywords: Sucrase-isomaltase, colorectal adenoma, constitution theory

1. Introduction
Intestinal cancer is one of the common malignant tumors in the digestive system. It has been gradually increased in the past 20 years. The latest information from the World Health Organization showed that in 2019, the number of new cases of intestinal cancer (ie, colorectal cancer) worldwide was more than 1 million, ranking fourth among malignant tumors [1]. The incidence of colorectal adenomatous polyps is gradually increasing, and there is a risk of cancer. 93% of intestinal cancers come from adenomatous polyps. Screening and stopping adenomas in the precancerous stage can improve the survival rate of patients.

Traditional Chinese medicine (TCM) believes that intestinal neoplasia is in the colorectal, which is based on multiple spleen deficiency, involving liver and kidney; fatigue, blood therapy, damp-heat, Qi stagnation, and so on, are the indicators [2]. The basic point of the TCM physique theory is: diseases are biased on constitution; positively change the body's own pathological environment, so that the body's Qi, blood, Yin and Yang reach a dynamic balance, and the disease will not bud and develop [3]. Therefore, according to the physical characteristics of patients with colorectal adenoma, it has important research value for the prevention and treatment of colorectal adenoma.

In humans, six enzyme activities (two α-amylase and four-glucosidase activities) are involved in the breakdown of dietary starches and sugars into glucose. The α-glucosidase activities are associated with two small intestinal membrane-bound
Enzymes: maltase-glucoamylase and sucrase-isomaltase (SI) (Fig. 1). SI is composed of duplicated catalytic domains: an N-terminal membrane-proximal domain and a C-terminal luminal domain [4]. The domains are anchored to the small intestinal brush-border membrane via an O-glycosylated stalk stemming from the N-terminal domain [5]. SI domains are members of the glycoside hydrolase 31 family (GH31). The four domains exhibit exo-glucosidase activities against α-1,4-linked maltose substrates but display different specificities for malto-oligosaccharides of various lengths [6]. Some subunits have additional activity for the α-1,6 linkages of starch branch point and the α-1,2 linkage of sucrose, respectively, and are historically referred to as isomaltase and sucrase.

**Figure 1.** Crystal structure of sucrase-isomaltase and N-terminal domain of SI

Some researchers have conducted studies on sucrase-isomaltase as a marker for the tendency of colorectal adenoma to become malignant. The activity of sucrase-isomaltase in adenoma increases with the increase in adenocarcinoma and dysplasia, indicating that the increase in sucrase activity is very helpful in predicting malignant transformation of adenoma [7]. It is also reported that the effect of aspirin on the expression of intestinal differentiation markers of human colon cancer HT-29 cells, in which the mRNA and protein expression of the markers sucrase-isomaltase and lysozyme were down-regulated [8, 9].

Therefore, SI could be used as an indicator to explore the relationship with colorectal adenoma. In this present study, human colorectal adenoma tissue was used as the research object, and analyzes the relationship between colorectal adenoma and SI through the difference of SI protein expression and SI biochemical characteristics, and then discuss the correlation between Enzyme-Disease- Constitution.

2. Experiment

2.1. Chemicals and biochemical reagents
α-pNPG, maltose, and sucrose were purchased from Sigma-Aldrich. Isomaltose, Glucose-6-phosphate dehydrogenase and hexokinase were purchased from Xi'an Hexin chemical reagent instrument co. LTD, China. All reagents were analytical-reagent grade.

2.2. Prepare colorectal adenoma tissue samples
Conduct a survey on the constitution of traditional Chinese medicine for patients with colorectal adenoma in the Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine through questionnaires.

According to the colorectal cancer screening program issued by the National Health and Family Planning Commission, those who are positive in any of the following 4 items are at high risk and need to undergo colonoscopy: ① The FOBT immunoassay is positive; ② First-degree
relatives have a history of colorectal cancer; ③In person A history of cancer or intestinal polyps; ④Patients with two or more of the following, chronic constipation, mucus blood in the stool, chronic diarrhea, chronic appendicitis, history of mental stimulation, chronic biliary disease.

2.3. Purification of protein
The brush border membrane vesicles were separated from the small intestine tissue samples, and then dissolved in 0.5% Triton X 100 solution. DEAE-Sepharose CL-6B weak anion exchange was developed with 0.1% Triton X100 solution. This was followed by a second solubilization by papain treatment. The hydrophobic Octyl Sepharose CL 4B gel and Ultrogel ACA 22 gel were used as stationary phases for gel filtration chromatography. Protein concentration was determined by the Bradford assay (Bio-Rad protein assay dye reagent) using BSA as protein standard.

2.4. Protein expression analysis by Western Blot
The protein of frozen tissue samples was lysed and collected by RIPA lysis buffer, and BAC quantification. Separate proteins by SDS-PAGE electrophoresis, electrotransfer to nylon membrane, seal the membrane with 5% dry powder milk overnight at 4° C, overnight at 4° C for the primary antibody, overnight at room temperature for the second antibody, detect the target band by chemiluminescence method, and expose with X-ray film.

2.5. Enzyme assays
The enzyme assays were performed at 30°C, pH7, in 1 mL solution mixing 500μL of citrate potassium phosphate buffer. Reactions were carried out continuously by using the thermostated Agilent spectrophotometer. The α-glucosidase activity was detected by using α-p-nitrophenyl-D-glucopyranoside (α-pNPG) as substrate and monitoring the release of p-nitrophenyl (pNP) at 405 nm. Activity of other substrates was measured the release of glucose and the production of NADPH at 340 nm by using a coupled spectrophotometric assay, with the simultaneous use in the assay of hexokinase and glucose-6-phosphate dehydrogenase. The assay was performed by the addition of 20μL freshly purified sucrase-isomaltase to the 1 mL reaction mix containing 5 mM MgCl₂, 1 mM ATP, 1 mM NADP, 5 units of hexokinase, 5 units of glucose-6-phosphate dehydrogenase and substrate at required concentration. Velocities were obtained as the initial reaction rates (v), which were proportional to the amount of sucrase-isomaltase.

3. Results and discussion

3.1. Constitution fitness
According to all the answers in the "Classification and Judgment Table of Traditional Chinese Medicine Constitution", each question is scored on a 5-level basis, the original score and conversion score are calculated, and the constitution type is determined according to the standard. Original points= The points of each entry are added. Peaceful constitution is normal constitution, and the other 8 constitutions are partial constitution.

Conversion score = [(original score-number of entries)/(number of entries×4)] ×100

In the final analysis of the results of the early screening of colorectal cancer, this present study, two constitutions of Yang deficiency type and blood stasis type were found to be high-risk constitution. The experiment was carried out with neutral type as a control.

3.2. Protein expression
Sucrase-isomaltase is expressed in colorectal adenoma tissues of patients with three constitutions (Fig.2). The difference in expression level is not significant. Among them, the expression of Yang deficiency type was the highest, followed by blood stasis type, and the expression of neutral type was lower.
3.3. Sucrase-isomaltase heat inactivation
The purified sucrase-isomaltase was enzymatically reacted in 1 ml of 10 mM potassium phosphate buffer (pH 6.0) at 40 °C. Within 10 minutes, samples were taken and analyzed for enzyme activity. Sucrase-isomaltase isolated from patients of different constitutions showed different thermal stability (Fig. 3). Among them, the neutral type is the most stable, and the blood stasis type is the most prone to inactivation.

3.4. Analyze the difference of sucrase-isomaltase
In conjunction with major databases, SI was used as the target for disease correlation and drug analysis. After analysis, it was found that sucrase-isomaltase and environmental factors play a more
important role in the process of enteritis to intestinal cancer. Nutritional factors, microorganisms, drugs, and other environmental factors can be used as biological signals to affect the body's homeostasis, affect the body's metabolism, immunity and other physiological processes, and also affect the intestinal mucosal immune function.

4. Conclusion
Based on the correlation and difference between patients with colorectal adenoma and healthy individuals, the role of physical factors in the occurrence of colorectal cancer is analyzed. The constitution susceptible to colorectal adenoma is mainly Yang deficiency type, followed by blood stasis type. In this study, combined with the analysis of the sucrase-isomaltase difference of traditional Chinese medicine, it was found that the two are correlated but not statistically significant, which may be related to the sample quantity investigated. If you can adjust the Yang deficiency and blood stasis type, there is a possibility to reduce the occurrence of colorectal adenomas. It is hoped that the later work can use sucrase - isomaltase as the target for disease correlation and drug analysis, establish related target prediction and component-target-pathway/disease network, and provide medication reference for patients with colorectal adenoma of different constitutions.

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