International Journal of Dental Sciences
www.dentaljournal.in
E-ISSN: 2663-4708, P-ISSN: 2663-4694
Received: 05-02-2022, Accepted: 20-02-2022, Published: 08-03-2022
Volume 4, Issue 1, 2022, Page No. 13-16

Role of exosomes in oral cancer–A review

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Abstract
Oral cancers constitute approximately 2% of all cancers. About 96% of oral cancers are carcinomas and remaining 4% are sarcomas .majority of oral carcinomas are squamous cell carcinomas. Its estimate d that 9 out of every 10 oral malignancies is oral squamous cell carcinomas. It is a disease of increasing age with 95% of the patients older than 40 years of age. OSCC has high recurrence rate and poor prognosis so exploring new therapeutic strategies has become an urgent priority. Exosomes are extracellular vesicles, released from different tissues in a living individual. They can be released from both the normal and diseased cells. Exosomes have been associated with tumorogenesis of OSCC, promote the proliferation, colonization and metastasis of OSCC. They play an important role in the diagnosis, prognosis, and therapeutic aspect of a disease.

Keywords: oral squamous cell carcinoma, exosomes, extracellular vesicles, tumour microenvironment, miRNA

Introduction

Communication between various cell types is by means of signal transmission, being favoured by factors such as cytokines and chemokines and exosomes. Exosomes are nanovesicles which are seen in almost all cell types [1]. According to the International Society for Extracellular Vesicles (ISEV), EVs are nano-size lipid bilayer vesicles released naturally from the cells to the ECM .EVs are categorized as exosomes, microvesicles, and apoptotic bodies [1]. Exosomes are small, membranous, extracellular microvesicles (30–150 nm in diameter) of endocytic origin. The formation of exosomes occur in a sequence of events including endocytosis, MVB creation, and finally exosom secretion [2]. They play an inevitable role in communication between the cells and the cellular function regulations, the amount of exosomes secreted and their composition [3]. Exosomes contain a variety of proteins, mRNAs, and non-coding RNAs, including microRNAs, long non-coding RNAs, piRNAs, circular RNAs, tsRNAs, and ribosomal RNAs, which are delivered to neighbouring cells or transported to distant sites [1]. To overcome the limitations of treatment with stem-cell therapy, the emergence of cell-free therapy using microvesicles is rapidly evolving.

History of Exosomes

Exosomes were first discovered in the maturing mammalian reticulocyte. Rose Johnstone in 1970 was the first to coin the term “exosomes”. The term “exosomes” refers to the extracellular vesicles (EVs) released from cells by fusion of the multivesicular body (MVB) with the plasma membrane. This fusion frees intraluminal vesicles (ILVs) into the external environment and these liberated particles are termed exosomes. Exosomes were earlier thought to be waste products liberated through shedding via plasma membrane [4].

Nowadays there has been a vast number of studies on exosomes, mainly because of

1. Their source of cell to cell signalling and intracellular mediation of macromolecules.
2. Their role in contribution toward the progression of diseases.
3. Its role as drug vectors, as they have a cell membrane that aids in better tolerance by the host unlike synthetic polymers
4. They are not immunogenic thereby achieving lesser chance of tissue rejection
5. and lower risk of tumorogenesis.
6. Their role in in tissue repair and regeneration.

Formation and Isolation of Exosomes

Biogenesis of exosomes are different from that of other variants of EVs., exosomes are formed by the invagination of endosomal plasma membrane during the process of maturation from early endosome to late

endosomes. The late endosomes,are known as the microvescicular bodies. These contain a population of ILVs that are called exosomes when released. MVBs when transported to the cell membrane, they fuse with the membrane and release their contents to the extracellular environment or they are transported to a lysozyme and are digested. The formation of exosomes from MVBs occurs by two different pathways:

1. "Endosomal Sorting Complex Required for Transport (ESCRT)-dependent mechanism"
2. "ESCRT-independent mechanism." [5]

Exosomes are identified by means of their characteristic saucer-like morphology—a flattened sphere that is limited by a lipid bilayer. Even though exosomes, are derived from different cell types, they share their common characteristic structure. Their shape is determined by the type of cells from which they are formed and they perform various functions based on origin cell-specific proteins.

Composition

Exosomes contains proteins, cytokines, lipid rafts, miRNA, mRNA, and small noncoding RNAs, rRNAs, tRNAs, and occasionally DNA [5].

Factors affecting exosomal release

- Oxidative stress, hypoxia etc are the major environmental factors that influence release of exosomes

The vasculature in the TME shows abnormalities, including leakage, a heterogeneous basement membrane, irregular vessel branching, and poor pericyte coverage. These changes may lead to a hypoxic TME (Hu and Polyak, 2008),

- Drugs like sitafloxacin, pentetrazole, fenoterol can act as activators in production of exosomes.
- Hyperthermia also plays a significamt role in the production of exosomes.
- The amount of exosomes secreted by cancer cells is greater than normal levels.

Isolation

Exosomes can be isolated from different body fluids including saliva, blood, urine, breast milk, semen, cerebrospinal fluid, amniotic fluid, ascetic fluid, and bile [6].

- Ultracentrifugation – widely used , gold standard
- Size based isolation- ultrafiltration and size exclusion chromatography
- Polymer precipitation
- Immune affinity
- Micro fluidic separation

Exosomes can be stored for a period of 6 months at –20°C or even lower without cryopreservative agents, without changes in size and structure of the exosomes.

The Biological Function of Exosomes

1. Tumerogenesis

Exosomes are important mediators of intercellular communication. They also play an inevitable role in regulating cellular activities during physiologic and pathologic conditions. During the progression of cancer, cells such as cancer cells, immune cells in TME generate exosomes that can transfer different proteins among cells and participate in the pathogenesis of tumor development and metastasis. These contents also regulate angiogenesis, sensitivity to chemotherapy, and immune evasion [7].

Among the bioactive components of exosomes, miRNAs are the ones which can alter gene function in the recipient cell and thus exert an essential regulatory function on gene expression [8]

Most of the miRNAs exist intracellularly, while some exist in body fluids, including a variety of extracellular biologic fluids, such as blood, urine, saliva, pancreatic juice, and breast milk [9].

The presence of transcription factors, miRNAs, and oncogenic proteins, plays a crucial role in tumorogenesis, reprogramming tumor microenvironment, immune tolerance, facilitating metastasis, and resistance to therapy [10].

The composition of TDEs differs from the exosomes of healthy cells in a way that they facilitate functional cargo delivery, which includes oncogenes and oncogenic proteins, which can exert biological activities. Thus the cancer progression and metastasis is mainly mediated by the TDEs in the tumour environment [10].

The presence of protein cargoes including different oncoproteins, growth factors, and immunomodulatory molecules acts as the tumorogenesis mediators

Exosomes derived from OSCC cells may influence cell motility and angiogenesis that, in turn, can affect OSCC progression.

The exosome suppresses tumour immunesurveillance by inhibiting the function of the immune cells and down regulating the activity of regulatory T cells and mesenchymal dental stem cells, interfering with the differentiation of dendritic cells, and polarization of tumor associated macrophages.

Exosomes derived from head and neck squamous cell carcinoma (HNSCC) promotes metastasis by increasing the proliferation of endothelial cells, epithelial mesenchymal transition (EMT), cancer associate fibroblasts (CAFs), and facilitating angiogenesis as well as stromal compartment reprogramming [10]. Alteration of the TME is the first step in forming a premetastatic niche.

TME consist of non-malignant cells, molecules, structural components, and chemicals that surround cancer cells. The extracellular matrix along with multiple non-malignant cells, including endothelial cells, pericytes, immune cells, fibroblasts and ECM, forms the supportive stroma of the tumour and it can manipulate the TME.
The "seed and soil" theory is widely accepted in cancer studies [11]. The pre-metastatic niche, considered as a fertile soil conducive to the survival and growth of metastatic seeds, consists of diverse cell populations, such as CAFs, immune cells, and multiple non-cell components of the ECM. These niche components influence the fate of disseminated tumor cells by cell proliferation and differentiation, and contribute to tumor angiogenesis, invasion, and metastasis [12].

2. Diagnosis and Therapeutics

Exosomes play a vital role in diagnosis, development, and treatment of some cancers. Exosomes are ideal biomarker for diagnosis and therapy as they bear the close resemblance to their parental cells, are stable in circulation, and their ease of collection from the body fluids. Thus, assessing the level of exosomes in patient's body fluids and their level as cargos can be used as a potential tool in diagnosis of disease or progression of disease, therapeutic response, and overall survival.
Exosomal miRNAs could serve as potential biomarkers for the treatment of OSCC. For example: MiR-24-3p released from salivary exosomes.
Exosomal miRNAs could serve as therapeutic targets for the treatment of OSCC. It can be achieved by anchoring nanoparticles to the EVs, which aids in suppressing the communication between tumor and recipient cells. This is achieved by various proteins, small interfering RNA, miRNAs, and targeted drugs, which can be incorporated into exosomes which results in enhanced activity and targeted delivery in patients.
Exosomes and microparticles can be packed with miRNA, resulting in transfer of miRNAs to body fluids by a passive release mechanism [13] They can also be released by tumor cells via shedding as an active secretion mechanism (Shah and Calin, 2013) [14].
The exosomes extracted from oral fluids in patients with oral carcinoma present different patterns of CD9, CD69, and CD 61.The marked reduction in the expression of CD81 and CD9 appears to be an indicator of oral cancer, even in the initial disease stage [15]. thus, the role of exosomes as biomarkers and as a therapeutic tool in HNSCC will be promising.
A number of studies shows that T-helper 17 cell (Th17)/Treg imbalance is the main etiological source for the development of autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, graft versus host disease, and multiple sclerosis. Exosomes derived from dental pulp stem cells can reduce Th17/Treg imbalance *in vitro* and are better when compared with that of bone marrow stem cell-derived exosomes. Hence, exosomes can be used as an aid in treatment of autoimmune diseases [16].

3. Drug vectors

Exosomes can be used as drug vectors because they can be fused with cell membrane with high reliability. Thus non coding RNAs or peptide drugs can be packed in to exosomes and delivered to the target tumour cells.
But their efficiency as a drug vector is still a challenging idea, because of their failure to cross the BBB and chances of degradation. Liu *et al*. (2017) first isolated cisplatin-resistant OSCC cells and used the conditional medium from resistant cells to treat parent OSCC cells. They later found that cisplatin-resistant OSCC cells could transfer miR-21 by exosomes targeting PTEN and PDCD4 to confer the cisplatin-resistance of the parental OSCC cells.Thus, exosomes may function as a vector for resistance transfer in cancer cells, and the resistance-related factors can be used as therapeutic targets for treatment of OSCC. Exosomes have the characteristics including biocompatibility, noncytotoxicity, low immunogenicity, simple to produce and store, long life span, and high cargo loading capacity also their small size confers resistant to lung clearance and passing through the blood–brain barrier effectively, all these factors contribute to function as drug vectors.

4. Tumour suppression

Even though miRNAs are said to help in tumour metastasis, there are certain miRNAs like miR223 and miR1013p are found to suppress the tumour progression. They act by inhibiting cell proliferation and inducing apoptosis thereby preventing tumour growth.
ROSENBERG *et al* discovered that some exosomal contents can inhibit angiogenesis by manipulating secreation of VEGF and thereby suppress the tumour growth.

Conclusion

Exosomes are a promising tool in the diagnosis, prognosis, and therapeutic aspects of various diseases. Dental professionals have an excellent opportunity in the field of regenerative dentistry and can play a major role in the therapeutic application using exosomes. Though the isolation, culture and application of exosomes are quite challenging, the rapidly progressing field of research can surpass these limitations, thereby making exosomes as a destined tool in all aspects of medicine.

References

1. Kalra H, Simpson RJ, Ji H, Aikawa E, Altevogt P, Askenase P, *et al*. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation, 2012, PLoS Biol. 10:e1001450

2. Keller Sascha, Sanderson, Michael, Stoeck, Alexander, Altevogt, Peter. Exosomes: From biogenesis and secretion to biological function. Immunology letters, 2006, 107. 102-8. 10.1016/j.imlet.2006.09.005.
3. Ohno S, Ishikawa A, Kuroda M. Roles of exosomes and microvesicles in disease pathogenesis. Adv. Drug. Deliv. Rev,2013a:65:398–401.
4. Thery C. Exosomes:secreted vesicles and intercellular communications. F1000 Biol Rep,2011:3:15.
5. Doyle LM, Wang MZ. Overview of extra cellular vesicles, their origin ,composition,purpose,and methods for exosome isolation and analysis.Cells.2019:8:727.
6. Avc1 E, Balci-Peynircioglu B. An overview of exosomes from Biology to emerging roles in immune response.Acta medica,2016;5:2-10
7. Raposo G, Stoorvogel W. Extracellular vesicles exosomes, microvesicles, and friends; J Cell Biol 2013.
8. Ridder K, Keller S, Dams M, Rupp AK, Schlaudraff J, Del Turco D. *et al*. Extracellular vesicle-mediated transfer of genetic information between the hematopoietic system and the brain in response to inflammation, 2014. PLoS Biol. 12:e1001874.
9. Wang Y *et al.* Integrative approach detected association between genetic variants of microRNA binding sites of TLRs pathway genes and OSCC susceptibility in Chinese Han population, 2014. PLoS One 9:e101695.
10. Weber *et al*. The microRNA spectrum in 12 body fluids. Clin. Chem,2010:56:1733–1741.
11. Xiao C, Song F, Zheng YL, Lv J, Wang QF, Xu N. Exosomes in head and neck squamous cell carcinoma. Front Oncol 2019:9:894.
12. Paget S. The distribution of secondary growths in cancer of the breast.. Cancer Metastasis Rev,1889:8:98–101.
13. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K. *et al*. Characterizationof microRNAs in serum: a novel class of biomarkers for diagnosis of cancer andother diseases. Cell Res,2008:18:997–1006.
14. Zlotogorski-Hurvitz A, Dayan D, Chaushu G, Salo T, Vered M. Morphological and molecular features of oral fluid-derived exosomes: oral cancer patients versus healthy individuals. J Cancer Res Clin Oncol,2016:142:101-10.
15. Ji L, Bao L, Gu Z, Zhou Q, Liang Y, Zheng Y, *et al*. Comparison of immunomodulatory properties of exosomes derived from bone marrow mesenchymal stem cells and dental pulp stem cells. Immunol Res,2019:67:432-42
16. Steinbichler TB, Dudas J, Skvortsov S, Ganswindt U, Riechelmann H, Skvortsova II. Therapy resistance mediated by exosomes. Mol. Cancer,2019:18:58.