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Review

Bioanalytical methods for metabolomic profiling: Detection of head and neck cancer, including oral cancer

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ABSTRACT

Metabolomics is an emerging field dealing with the measurement and interpretation of small molecular byproducts of biochemical processes, or metabolites, which can be used to generate profiles from biological samples. Promising for use in pathophysiology, metabolomic profiles give the immediate biological state of a sample. These profiles are altered in diseases and are detectable in biological samples, such as tissue, blood, urine, saliva, and others. Most remarkably, metabolic profiles usually are altered before symptoms appear in a patient. For this reason, metabolomics has potential as a reliable method for an early diagnosis of diseases through disease biomarker identification. This application is most prevalent in cancer, such as head and neck cancer (HNC). Metabolomic studies offer avenues to improve on current medical techniques through the application of mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), and statistical analysis to determine better biomarkers than those currently known. In this review, we discuss the use of MS and NMR tools for detecting biomarkers in tissue and fluid samples, and the appropriateness of metabolomics in analyzing cancer. Advantages, disadvantages, and recent studies on metabolomic profiling techniques in HNC analysis are also discussed herein.

1. Introduction: metabolomics and cancer

Metabolomics is the study of the entirety of a metabolic profile of a given biological structure or compartment, or a representative subgroup therein. It harnesses the chemical fingerprints of cellular processes and uses them to develop real-time profiles of the state of an organism, tissue, or cell through its metabolome, which represents the total unity of the metabolites and chemical byproducts in a cell, tissue, organ, or organism, depending on the scale under study, providing an instantaneous snapshot of the physiological state of a biological compartment at the moment of sampling. Although proteomics and genomics aid metabolomic analysis, metabolomic approaches give the most holistic analysis, bearing significant promises for medical applications, from tuberculosis [1–3] to bipolar disorders [4,5].

Bioanalytical approaches utilized in metabolomics studies could potentially be developed into new diagnostic tests that give a more detailed understanding of disease pathogenesis, as well as better prognostic and diagnostic approaches for patient care, and have already been well-established in cancer, in general, more specifically in head and neck cancers (HNCs). In order to reach the goal of having clinical tests being replaced by metabolomics related exams for HNC, all aspects of the metabolomic process, including the selection, preparation, analysis, and interpretation of samples, must be examined, perfected, and standardized to ensure uniformity and remove variation. Detailed attention should be focused on the type, number, and time of collection of samples, as well as the storage conditions, type and stage of cancer, medications ingested, and the characteristics of the patient, such as age, diet, sex, and other extenuating circumstances [6,7]. To eliminate statistical anomalies [8], a large number of control and afflicted patients must be used. All researchers need to be fully aware that the safety and the rights of the human subjects under study need to be protected and invariably occupy the highest priority. This is ensured by following the provisions of Good Clinical Practice, the international quality standard for clinical trials.

Metabolomics, however, can be a challenging field for this kind of analysis. The intrinsic nature of metabolites themselves is varied and ever-changing, with a large dynamic range, changing based on time

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dependency, inter-individual variation, and others [9]. Even further, meaningful changes in metabolite profiles can indicate diseases, natural physiological fluctuations, and variety in aspects such as diet, or even sample transport, freezing, and storage procedures that can change between two research groups [10]. Usable data could be obscured by these phenomena as well. Due to these issues, studies need to be under strict and reproducible conditions. Analysis is usually done using spectrometric and spectroscopic techniques for this reason, usually by mass spectrometry, nuclear magnetic resonance spectroscopy, and fluorescence spectroscopy [5]. It is crucial to painstakingly establish and follow the detailed collection and preparation protocols to standardize the data. These standard operating procedures (SOPs) are necessary for the highest accuracy of metabolomic analysis.

To improve on current metabolomic methods, a comprehensive technique would be useful as discussed herein in relation to HNC. A successful application to HNC would indicate feasibility concerning other cancers and diseases as well. An ideal technique would detect HNC early, before other symptoms used in current diagnoses appear. This would be beneficial in treating these cancers, preventing metastasis and other complications. A screening test would be best, which would encompass the benefits and sensitivity of current diagnostic techniques into a more cost-effective and less invasive procedure. It must have a high sensitivity, specificity to HNC, reproducibility, and ease of performance that matches current techniques and allows to be used by all. This must depend on small-molecule metabolic profiles, specific to HNC and detectable through fluids such as urine, saliva, and blood, or tissue, among others. Changes in the profiles and concentrations of these markers come from differing pathological conditions. Monitoring such changes allows for diagnoses and tracking treatments, due to its to-the-minute profiling.

Currently, most cancer diagnostic tests use the principles of biomarkers [11] such as the prostate specific antigen (PSA), upon which metabolomics improves. Biomarkers are metabolites whose concentration is correlated to certain complex physiological parameters or states, such as diseases. Newer than the other "omics", genomics and proteomics [12], metabolomics uses small molecules as biomarkers instead of proteins and nucleic acids, offering new routes to identifying and quantifying biomarker metabolites. Complex physiological states may not be detectable by an individual biomarker alone, but in the concentration patterns of a group. These fingerprints found through metabolomics could greatly influence the field of oncology [13]. The most powerful technique would be a combination of all the major "omics" analytical data [14], but metabolomics on its own provides a more holistic offering of data as an individual group. Since this research is relatively new in the "omics", recent advances are only beginning to make an impact in the diagnosis and management of diseases at a clinical level.

Before medical applications can be fully realized, basic principles of metabolomics must be established. Metabolomics searches for metabolite variations that differentiate between healthy and diseased samples, providing diagnoses. It also allows a better understanding of the underlying mechanisms of a disease through determining changes in metabolites, and discovering the reasons for these changes, monitoring the progress of the disease and giving insight into pathology while providing biomarkers that can diagnose the disease. Once these principles are well-established, metabolomics can progress to clinical implementation and usage.

2. Head and neck cancer (HNC) and head and neck squamous cell carcinomas (HNSCC)

HNCs encompass cancers of the mouth, throat, larynx, lip, squamous cell carcinoma, and other related diseases, including leukoplakia and periodontal disorders [15]. Head and neck squamous cell carcinomas (HNSCC) are sometimes distinguished from general HNCs. HNSCCs are cancers of the epithelium of the head and neck area. Epithelia include the outer layer of cells that make up the covering of the body surfaces, lining the cavities and surfaces of structures throughout the body, including many glands. They function in secretion, like sweats and oils for the skin, selective absorption, like the reabsorption of water, protection from bacteria and other damaging substances, and sensation. They are avascular, nourished from cells below. Many are made up of squamous cells, flat cells that are wider than they are tall, named for its scale-like appearance. Examples in the head and neck area include exterior skin, the outer layer of the lip, the visible mouth, and the outer throat. Epithelia are targets for cancer; as the outer layer, they contact carcinogens, such as UV light, cigarettes, and others, first. HNSCCs constitute 90% of all HNCs [16]. HNCs are the eighth most common cancers in the United States of America, accounting for about 3% of all new reported malignant tumors and 2% of deaths [17]. About 42,440 new cases and 8390 total deaths, from new as well as former cases, were predicted for 2014 [17].

Risk factors for HNC include tobacco and alcohol consumption [18]. Current diagnoses depend on invasive procedures, such as needle biopsies. However, such a procedure is only feasible in certain cases, and is based on previous symptoms of HNC, including halitosis, neck pain, sinus infections, and weight loss [19], all of which may also represent other diseases. More specific symptoms, such as detectable lumps in the throat or neck, are not always present in HNC. Furthermore, invasive procedures such as biopsies carry a risk of causing damage to the patient, as well as involve large costs.

Despite advancements in the detection and treatment of HNC, prognosis depends on the stage and site of the tumor, and the rate of metastasis, giving a poor survival rate [20]. Although tumor biology has become better understood, the effectiveness of chemotherapy is limited and disease resistance remains high [21]. Most current studies use tumor cells and tissue *in vitro* [22,23] and *in vivo* [24,25], to combat HNC. In contrast to a cellular approach, a global metabolomics study provides more information to understand HNC and identify biomarkers to diagnose it [26].

Current HNC diagnosis depends on a physical examination, with blood and urine tests, endoscopies, X-ray, computed tomography, and magnetic resonance imaging (MRI), normally in response to symptoms already present in the patient. Generally, diagnoses are confirmed by procuring a physical sample of tissue through a needle biopsy of the potential tumor, which is then examined for cancer, confirming or dismissing diagnosis. However, metabolomics can greatly improve on this procedure. HNC, as with all diseases [27], has a specific and detectable metabolic signature, separate from cancers and normal cells [28]. Therefore, metabolomic based analysis of head and neck cancer would lend itself easily to diagnosing this disease. This has already been seen with genomics and proteomics through mass spectrometry [29–35]. Metabolomics, giving an earlier diagnosis than even genomics and proteomics, would help fight HNC, removing the need for harsh treatments, involving surgeries and chemotherapy [36–38], and easing suffering on the road to recovery. Also, being non-invasive, HNC would cause less distress in the diagnostic process, ensuring that the patients' quality of life during treatment will improve.

3. Metabolomics methodology

Due to the chemico-physical variation in metabolic mixtures, analysis is extremely complicated. Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) have the necessary analytical power to overcome this, especially when