The Roles of Exosomes Related to Some Oral Diseases

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Abstract. Exosomes are nanometer-sized, membranous vesicles secreted by many cell types into surrounding extracellular space and body fluids. In recent years, studies have shown that exosomes play important role in many physiological and pathological processes such as immune regulation, cell differentiation, infection and cancer. As an efficient mediator of intercellular communication, exosomes have been demonstrated to widely exist in oral fluid and supernatants. Meanwhile, the important roles of exosomes in some oral diseases have also been revealed. This review aims to introducing characteristics of exosomes in oral cavity, summarizing their functions in different physiological and pathological conditions and provides the further insight into potential application of exosomes to disease diagnosis and treatment.

Introduction

As one of the most important part in our body, oral cavity bears many vital functions such as speaking, chewing and swallowing. Oral diseases not only influence the living quality of patients but threaten their life in certain circumstance also. Oral cancer, oral lichen planus (OLP), external root resorption etc. still have a series of uncertain factors in their diagnosis and treatment. Therefore, more sensitive, safer indicators are needed for prognostic and diagnostic method to detect lesion in an early stage, making this method more favourable for clinical therapy. Exosomes, as a crucial mediator of intercellular communication, have been widely researched in multiple physiological and pathological processes [1-3]. Their applications as biomarkers for disease diagnosis or as carrier for drug delivery were being explored as well [4-6]. Thereafter, this review is focusing on the role of exosomes related to oral diseases, and the potential application to disease diagnosis and treatment will be discussed simultaneously.

Exosomes in Oral Cavity

Almost all tissues and cell types produce and release exosomes that are distinct population of microvesicles ranging from 30 to 150 nm in size. Exosomes are excreted into various body fluids after the fusion of multivesicular bodies with the plasma membrane under normal and pathological conditions [7-11]. Relative to the composition of the cytoplasm, exosomes contain abundant components such as lipids, nucleic acids, and proteins and these contents can serve as cargo for intracellular communication [12-15]. Studies in recent decades indicate...
that exosomes’ roles exert in several aspects including immune response, tumor, and infectious diseases [16-22]. In the field of stomatology, abundant exosomes were detected and their characteristics were demonstrated in several studies. Kapsog Georgou EK et al confirmed that non-neoplastic salivary gland epithelial cells (SGECs) secret abundant exosomes and contain RNPs, which are major autoantigens in systemic rheumatic diseases [23]. Ogawa et. al. found exosome-like vesicles in saliva [24], which are exosomes I and II, differing in size and protein composition [25], and the average diameter of exosome I is 83.5 nm and the exosome II is 40.5 nm: Proteomic analyses indicated that immunoglobulin A, polymeric immunoglobulin receptor and some exosomal biomarkers (Alix, Tsg101, and Hsp7) can be found in both of the exosomes but DPP IV, carbonic anhydrase 6, and cystatin family proteins are expressed more abundantly in exosome II, while ezrin, moesin, radixin, Rab GDP dissociation inhibitor beta, alpha enolase, guanine nucleotide-binding protein Gi/Gs/Gt subunit beta-1, and annexins are expressed only in exosome I. Palanisamy V et al [26] obtained saliva exosomes and found 509 mRNA core transcripts in these exosomes by microarray assessment, and saliva exosomes can be taken up by oral keratinocytes and alter gene expression by transferring genetic information to recipient cells.

The Roles of Exosomes Related to Oral Diseases

Reflecting Oral Pathophysiology Change via the Variation of Characteristics

Usually, exosomes are thought with specific structure and some common exosomal biomarkers, however, under certain circumstance, exosomes can present characteristics aberrations. Sharma S et al[27] found that oral cancer derived exosomes displayed a higher level in particle concentration, a wider range in size distribution (normal: 40–80 nm, oral cancer: 20–400 nm ), a more diverse in shapes by using AFM, which in accordance with the results by nanoparticle tracking analysis (NTA)[28]. Not only have these differences in size and shapes been confirmed to be different between oral cancer (OC) patients and health individuals (HI), but some exosomal biomarkers such as CD63, CD81 and CD9 also. The concentration of CD63 was higher in the OC (OC:234 ± 79 pg/ml, HI:176.2 ± 42.3 pg/ml) and in addition to the CD63 bands at 25 kDa , a more prominent glycosylated form of the CD63 band at ~53 kDa in OC also was found. However, the expression of CD81 and CD9 in OC, compared that in HI, was considerably lower, which may be due to their known functions of regulating cellular adhesion and motility [29-31].

In terms of contents of exosomes, exosomal proteins and mRNA were comparable between patients and health individuals. Rody et al found 37 upregulated and 59 downregulated root resorption proteins in gingival crevicular fluid between resorbing deciduous molars and nonresorbing permanent teeth, many of which are characteristically found in exosomes [32]. In the exosomes of hand, foot, and mouth disease (HFMD) and oral lichen planus (OLP), differential expressed exosomal miRNAs were found between patients and healthy controls. Moreover, several special miRNAs such as miR-4484, miR-671-5p, miR-16-5p, and miR-150-3p were identified in further verification [2, 33]. Based on the above-mentioned fact, we can speculate that except exosomal proteins or miRNAs, other exosome contents such as mRNA, lncRNA etc. may seem variable under different biological conditions. The research about the exosomes components may open a new perspective for diagnosing and elucidating the pathogenesis of oral diseases.
The changes of exosomes structure, morphology and contents in these diseases clearly indicated the influence of subject patho-physiological condition reflected in exosomes characteristics aberrations. These results suggested the possibility to distinguish an individuals’ patho-physiological condition via observing corresponding exosomes in the future, which maybe will establish a rapid disease screening method.

**Influencing Oral Diseases Progression via Transmitting Exosomal Contents**

Since the initial studies showed that exosomes can mediate the intercellular transfer of RNAs and proteins, many studies have focused on the contents of exosomes involved in intercellular communication [34]. In oral cancer, exosomes were reported to facilitate tumor progression by transferring miRNAs or proteins. For example, OSCC-derived exosomes delivers miR-21 between normoxic and hypoxic cancer cells, which can remodels the tumor microenvironment of OSCC and drive normoxic cells toward a prometastatic phenotype [1]. Sakha S et al reported that a highly metastatic human OSCC subline (HOC313-LM)-derived exosomes can deliver miR-342–3p and miR-1246b to HOC313 parental cells (HOC313-P), which contributes to increasing the migration and invasion ability of HOC313-P cells [35]. Similarly, the release of miR-142-3p via SEVs (including exosomes) was reported to increase the growth of oral cancer cells and enhance tumor supporting potential in cells in associated stroma, [36-38]. In terms of proteins, Caveolin-1 (CAV1), a protein associated with several tumors progress [39-41] was reported to be delivered to tumor microenvironment (TME) of tongue squamous cell carcinoma (TSCC) via oral cancer cell-derived exosomes. The accumulation of CAV1 in TME was reported to have a negative prognostic value [42]. In addition, CD109, a glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein, which was reported to play a role in the development of oral cancers, was found as a novel exosomal protein. Researchers speculated that exosomal CD109 might be transmitted to adjacent cancer cells or mesenchymal cells and regulate TGF-β1 signaling in these cells [43].

Besides the involvement in oral cancer, exosomal contents also play roles in viral infections. There is evidence demonstrated that human oral epithelial cell line (OKF6) - derived exosomes contain miR-200 family members, which can be transmitted to proximal EBV-positive B cells via exosomes. This process leads to the disruption of latency and production of new viruses, which facilitates the exchange of virus from the peripheral B-cell stores to the oral epithelium. Once the virus is transmitted to epithelial cells, the highly permissive nature of this cell type for lytic replication allows virus amplification and exchange to other hosts [44]. In addition, two HSV miRNAs (miR-H28 and miR-H29) made late in productive infection were reported to export in exosomes and they were confirmed to restrict HSV-1 replication and spread in recipient cells [45].

These evidences described above give us hopes that exosome inhibition has therapeutic value for treatment of oral diseases. However, a lot of mysteries remain to be unsolved. For example, what happened with the recipients when they received these exosome contents? How did these contents influence the recipients’ fate? Are there other obscure exosomal contents to be involved in the progression of oral diseases? A number of further researches will be needed to contribute to explaining pathogenesis of diseases.

**Promoting Oral Diseases Progression via Activating Signaling Pathways**

Several studies have been carried out in order to explain the mechanism of exosomes action. Sento S et al demonstrated OSCC cell-derived exosomes were taken up by OSCC cells themselves and significantly promoted proliferation, migration, and invasion through the
activation of the PI3K/Akt, MAPK/ERK, and JNK-1/2 pathways [46]. Sakha S et al reported that a highly metastatic human OSCC subline (HOC313-LM)-derived exosomes (LM-exosomes) can induce cell growth when co-cultured with HOC313 parental cells (HOC313-P) and the phosphorylation status of cell survival signaling proteins EGFR, ERK and AKT was elevated in HOC313-P cells upon treatment with LM-exosomes[35]. These results mentioned above indicated that exosomes have emerged as novel subcellular transduction materials for signal pathways and regulated malignant transformation of recipient cells in oral cancer. In addition to the involvement in cancer, PI3K/Akt and MAPK/ERK pathways have been reported to be critical ways for virus infection, cell progression and drug resistance [47-49]. Therefore, more studies are needed to make clear if Akt, ERK, and JNK signaling pathways were essential for the exosome-induced effects in other oral diseases, or if there are other pathways are functional in oral diseases.

In conclusion, exosomes play vital roles in oral diseases, and may be candidates for diagnosis and treatment. However, these studies only involved in several diseases, there are still some common oral diseases were not addressed. For example, the research of exosomes in periodontitis hasn’t been touched to date. Even so, several studies have presented the potential of exosomes in bone metabolism and inflammation regulation. Osteoblast-derived MVs (including exosomes) were reported to facilitate the osteoclast formation by transferring of RANKL protein, which may represent a novel mechanism of bone modeling and remodeling [50]. Kagiya T et al demonstrated that exosomal miRNA including let-7e, miR-21, miR-33, miR-155, miR-210, miR-223, miR-378, and miR-1224 were reported to be critical for osteoclast differentiation [51]. Alexander et al. found miR-155 and miR-146a can be delivered to recipient dendritic cells via exosomes, which mediate target gene repression and reprogramme the cellular response to endotoxin. This report indicated that miRNAs within exosomes may be transferred between immune cells in periodontal tissues [52].

Given these previous evidences about the connection between exosomes with bone metabolism and inflammation, we can boldly speculate that exosomes may play an important role in periodontitis.

**Exosomes Application in Oral Regenerative Medicine**

Regenerative medicine, an interdisciplinary field that applies engineering and life science principles to promote regeneration, can potentially restore diseased and injured tissues and organs. In oral and maxillofacial region, the subject is developing rapidly because of the increasing requirements of tissue regeneration. For example, firstly, tumor, trauma or craniofacial anomalies induced bone defect needs bone repairmen or bone reconstruction; Secondly, a significant portion of people need bone graft surgeries when perform implant placement or perform periodontal surgery; Thirdly, dental caries induced inflammation and necrosis of dental pulp tissue also needs restoration in order to maintain the its functionality. At present, although a few of therapy strategies including autografts, allograft and growth factors are used for bone regenerative, their deficiencies such as donor site morbidity, lesser osteogenic capacity and biosecurity concerns limit the usage of them. In addition, root canal therapy, the most popular treatment for pulpitis, will make the teeth lose its vitality, sensitivity and immune response, which influences integrity and longevity of teeth. Therefore, researchers are trying to look for a safe and reliable regenerative procedure. In recent years, exosomes-mediated tissue regeneration as a novel approach is arousing more and more attentions in the study of oral and maxillofacial regenerative medicine.
Narayanan et al[53] reported HMSC-derived exosomes can be endocytosed by undifferentiated primary HMSCs, and the endocytosed exosomes can induce the osteogenic differentiation of HMSCs both in 2D and 3D cultures. Several representative genes of induction osteogenic differentiation including BMP9, TGFβ1, runx2 and Osterix were upregulated significantly. In vivo, scaffolds containing HMSCs and exosomes were implanted subcutaneously on the back of athymic nude mice, and the ‘HMSCs+exosome’ scaffolds showed more robust vascularization and calcium phosphate nucleation than ‘HMSCs’ scaffolds, which indicated the exosome have a higher potential for osteogenesis. Another similar experiment [3] on the aspect of dental pulp regeneration showed that exosomes derived from dental pulp cell could be endocytosed by human dental pulp stem cells( DPSCs) and then triggered odontogenic differentiation of DPSCs by affecting the expression of regulatory genes. In a tooth root slice model with DPSCs, dental pulp cell-derived exosomes triggered regeneration of dental pulp-like tissue, in which odontogenic differentiation marker proteins DMP1 and DPP, growth factors BMP2, TGFβ, the pro-vasculogenic factor PDGF etc. showed an increase level of expression. In addition, the results of the two studies revealed that exosomes can bind to matrix proteins such as type I collagen and fibronectin, indicating maybe exosomes can be used to bind to ECM proteins as therapeutic agents during regenerative procedures in the future application.

Overall, the two studies showed the potential of exosomes in tissue regeneration, but they only suggested the role of exosomes in inducing lineage specific differentiation of DPSCs or HMSCs, further studies are required to investigate the mechanism by which exosomes control stem cells fate and the exosomal miRNAs and proteins that contribute towards this process.

Conclusions and Prospects

The rapid expansion of the number of published studies on exosomes clearly shows that research on exosomes and their functions is now a very exciting field. As important means of cell communication, exosomes roles in various physiological and pathological processes have been gradually realized. In this review, several aspects of exosome biology associated with some oral diseases were discussed. As detailed in our article, the recent approaches of exosomes related to oral diseases have elucidated great progress toward clinical application. Nevertheless, many questions regarding application of exosomes remain to be addressed. For example, the relationship between periodontitis and exosomes still need more direct evidences to verify in vitro and in vivo. In addition, although much information concerning the functional role of exosomes in regenerative medicine has been demonstrated using cultured cells and animals, further investigations on patients are needed to expand our understanding. Exosomes would be ideal biomarkers for disease diagnosis and targeted therapy because they closely represent the state of their parental cells and are relatively stable in the circulation and could be easily collected from body fluids. We truly believe that integration of better understanding of exosomes will open new approaches for the diagnosis and therapeutics of oral diseases.
Reference


