

Application of Metabolomics in Oral Cancer

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Abstract: As an extension and terminal of genomics and proteomics, metabolomics has recently become a research focus of disease prevention, pathogenesis and disease diagnosis. With the development of metabolomics, its research in oral cancer and potential malignant lesions of oral cavity can provide important reference materials for laboratory and clinical practice. This paper reviews the theoretical basis of metabolomics, detection methods and analysis methods in oral cancer and potential malignant lesions of oral cavity.

Keywords: metabolomics; oral cancer

1. Introduction

Oral cancer is one of the most common malignant tumors in the world. It occupies the sixth place in the whole body malignant tumors and shows an increasing trend year by year^[1]. WHO reported that the diagnosis of Oral squamous cell carcinoma (OSCC) begins and survives for 5 years. The rate is about 55%, while the survival rate of advanced disease is only 30%^[2]. It has been reported that early diagnosis of OSCC can lead to a 5-year survival rate up to 80% ~ 90%^[3]. Therefore, early detection of cancer and potential evil venereal lesions are important for cancer prevention, early diagnosis and treatment meaning.

At present, the gold standard for the diagnosis of oral squamous cell carcinoma is pathological examination, but at present, the preoperative auxiliary examination is basically only imaging examination (such as CT and MRI), and the means are relatively simple. Now we can study more non-invasive and simple methods to assist diagnosis.

2. Metabolomics

Metabolomics is a newly developed discipline after genomics and proteomics. It is a part of systems biology to quantify all kinds of metabolites in an organism and find the relative relationship between metabolites and physiopathological changes. Most of the research objects are small molecular substances with relative molecular mass within 1000. Instead of genomics, transcriptomics, and proteomics telling you what might happen, metabolomics directly and accurately reflects the current state of an organism and tells you what actually happened.

Metabolites are the basis of phenotypes of organisms and can help in a more intuitive and effective understanding of biological processes and their mechanisms. Metabolomics can be used for the analysis of metabolic pathways or metabolic networks, and the metabolic basic research of the macroscopic phenotype phenomena of different biological individuals is widely used in clinical diseases, biomedicine, agriculture, forestry and animal husbandry, Marine aquatic products and other fields.

Untargeted metabolome is a commonly used method of metabolomics research, the main research idea to compare the experimental group and control group, detect all metabolites in the sample, and obtain quantitative information, find statistically significant differences between different groups metabolites, can explain the found metabolites and biological process or biological state.

The metabolites generated by the organism's metabolism are closely related to the biochemical state of the organism. When the body has pathological changes, its homeostasis will be broken and then cause the change of metabolite concentration. Studies have found that changes in metabolite concentrations have been observed in the urine, blood, saliva and tissues of patients with oral cancer

and potentially oral malignant diseases, which helps to understand the pathogenesis of oral cancer and potential malignant lesions of the oral cavity.

3. Methods for studying metabolomics

Metabolomics research process generally includes the collection and pretreatment of biological samples, on-based detection, data collection and processing, identification of metabolic markers, biochemical pathway analysis, etc.

3.1. Biological sample collection and pretreatment

Metabolomics samples in tumor research usually include biological fluids (plasma, serum, saliva, urine, ascites, bile, etc.), cells (extracts, culture media), and tissues. In the field of oral cancer, OSCC starts from squamous epithelial cells, and through body fluid transfer and diffusion, easily accessible blood and saliva is the most common sample, and tissue and cell samples are becoming more common. Because metabolites are easily affected by external factors, the differences between samples (such as age, gender, location, living environment, physical condition, medication history, etc.) should be considered to determine the inclusion conditions, and maintain the consistency of operation and preservation conditions. The purpose of biological sample pretreatment is to maximize the removal of impurities and interfering substances. According to the purpose of the experiment, non-targeted metabolomics requirements as far as possible to retain the complete information of metabolite components, using the pretreatment method is mainly using organic solvent (methanol, acetonitrile, etc.) to extract small molecule metabolites, targeted metabolomics extract target metabolites for accurate analysis, research in addition to using organic solvent extraction, specific metabolites can also take solid phase extraction, solid phase micro extraction for special treatment.

3.2. Analytical technology platform

After the sample is processed for pretreatment, the metabolites are detected on the appropriate analytical technology platform. At present, the most commonly used techniques in metabolomics research are divided into two categories: nuclear magnetic resonance (NMR) based and mass spectrometry (MS) based methods. NMR analysis covers a wide range of samples ranging from bulk fluids to whole organs without treatment. The advantages are that the sample pretreatment is simple or even directly injected, the analysis cost is low, good repeatability. However, it has low sensitivity and narrow dynamic range, making it difficult to conduct quantitative analysis. In NMR methods, high-resolution NMR can solve the defect of low sensitivity^[27]. Somashekar used high-resolution magic angle rotating nuclear magnetic resonance (HR-MAS NMR) spectroscopy and found significant differences in their metabolites, and successfully distinguished normal and cancerous tissues^[28]. Compared with the disadvantages of NMR, MS has high sensitivity and can improve its separation ability with chromatography, including temperament (GC-MS), liquid mass (LC-MS), capillary electrophoresis mass spectrometry (CE-MS). GC-MS has high sensitivity and separation ability, and comes with structure identification spectrum library, which facilitates qualitative analysis of metabolites. However, macromolecules and difficult volatile metabolites in organisms are not suitable for direct meteorological analysis, and more complex treatments are needed to convert them into volatile derivatives. In recent years, the research hotspot of GC are full two-dimensional gas chromatography (GC/GC), and fast, micro GC instrument. In contrast, LC-MS avoids the complicated sample pretreatment in GC-MS, has high sensitivity and wide dynamic range, and is good at qualitative and quantitative analysis, which has become the most important metabolomic analysis platform. However, it also has corresponding disadvantages, such as the need for standards and independent databases, high cost relative to GC-MS analysis, and matrix effect. Recently, with the application of supercritical fluid chromatography and ultra-efficient liquid chromatography, the separation capacity and separation speed have been further improved, and the research scope has also been expanded. CE-MS can separate polar or ionized compounds well, and it has the advantages of small sample size, fast analysis speed, high resolution, and many operation modes. However, its concentration sensitivity is not as low as that of LC-MS, and MS will limit the application of CE separation buffer. Buscher Comparing GC-MS based, LC-MS and CE-MS, using 91 metabolites including glucose metabolism, amino acid metabolism and nucleotide metabolism pathway, and found that LC-MS can separate strong polarity, weak polar and non-polar compounds, which are most suitable for metabolomics research in the comparison. However, to detect all the metabolites in the sample at the same time, it is difficult to

use only one method. Multiple methods can help measure more metabolites and get more relevant information.

4. Salivary metabolites in the study

Saliva is an important physiological humour, rich in serum products and various immune cells. Because of its non-invasive, simplicity, timeliness, popularity, reliability and other advantages, it has been widely used. In recent years, with the development of salivary omics, there has been a potential to become blood and urine substitutes and is further defined as a "potential pool of biomarkers". Keiingishu University in Japan used the whole spectrum amino acid metabolomics technology to analyze the metabolites in the saliva of cancer patients and healthy patients, and found that there were obvious differences in amino acid content, and the accuracy of distinguishing the two was as high as 80%^[29]. OLP is chronic non-specific inflammation of the oral mucosa with a cancerous rate of 0% to 12.5%. Yan SK et al. used LC-MS technology to study the saliva metabolomics of OSCC: OLP and OLK patients and normal population, and showed that this method can effectively distinguish all kinds of OSCC, OLP and OLK 3 diseases, and the accuracy of the method reached 100%, indicating that this method can be used for the screening of oral cancer and potential malignant lesions^[30]. A significant difference in salivary metabolite content in OSCC patients, OLK patients and healthy people, Wei J et al^[31]. The OSCC and OLK groups had increased lactate content and decreased valine and phenylalanine content compared with the normal control groups. It is suggested that salivary metabolite detection can identify OSCC and potential malignant lesions of the oral cavity.

5. Studies of other tissue metabolites

5.1. Blood

Blood contains a variety of cellular metabolites, hormones and enzymes and other components, nutrition, regulation and defense functions. The blood not only stores human genetic information, but also carries human metabolic information, which is also a commonly used sample for metabolomics research. OLK is a white plaque or plaque on the oral mucosa that cannot be diagnosed clinically by clinical and histopathological means. As the most common potentially malignant lesion of the oral cavity, the cancer change rate was 1.58% to 27.27%. Studies have shown that the screening of people at high risk of OLK allows for the early detection of OSCC. Ashish Gupta And others^[32] using the NMR hydrogen spectrum (1H-Nuclear Magnetic Resonance Imaging, 1H-MRI) combined with OPLS-DA method analyzed the metabolites in OLK patients, OSCC patients, and the serum of healthy population, Eight differential metabolites were obtained, Some of these metabolites such as glutamine, propionate, acetone and choline can distinguish OLK from the other two with as high as 93.5% accuracy, While glutamine, acetone, acetic acid and cholinergic distinguish OLK and OSCC with an accuracy of 92.4%, It suggests that metabolomics techniques can accurately distinguish oral cancer from oral potential malignant lesions, This provides a new direction for the early detection and diagnosis and treatment of oral cancer.

Tiziani S et al^[33] showed that metabolite profiling can distinguish OSCC patients from healthy populations and can also distinguish between early and late OSCC patients. The amino acid, fat and glucose metabolism in plasma of OSCC patients, more pronounced in late stage than in early patients. It suggests that the metabolomics technique can distinguish oral squamous cell carcinoma and its stage lesions.

5.2. Urine

Urine, as the "scavenger" in the body circulation, is closely related to the body metabolism. He Hongbing et al^[34]. Through the urine of oral good, malignant tumor patients research shows that: including benign and malignant lung cancer patients with energy, lipid metabolic disorders, oral malignancy patients in TCA cycle, inositol metabolism disorder, prompt metabolomics technology for oral good, malignant tumor early diagnosis and treatment to provide important basis.

5.3. Other organizations

The incidence of oral cancer in India is the highest in the world, with the incidence rate of OSF

ranging from 7% to 13% [35]. Musharraf et al.[36] received a total of 51 mucosal tissue biopsies, including 15 OSF patients, 21 OSCC samples, and 15 healthy population control samples (healthy population from third molar flap extraction). GC-MS technology was then used to study the samples, which showed that metabolomics technology can identify biomarkers and can significantly distinguish oral cancer from normal population. In this study, oral cancer and potentially malignant lesions of OSF had reduced amino acid levels compared with controls. It suggests that the metabolism of amino acids can be a new direction for early diagnosis and treatment of oral cancer and potential malignant lesions of oral cavity. The Warburg effect points out that the vast majority of tumor cells produce energy mainly through glycolysis[37]. Under aerobic conditions, glycolysis is the pathway of glucose catabolism into the TCA cycle. The TCA cycle acts as a sugar, lipid and amino acid metabolic hub and is also their final metabolic pathway. It can be seen that tumor cells further affect the metabolism of energy, lipids and amino acids by affecting glycolysis.

6. Discussion

Metabolomics, as a research method in recent years, is a technique to study the metabolic pathways of biological systems by observing the changes of their metabolites or perturbation. It is more closely related to the phenotype characteristics of tumors than genomics and proteomics. At present, many scholars to explore the metabolomics is very active, and is trying to apply to a variety of metabolic biomarkers in the process of tumor development, through metabolomics capture and analysis of disease early characteristic metabolites, understand the cause of the formation of disease in order to realize the cancer early screening, early detection, early diagnosis, early treatment, so that the cancer incidence and mortality is effectively reduced.

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