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Review

Emerging role of non-coding RNA in oral cancer☆

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is characterized by genomic and epigenomic alterations. However, the mechanisms underlying oral squamous cell carcinoma tumorigenesis and progression remain to be elucidated. Long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and extracellular RNAs (exRNAs) are emerging groups of regulatory RNAs, which possess low or no protein-coding potential. Emerging lines of evidence indicates that deregulated expression of lncRNAs and circular RNAs are associated with the induction and progression of various cancers, including oral cancer, through epigenetic, transcriptional, and post-transcriptional alterations. In this review, we highlight the expression and functional roles of extracellular RNAs, lncRNAs, and circular RNAs in oral squamous cell carcinoma and discuss their potential clinical applications as diagnostic or prognostic biomarkers, and therapeutic targets.

1. Introduction

The discovery of non-coding RNA added a new layer to our understanding of biological processes. The term non-coding RNA (ncRNAs) encompasses microRNA (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and intronic RNAs [1]. The literature indicates that there may be a lack of potential for these RNAs to encode proteins or peptides. Although ncRNAs do not encode proteins, they are master regulators of gene expression through various mechanisms [1–3]. In the field of cancer research, it has become apparent that aberrations within the noncoding genome drive fundamental cancer phenotypes in addition to the best-known protein coding mutations [4,5].

Oral squamous cell carcinoma (OSCC) is a major global health problem [6]. Some of the most widely recognized risk factors of OSCC are tobacco use and alcohol consumption [6]. Recently, infection of high risk HPV was introduced as a novel risk factor in subsets of patients who were exposed to the disease [7]. Traditionally, the expression of protein-coding genes [messenger RNAs (mRNAs)] has been the focus of cancer studies for decades. Recently, this approach has been challenged due to the discoveries that small proportions of human transcriptome are protein-coding genes [8]. Specifically, Ensemble1 (v76) statistics reveals that only 34% of human transcriptome are protein coding genes and 66% are non-coding genes including long intergenic non-coding RNAs, antisense RNAs, pseudogenes, and microRNAs (miRNAs) [8]. Recent evidence has also indicated a fundamental role of non-coding RNAs in almost all stages of gene expression process such as at the levels of cellular physiologic processes, and in the development of different human diseases including cancers [9]. Thus, an understanding the function of different non-coding RNAs including lncRNAs, circRNAs, and ExRNAs provides an opportunity to understand underlining biological events involved in different cancers including OSCC. This understanding might ultimately lead to development of novel new therapies and diagnostic tools. Here, we aim to review the characteristic and functions of oral cancer related non-coding RNAs. In this perspective, we provide an overview of the current state of non-coding RNA biomarker identification in cancer phenotypes, catalog the molecular roles for lncRNAs in cellular processes, and review the emerging roles for lncRNAs, circRNAs, and extracellular RNAs in oral cancer pathophysiology.

2. Extracellular RNA and exosome-associated RNA in oral squamous cell carcinoma

Exosomes are the smallest (30–100 nm) vesicles and most heavily studied subpopulation of extracellular vesicles [10,11]. These particles are generated by the exocytosis of multivesicular bodies (MVBs) [12] (Fig. 1). Early endosomes can be targeted for ubiquitin-dependent

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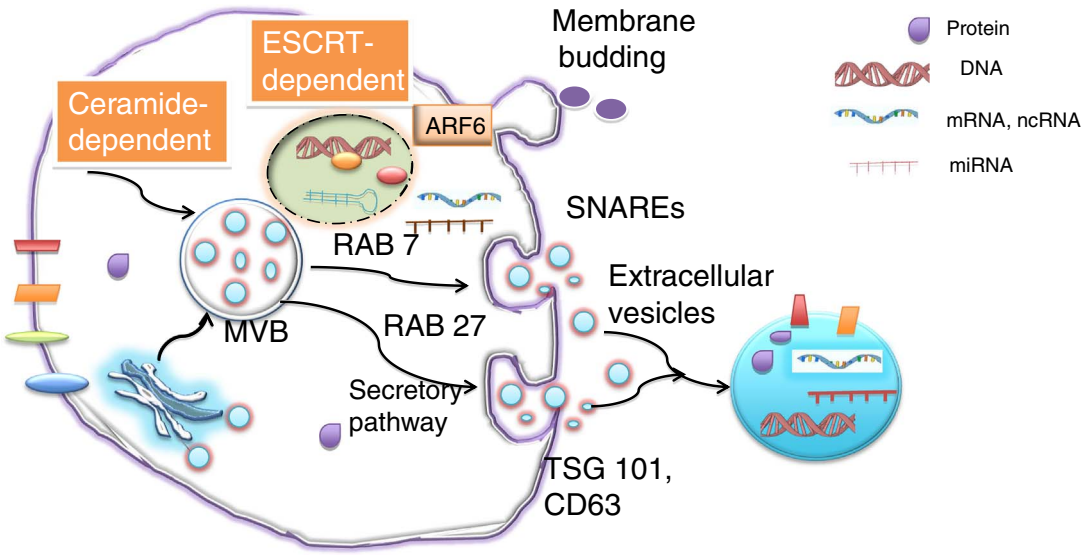

Fig. 1. Biogenesis of extracellular vesicles. Extracellular vesicles (EVs) are comprised of various types of vesicles including exosomes, microvesicles, macrovesicles. Exosomes are formed by internalization of the endocytic membrane and formation of multivesicular bodies (MVB inside the cell). The fusion of the MVBs with plasma membrane results in their release. Microvesicles are formed and released by plasma membrane. Exosomes/microvesicles carry various mRNA, miRNA, ncRNA, DNA, proteins and are emerging as a new inter or intra (cell-cell/organ) communicatior.

interactions in one of three endosomal sorting complexes required for transport (ESCRT-0, ESCRT-I and ESCRT-II), which lead to the recycling of the endosome or, alternatively, its progression towards a late endosomal pathway [12]. Late endosomal pathways are dependent on MVBs, are ubiquitin-independent, and lead to the formation and sorting of exosomes [13]. Exosomes have been isolated from almost all cell types as well as mucosal and endogenous biofluids and have been implicated in key processes like growth and development, immune response, blood coagulation, and various stages of tumorigenesis [14–17]. Exosomes carry various molecular cargos including nucleic acids, proteins, and lipids and provide a snapshot of cells at the time of release [18]. The lipid bilayer structure of exosomes protects cargo from degradation enzymes such as RNases [19]. Low-abundance molecular analytes specific to the disease can be enriched in exosomes and have been recovered from exosomes [19]. These characteristics position EVs as a new, highly appealing class of biomarkers with strong diagnostic potential in the context of personalized medicine [20,21]. Up to 76% of all map able reads generated by RNA-Seq on exosomes were miRNA transcripts [22]. miRNAs demonstrated fundamental roles in normal development, differentiation, growth and in pathogenesis of different diseases [23]. They also play pivotal roles in cancer initiation and progression [23]. PCR based array methods identified the role of miRNA-26a and miRNA-26b in OSCC cells [24]. In OSCC, loss of tumor-suppressive miRNA-26a/b enhances cancer cell migration and invasion *via* regulation of TMEM184B [24]. This study provided new insights into the potential role of miRNA-26a/b in OSCC oncogenesis and metastasis [24]. miRNAs were also detected in the extracellular vesicles in OSCC. In this context miRNA-21 was detected in exosomes derived from OSCC under hypoxic conditions and significantly enhanced snail and vimentin expression while significantly decreasing E-cadherin levels both *in vitro* and *in vivo* studies [25]. Moreover, circulating exosomal miRNA-21 levels were associated with HIF-1α/HIF-2α expression, T stage, and lymph node metastasis in patients with OSCC [25]. These findings suggest that the hypoxic microenvironment may stimulate tumor cells to generate miRNA-21-rich exosomes that are delivered to normoxic cells to promote prometastatic behaviors [25]. Further investigations into the therapeutic value of exosomal miRNA inhibition are needed for oral cancer treatment.

Saliva is a readily available biofluid, therefore salivary exosomes/Ex-RNAs biomarker approaches are emerging. Interestingly, most miRNAs are shown to be enriched in the exosomal fraction of saliva and serum rather than the exosome depleted fraction [26]. Momen-Heravi et al. reported the differential expression of extracellular miRNA in saliva of patients with OSCC, patients with OSCC in remission (OSCC-R), patients with oral lichen planus compared to healthy subjects [27]. Using genome wide study and NanoString nCounter miRNA expression assay and real-time quantitative polymerase chain reaction, we found miRNA-27b was significantly upregulated in saliva of patients with OSCC compared to other groups [27]. Furthermore, miRNA-27b showed higher sensitivity and specificity in detecting OSCC compared to other miRNAs tested [27]. Another study found that salivary miRNA-31 was significantly elevated in all the stages of OSCC irrespective of the tumor size [28]. Moreover, the levels of miRNA-31 were higher in saliva as compared to plasma, suggesting local production of miRNA-31 at the tumor site [28]. Interestingly after excision of oral carcinoma, salivary miRNA-31 was amazingly reduced, signifying that most of the upregulated salivary miRNA-31 came from tumor tissues [28]. Zahran et al. reported a highly significant increase in salivary miRNA-21 and miRNA-184 in saliva of OSCC patients when compared to healthy and disease controls [29]. Conversely, miRNA-145 levels showed a highly significant decrease in OSCC. Where as recurrent aphthous stomatitis (RAS) cases showed no significant difference from normal controls in any measured miRNA ($P > 0.05$). Interestingly, the only microRNA to discriminate between OSCC and oral potentially malignant disorders was miRNA-184 [29].

Transcriptomic analyses of human saliva derived exosomes revealed that 509 mRNA core transcripts were present [30]. Experimentally, *in vitro* co-culture of salivary exosomes with human oral keratinocytes altered the gene expression of the recipient cells, indicating a crucial role of exosomes in horizontal gene transfer [30]. Another study showed that exosome number, exosome size, and inter-exosome are increased in the saliva of patients with oral cancer [31]. Interestingly, oral cancer exosomes exhibited significantly increased CD63 surface densities and displayed irregular morphologies [31]. These studies suggest that RNA content in the exosomes might be a potential resource for oral cancer diagnostics and also as new oral cancer biomarkers. However, precise mechanisms *via* which exosomes play role in OSCC initiation and progression have yet to be determined.