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Review

Membrane transporters in salivary exosomes and microvesicles as biomarkers of systemic or oral disease

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ABSTRACT

Background: saliva is useful to assess health or disease states. Recently, proteomic technologies have allowed rapid progress in saliva analysis.
Highlight: (1) saliva contains three main types of extracellular vesicles; (2) the vesicles are exosomes, microvesicles, and apoptotic bodies; (3) proteome is analyzed in saliva, salivary exosomes, and salivary microvesicles; (4) membrane transporters are in saliva, and salivary exosomes and/or microvesicles; (5) biomarker discovery in exosomes and microvesicles of saliva is progressing.
Conclusion: membrane transporters such as aquaporin, ion channels, carriers in saliva, and salivary exosomes or microvesicles, might be valuable biomarkers of systemic or oral health.

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1. Introduction

Saliva is an important body fluid that reflects systemic and oral conditions. Because saliva sampling is non-invasive, painless, stress-free, and relatively simple when compared with blood collection, saliva is a useful tool for assessing health or disease states. Because of these significant advantages, the discovery of salivary biomarkers associated with health and diseases is desirable.

Saliva is majorly (99%) made up of water, and the other 1% is composed of electrolytes, proteins, peptides, polynucleotides, hormones, enzymes, cytokines, antibodies, and other substances [1]. In recent years, proteomic technologies have rapidly progressed the

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analysis of saliva, confirming that 20–30% of the salivary proteome overlaps with the plasma proteome [2,3].

In body fluids, such as saliva, there are three main classes of extracellular vesicles: exosomes, microvesicles, and apoptotic bodies [4–6]. In contrast to microvesicles, which are generated by shedding from the plasma membrane, exosomes are derived from multivesicular bodies that are components of the endocytic pathway [7]. Biomarker discovery is now progressing with regard to analysis of salivary exosomes and microvesicles.

2. Traditional biomarkers

Salivary unbound steroids, such as cortisol, estradiol, and testosterone, are correlated with their free-form serum concentrations [8], while conjugated steroids, such as dehydroepiandrosterone sulfate and estriol-3-sulfate, are present in saliva at only 1% of their unbound serum concentrations. To evaluate serum hormone status, salivary unbound hormone levels are measured.

2.1. Cortisol

Salivary cortisol appears to be independent of transport proteins, such as albumin and cortisol-binding globulin, and therefore reflects the bioactive, free molecule [9]. Salivary cortisol levels are used to assess Cushing's syndrome [10], adrenal insufficiency [9], exercise-related stress [11], and mental stress [12].

2.2. Aldosterone

Aldosterone is lipid-soluble and enters saliva by passive diffusion through cells of the salivary gland; thus, the concentration in saliva does not depend on flow rate, giving a clinically useful index of unbound plasma levels [13]. Salivary aldosterone is used to assess primary aldosterone associated with cardiac fibrosis, ventricular and vascular remodeling, and increased cardiovascular morbidity and mortality [13,14].

2.3. Insulin

Salivary insulin concentration is positively correlated with plasma free insulin levels [15]. After glucose loading, plasma and salivary insulin can be correlated using an immunoreactive measuring method [16].

2.4. Adiponectin

Adiponectin is released from adipose tissue into blood and is abundantly present in healthy human plasma at 5–30 μg/mL [17]. Plasma adiponectin levels are inversely associated with visceral fat accumulation [18]. Adiponectin has anti-diabetic, anti-hypertensive, anti-inflammatory and anti-oncogenic functions. Hypoadiponectinemia induced by visceral fat accumulation is a strong risk factor for metabolic and cardiovascular disease, as well as some types of cancer.

Salivary adiponectin levels range from 0.37–6.42 ng/mL [19]. There is a significant correlation between plasma and salivary adiponectin levels. This suggests that salivary adiponectin may be a marker of increased risk of non-insulin-dependent diabetes mellitus or cardiovascular disease.

3. Salivary proteome and diagnosis

Proteomics is the large-scale study of proteins, and their structure and function. Although proteins are products of genes, multiple distinct protein isoforms can be created from a single gene. Because proteins are also susceptible to posttranslational modifications, proteomics more accurately reflect cellular processes. Secreted proteins released from diseased cells contain important biological information. Comparison of protein patterns in biological fluids between healthy individuals and patients with a given disease can be used to discover biological disease markers. The major analytical strategies in proteomics are fingerprint analysis and shotgun analysis.

Fingerprint protein analysis refers to bottom-down proteomics [20]. Proteins are separated first by two-dimensional gel electrophoresis and are then visualized by staining. To identify proteins, spots are excised and digested with trypsin. The resulting peptides are extracted and subjected to liquid chromatography–tandem mass spectrometry (LC–MS/MS) or matrix-assisted laser desorption/ionization-time of flight (MALDI-MS/MS) [21] for identification.

Shotgun protein analysis refers to bottom-up proteomics [22,23]. Proteins are first cleaved by enzymatic or chemical methods, and are then subjected to LC–MS/MS, MALDI-MS/MS or ESI–MS/MS [24] to recover peptides.

Using fingerprint analysis, 2340 proteins were identified in whole human saliva, and approximately 20% of total salivary proteins are seen in plasma [2]. On the other hand, 1939 proteins were identified in human saliva by the shotgun method and 27% of these have been observed in plasma [3].

Proteomic technologies are currently being used to discover disease biomarkers, particularly for oral cancer [25,26], lung disease [27], Sjögren's syndrome [28,29], and gingivitis [30].

4. Salivary exosomes and diagnosis

Exosomes are small membrane vesicles (40–100 nm in diameter) of endocytic origin that are secreted by most cell types upon fusion of multivesicular bodies with the plasma membrane [7]. Exosomes have been identified in body fluids, such as breast milk, blood, urine, semen, amniotic fluid, ascites fluid, and saliva [4]. Exosomes participate in intercellular communication [31–33], immune regulatory functions [34], transport of morphogens and RNA [35–37], and tumor metastasis [38].

Gonzalez-Begne et al. cataloged 491 proteins in the exosome fraction of human parotid saliva using the shotgun approach [39]. Forty-three percent of 491 proteins were of cytosolic origin, 26% were integral plasma membrane proteins, and 13% were associated/peripheral plasma membrane proteins. Integral plasma membrane proteins were annotated according to their function: pumps (2%); channel protein (5%); and solute carriers (14%).

Ogawa et al. identified two types of exosome in whole human saliva; exosome I (mean diameter: 84 nm) and exosome II (mean diameter: 40 nm) [40]. Exosomes I and II contain numerous plasma membrane proteins: single-pass transmembrane proteins, such as type I and type II membrane proteins; tetraspanins; pentaspans; the six-pass membrane protein, aquaporin-5; and seven-pass membrane proteins. Alpha-amylase (about 20% of salivary proteins) and proline-rich proteins (about 40% of salivary proteins) were also detected in exosome I and exosome II. Exosomal proteome studies are listed in Table 1.

Salivary exosomes have been shown to be useful in detecting diseases, such as Sjögren's syndrome [41], as well as head and neck cancer [38].

5. Salivary microvesicles and diagnosis

Microvesicles are generally larger (100–1000 nm in diameter) than exosomes [4–6]. Microvesicles shed from the surface of