

Review Article

Exosomes: Salivary Biomarkers?

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Abstract

Saliva is a bio-fluid considered similar to blood in that it contains various DNAs, RNAs and proteins. Therefore, it is a fluid with diagnostic potential. In recent time, exosomes are emerging as nano-vesicles which enhance intra-cellular communication. Exosomal content, which is dependent on the cell of origin, reflects physiological status of cells. Exosomes have potentials for use as biomarkers for variant diseases, based on their stability and availability in various body fluids. Current studies have proposed the role of exosomes as immuno-modulators in the etiology of auto-immune diseases and cancers. The present study focused on the role of exosomes as biomarkers and their therapeutic potentials in particular diseases related to the oral cavity.

Keywords: Exosomes, Auto-immune, Biomarkers, Saliva, Diagnosis

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INTRODUCTION

Human saliva is a clear and complex oral fluid which tends to coat the oral tissues. The saliva originates from different glandular and non-glandular secretions. The glandular secretions are produced from salivary glands, whereas the non-glandular secretions include crevicular fluids rich in oral microorganisms and host cells [1]. The salivary glands in human body are classified according to their sizes as major- and minor glands. The first category of major glands includes the paired parotid, and submandibular and sublingual glands [1]. The normal pH of human saliva ranges from 6.0 to 7.0, indicating

that if it is slightly acidic or neutral. However, the salivary pH can vary in large range from 5.3 to 7.8 depending on its flow rate, lowest pH at low flow rate whereas higher values at peak saliva flow [3]. The amount of hydrogen bicarbonate in saliva determines salivary pH and buffering capacity [4].

Saliva is 99% watery fluid, but becomes viscoelastic (thick and sticky) depending on the amount of protein content [2-4]. The average flow rate of unstimulated saliva in normal, healthy individuals is about 600 mL per day [5]. In a state of stimulation such as during meals, saliva secreted by parotid glands is about 60 -

65 % of whole salivary volume, with 20-30 % from submandibular and sublingual glands [6], and approximately 10 % from minor salivary glands. In the resting state, the situation is different: approximately 50 % of total saliva is secreted by submandibular glands [6,7].

The saliva has a capability to provide links to local- and systemic diseases, since it contains molecules of RNA, DNA, salivary proteins, and metabolites, as well as microorganisms. Any alteration in the molecular content is considered as biomarkers for detection and monitoring of diseases [8].

Exosomes are bi-layer, lipid-enclosed nano-vesicles of 40 – 100 nm in diameter [9]. They are formed by endosomal endocytosis, resulting in multiple vesicular bodies (MVBs) containing intra-luminal vesicles (ILV). Being endosomal in origin, they act as carriers for fusion proteins, MVB biogenesis proteins, heat shock proteins, lipid-related proteins, integrins, phospholipases and tetraspanins [10]. Depending on the cells of origination, exosomes also carry nucleic acids, lipids and the proteins [11]. Different cells like hematopoietic cells, cells of intestinal epithelium, adipocytes, tumor-like cells, fibroblasts and Schwann cells secrete various exosomes in body fluids (plasma, urine human milk and cerebrospinal fluid) [12]. Willms *et al* [13] have reported that inconsistent exosomal sub-populations have various special effects on the recipients' cell gene expressions. In particular, the transporter endosomal sorting complex (ESC) is composed of proteins active in budding endosomal membranes [14]. These proteins are marked liable for sorting and biosynthesizing of MVB involved in exosome formation [14].

The exosomes tend to fuse with the cellular membrane of the targeted cells, prior to emptying their contents in the cytoplasm, followed by endocytosis. The exosomes enter via various pathways such as macro-pinocytosis, endocytosis dependent on the clathrin–dynamain–caveolae, and the phagocytosis relying on the recipient cells [15]. These exosomes are thought to be responsible for cellular communication, regulation of gene expression, alteration in cellular signaling, and functional modification of target cells [16]. Alterations in the molecular contents of exosomes have been observed during pathophysiological, cellular or tissue changes. Thus, exosomes are expected to act as diagnostic markers.

Moreover, exosomes are involved in the pathogenesis and progression of diseases such as metastasis of cancer, neural degradation,

cardiac hypertrophy, renal and auto-immune disorders [17]. Recent studies have shown the diverse potential of exosomes such as induction of immunity for cancer cells, bacterial infections, evasion of host immunity, and reduction of inflammation, indicating their potential as smart therapeutic-vehicles for various diseases [18]. The major purpose of this review was to present contemporary and updated information regarding the use of exosomes in systemic auto-immune diseases with oral manifestations, as well as its biomarker and remedial potential

Potential role of exosomes in oral manifestation of auto-immune disorders

Exosomes are involved in various processes such as immuno-modulation and immune-regulation, and they have potential for intercellular communication by transporting regulatory molecules to adjacent and the distant cells [19]. Various investigators have reported the potential of exosomes for direct and indirect activation of T cells by dendritic cells [20]. Exosomes tend to arbitrate transportation of antigens directly by transportation of major histocompatibility complex and co-stimulatory molecules [21]. Indirectly, exosomes help in transfer of antigens to antigen presenting cells [22]. Mature dendritic cells which give rise to exosomes have immune-stimulatory effects, whereas immature dendritic cell exosomes (DCex) have immune-suppressive potential [23]. Mature DCex, in contrast to those produced by immature DCex, have extensive number of co-stimulatory and adhesive ligands expressing immune-suppressive ligands [tumor growth factor- β (TGF- β), NKG2D, and galectin-9]. Moreover, they tend to secrete death ligands e.g. CD95L [24].

Exosomes in autoimmune salivary gland disorder

Exosomes have been studied in Sjogren's syndrome (SS), an autoimmune salivary gland disorder which was first described as an auto-immune condition characterized with dry mouth and dry eyes in 1933 by a physician, Henrik Sjogren [25]. A chronic, multi-system autoimmune disease can be identified from peri-epithelial infiltrates of lymphocytes which lead to inflammation of the exocrine glands. The auto-antigens linked to SS and other auto-immune disorders are Anti-Ro (SSA)/ La (SSB). Kapsogeorgou and co-workers [26] have reported secretion of Ro/SSA, La/SSB and Sm from non-neoplastic salivary gland epithelial cells (SGECs), showing auto-immune exosomal regulatory response to SS. Other than the

transportation of auto-antigens, exosomes tend to transport conserved, small, non-coding RNA tangled in post-transcriptional modulation of genes known as microRNA (miRNA). Researchers have reported the effect of miRNA in regulating immune response, and have shown crucial regulatory mechanisms relative to auto-immunity [27]. Gallo and co-workers [28] have reported various miRNAs associated with exosomes in saliva.

Researchers have also reported the isolation of microRNA from the exosomes of parotid gland of patients suffering from primary SS and healthy volunteers [29]. The occurrence of three miRNAs (has-miR-203, has-miR-786-3p and has-miR-574-3p) has been reported, using Taq-Man miRNA quantitative amplification. Similar miRNAs have also been observed in the saliva produced by the minor salivary gland and whole saliva in patients suffering from primary SS. It has been reported that miRNA has-miR-786-3p and has-miR-574-3p can be used to identify patients with primary SS, based on salivary gland focus scores [30]. Recently, has-miR-786-3p, a small nucleolar RNA (snRNA) has been identified as a biomarker for primary SS. In the near future, research on exosomes may lead to a detailed understanding of the etiology and pathogenesis of SS.

Exosomes produced by saliva contain non-invasive diagnostic information regarding the patho-physiological condition of the salivary gland. The miRNA of exosomes are more stable and more impermeable to degeneration in long-term storage and freeze/thaw cycles than the circulating RNAs [31,32]. This is likely due to lipoproteins in the exosomes which protect them from degradation. A false positive result may be observed due to circulating miRNA derived from dead cells or inflammatory cells. The stability of exosomes and their cargo increases the reliability of exosomes as potential biomarkers.

Exosomes in oral lichen planus

Oral lichen planus is an inflammatory mucosal condition which is idiopathic in origin. Various studies have been conducted on the extraction of exosomal miRNA from whole saliva of individuals suffering from chronic oral lichen planus (OLP), an auto-immune disease. Oral lichen planus (OLP) can be identified from the presence of keratotic/erythematous and ulcerative lesions [33]. Byun and co-workers [33] demonstrated up-regulation of has-miR-4484 in the salivary exosomes of patients with OLP. Thus, has-miR-4484 may be a potential biomarker for OLP. Statistically significant

difference in exosomal expression between diseased and healthy individuals makes exosomes attractive biomarkers for OLP. Moreover, miRNAs are linked with variations of cytokine expressions in OLP, indicating its pathogenesis [34].

Salivary exosome as a potential biomarker for oral cancer

As a first line of defense, saliva contains various enzymes, proteins and immunoglobins. Proteomic analysis has revealed that saliva contains high levels of immunoglobulin A (IgA) with anti-inflammatory effect [35]. The oral squamous cell carcinoma (OSCC) is one of the most widespread oral cancers which accounts for more than 90 % of various cancers in oral region with poor prognosis [36]. It is usually diagnosed at an advanced stage, with more than 50 % of patients presenting with lymph node involvement [37,38]. Although many advances have been made in treatment approaches for the cancers, the survival of the patients (less than 50 %) has not significantly improved [39]. A group of researchers demonstrated differences between exosomal morphology and molecular features of healthy individuals, and those of patients suffering from oral cancer, which led to marker for early diagnosis of malignancy changes in patients with higher risk of cancer [40].

Another group of researchers observed that exosomes derived from the hypoxic cells of OSCC tended to increase migration and invasion in an HIF-1 α and HIF-2 α -dependent pattern [41]. The miRNA derived from exosomes of normoxic and hypoxic OSCC cells displayed different expression levels of 108 miRNAs, with miRNA-21 up-regulated significantly in hypoxic OSCC cells. This suggests that hypoxic environment has potential to stimulate tumor cells for production of miRNA-21-enriched exosomes, which in-turn, are delivered to normoxic cells for the promotion of pro-metastatic behavior. Furthermore, the exosomes rich in miRNA-21 target the phosphatase and tensin homolog (PTEN) and protein 4 for programmed cellular death (PDCD4) [42]. Kawakubo-Yasukochi and co-workers [43] reported that highly-invasive OSCC cell exosomes containing miRNA-200c-3p can lead to a similar type in the non-invasive regions. On salivary profiling analysis 381 proteins have been detected from extracellular vesicles (EVs) of both OSCC and healthy individuals, but only 8 were expressed differently, i.e. alpha-2-macroglobulin, haptoglobin alpha chain, mucin 5B, galectin-3-binding protein, immunoglobulin alpha-1 chain c region, prolactin-inducible protein, pyruvate

kinase isozymes M1/M2, and glyceraldehyde-3-phosphate dehydrogenase [44].

Therapeutic exosomes

Besides being biomarkers, exosomes have potential of being therapeutic agents for various diseases. The deterioration of exosomal content is controlled by a biogenic non-synthetic lipid bilayer [45]. Being small and flexible, the exosomes have potential to pass through the membranes and prevent phagocytosis/deterioration by macrophages. Being stable in various biological fluids, exosomes can send their contents to a variety of cells present throughout the body. Exosomes are endogenous vesicles with higher bio-safety than synthetic liposomes [46]. Ohno and group of workers [47] demonstrated suppression of cancer growth in xenografted mice through delivery of tumor-suppressing micRNA via exosomes. Moreover, exosomes with siRNA can be used for suppression of cancer cells in xenografted mice [48]. Exosomes can be contrived to explicit cancer-specific antigens, which in turn, stimulate anti-tumor immune response [49].

Exosomes can be used as nano-carriers since they exert little or no toxic effects. These endogenous vesicles are used for immune-therapy of various diseases such as rheumatoid arthritis, cancer, multiple sclerosis and inflammatory bowel disease [50]. Munich and co-workers have demonstrated that exosomes derived from the dendritic cells (dexosomes) can stimulate caspase activation and tumor cell apoptosis in a murine model [51]. During initial clinical trials (Phase I) for treatment of melanoma and lung cancer in humans, immune-therapy with dexosome promoted innate, disease stabilization and adaptive immune response, thereby increasing survival of patients [52].

Dexosomes have shown potential for use in the treatment of Alzheimer's disease through delivery of RNA interference (RNAi) to specific parts of the brain [53]. Kim and co-workers [54] have reported IL-4-containing dexosomes with potent effects on collagen-induced arthritis in a murine model. Furthermore, various studies in murine models have demonstrated that exosomes generated by mesenchymal cells have potential to reduce myocardial ischemia and reperfusion injury [55], enhance neurovascularization [56], and confer protection from acute-tubular injury [57, 58]. Thus, it can be concluded that exosomes have potential for treatment of various diseases. However, till date,

studies on this subject have been limited to animal models.

CONCLUSION

In healthy individuals, the main function of exosomes is regulation of cellular function and intracellular communication. On the other hand, under abnormal conditions, exosomes increase with severity of illness, invade host immune-system, and mediate cancer metastasis. Furthermore, due to their availability in various body fluids/cell types, exosomes serve as potent biomarkers for disease diagnosis, pathogenesis and prediction of treatment response. Further research is required to unravel the regulation and effect of exosomes in recipient cells.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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