Journal of Oral Biology and Craniofacial Research 14 (2024) 461–464

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

# Targeting compensatory proliferation signals in oral cancer

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A B S T R A C T

Apoptosis is an orchestrated phenomenon that regulates cell populations in physiological and pathological conditions. Carcinogenesis involves a state of disequilibrium between cell proliferation and cell death. The resistance to conventional therapeutic modalities of cancer, including surgery, radiotherapy, and chemotherapy, can be explained by the compensatory repair and regeneration that occurs in the tumor microenvironment following apoptosis through the apoptotic compensatory proliferation signaling microvesicles (ACPSVs) or apoptotic extracellular microvesicles (ApoEVs). These microvesicles provide proliferative signals and act as mutagens, triggering cell proliferation, angiogenesis, immune evasion, metastasis, and invasion. This review discusses the phenomenon of apoptosis-induced proliferation and the role of ApoEVs in establishing an oncoregenerative niche, resulting in therapeutic resistance and recurrence of malignancies.

## 1. Introduction

**Oral cancer constitutes the 16th most common malignancy, accounting for 389,485 new cases, and is the 15th leading cause of mortality across the globe.**[1] Oral squamous cell carcinoma (OSCC) constitutes more than 90% of oral malignancies, with a higher incidence in males than females. OSCC, in the majority of the cases, is preceded by a group of conditions known as oral potentially malignant disorders (OPMDs).[2] The WHO 2020 working group defines OPMDs as "any oral mucosal abnormality that is associated with a statistically increased risk of developing oral cancer." The OPMDs include leukoplakia, erythroplakia, proliferative verrucous leukoplakia (PVL), oral lichen planus, oral submucous fibrosis, palatal lesions in reverse smokers, lupus erythematosus, epidermolysis bullosa, and dyskeratosis congenita.[3]

The complex and multifactorial oncogenic process in oral cancer may evolve as a continuum of the spectrum of OPMDs, involving genetic alterations, epigenetic modification, and a dysregulated tumor microenvironment. **Persistent exposure to the common risk factors, including tobacco (smoked and smokeless) with or without areca nut use and chronic alcohol consumption, leads to the development of OPMDs and OSCC.**[4] **OPMDs are red or white patches (homogenous or non-homogenous; solitary or diffuse) as seen in leukoplakia, erythroplakia, erythroleukoplakia, and PVL. They are usually asymptomatic and may present with mild discomfort. Dense submucosal fibrosis resulting in blanching and restricted mouth opening is characteristic of oral submucous fibrosis.**[2] **OPMDs, when overlooked and left untreated, may progress to OSCC. Early lesions of OSCC may present as red and white lesions with irregular margins that are typically painless (similar to OPMDs) and eventually develop into an ulcer/ulceroproliferative growth, lump, or nodule as the disease progresses in advanced stages**. The progressive malignant phenotypes arising from OPMDs - stemness, resistance to chemotherapy, immune evasion, extensive metastasis, and invasion, confer unique properties to the cancer cells and evolve as potential causal factors for relapse or recurrence. **The conventional treatment modalities include surgery, chemotherapy, and radiotherapy. Despite recent advances in cancer therapeutics, the 5-year overall survival of OSCC patients remains about 60 % due to tumor metastasis and subsequent recurrence.**[3] A significant reduction in the overall treatment outcome and survival probability of patients with substantial morbidity affects their physical, psychological, and social well-being.[2]

The steep increase in cancer incidence underscores the high demand for early diagnostic and therapeutic markers for combating oral cancer.[5] A myriad of research is conducted globally to augment our comprehension of the intricate molecular networks and mechanisms involved in oral carcinogenesis. Numerous exosomal biomarkers intended for the diagnosis, prognosis, and therapeutics have been identified and targeted

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https://doi.org/10.1016/j.jobcr.2024.05.014
Received 17 March 2024; Received in revised form 12 May 2024; Accepted 16 May 2024
Available online 7 June 2024

to provide early detection and improved treatment options for oral cancer patients.[6] **For example, the upregulation of circular RNA molecules derived from the circulating exosomes, circ_0000199, has been associated with the worst prognosis among OSCC patients.[7] The exosomal microRNA, miR-382-5p, from cancer-associated fibroblast was demonstrated to facilitate OSCC progression. This microRNA can be targeted further for therapeutic purposes.[8]** In line with these facts, a recent study on microvesicles derived from apoptotic cells provides novel perspectives on how compensatory signals drive pathogenic pathways associated with tumor development.

## 2. Apoptosis-induced proliferation (AiP)

Apoptosis, or programmed cell death, is a biological process promoting cellular turnover and eliminating infected or damaged cells. The cysteine-dependent proteases, called caspases, drive pathways leading to cell death and concurrent proliferation of neighboring surviving cells to ensure the regeneration of damaged tissues and wound healing. The proliferative signals are diffusible mitogenic proteins delivered by apoptotic cells. These signals are received by the nearby cells to trigger their proliferation. This process of caspase-mediated compensatory cell proliferation is termed as "apoptosis-induced proliferation" or "AiP".[9] Mitogenic response following apoptosis was first studied in *Drosophila* during development and in wounds. Programmed cell death can result in reparative and regenerative responses such as angiogenesis and compensatory proliferation in tissues significantly associated with tumor progression. Apoptotic caspases promote inflammation, sustained proliferation, and tumor initiation in the tumor microenvironment, forming an oncoregenerative niche (ORN). Caspase-3 and caspase −7 activate the calcium-insensitive phospholipase $A_2$ ($iPLA_2$) by cleaving them and producing various phospholipid signals associated with migration of phagocyte, prostaglandin E2 (PGE2) synthesis, detection, and clearance of apoptotic cell. It generates signals that promote angiogenesis, stem cell and progenitor cell proliferation, and suppresses adaptive immunity. The production of $PGE_2$ by caspase-3 is reported to support tumor recurrence in mice following apoptosis induced by therapy in breast carcinoma, urinary bladder carcinoma, melanoma, and in glioblastoma models.[9–11]

### 2.1. Reactive oxygen species and AiP

In Drosophila, apoptotic caspases are reported to mediate the generation of ROS for promoting AiP. Dronc, the initiator caspase in Drosophila, serves as the major inducer of AiP by the generation of extracellular reactive oxygen species (eROS), which activate Jun-N-terminal kinase (JNK) signaling. JNK functions in a positive feedback loop in AiP as it transcriptionally activates hid and reaper (inhibitors of apoptosis, IAP), and amplifies the AiP process. Downstream of JNK signaling, undead cells produce and secrete several mitogens – including Wingless (Wg), a WNT-family member, Decapentaplegic (Dpp), a TGF- family member, and Spitz (Spi), an EGF homolog to the neighboring cells to initiate proliferation.[12]

### 2.2. Metabolite secretome and AiP

Lymphocytes and macrophages produce metabolites like adenosine monophosphate and triphosphate (AMP and ATP), guanosine monophosphate (GMP), creatine, spermidine, and glycerol-3-phosphate mediated by caspase-activated opening of pannexin I channel. This apoptosis-induced 'metabolite secretome' triggers anti-inflammatory signals and activates proliferative and reparatory responses in the neighboring healthy cells.[9,13] Cancer tissues involve apoptotic processes turned on constitutively. The outpacing of cells, supply of nutrients, survival insufficiency, anti-tumor immunity, and removal of toxic metabolites as a part of chemo- and radiotherapies are some factors related to apoptosis in cancer cell mass. This kind of cell death is critical in promoting tumor growth and repopulation of tumors, causing relapse.

### 2.3. Oncoregenerative niche and AiP

Apoptosis activates cells in the innate immune system and orchestrates a tumor microenvironment (TME) with pro-oncogenic properties, resulting in the evasion of cancer treatment. Apoptosis in the tumor-cell population promotes cell survival and resistance to treatment by regulating the TME. Thus, proliferative signals in a tumor microenvironment adequately maintain the oncoregenerative niche.[14] The ORN encompasses the intercellular communications in the TME through regenerative and tissue repair mechanisms driven by apoptosis. It promotes tumor expansion and invasiveness coupled with suppression of anti-tumor immunity. Apoptosis and angiogenesis provide a dynamic feedback loop in the ORN, supporting tumorigenesis through apoptotic extracellular vesicles (ApoEVs). The intercellular signaling caused by apoptosis in ORN of the TME involves (1) the accumulation of reparatory phenotype M2-like tumor-associated macrophages (TAMs) (2) activation of TAMs by angiogenesis induced by apoptotic cell-derived factors, soluble mediators, direct intercellular contact and in the presence of Apo-EVs (3) activation of endothelial cells and their progenitors by soluble factors, intercellular signals, and EVs.[10]

### 2.4. Extracellular vesicles and AiP

Extracellular vesicles (EVs), including microvesicles (MVs), exosomes, and apoptotic bodies, are crucial mediators of cellular differentiation and reprogramming, proliferation, apoptosis, intercellular communication, and many more. They have distinct biogenesis and cargo compositions. While protein content is a primary focus, nucleic acids and lipids also contribute significantly. EVs are identified by a set of shared markers, including CD9, CD63, and CD81.

•**ApoEVs**: ApoEVs are large 1–5 μm extracellular vesicles derived from apoptotic cells during programmed cell death. Apo-EVs are also known as Apoptotic Compensatory Proliferation Signaling microvesicles (ACPSVs), apoptotic blebs, apoptotic vesicles, apoptotic microparticles, and apoptotic bodies.[15,16]

- **Biogenesis of** ApoEVs: The biogenesis of ApoEVs is a sequential process initiated in the plasma membrane by the formation of blebs. The plasma membrane blebs extend as protrusions and eventually undergo fragmentation (Fig. 1). **They are made via PANX1-dependent mechanisms and membrane protrusions known as "apoptopodia". They contain lipids, proteins, and nuclear substances[16].**
- **Markers of** ApoEVs**: The apoptotic vesicles are characterized by specific markers such as phosphatidylserine and C1q, CD44, CD90, Fas, Integrin alpha-5, Syntaxin-4, Cavin 1 and Caveolin-1[16].** Recently, a unique population of apoptotic cells releasing Crkl-containing microvesicles distinct from exosomes or apoptotic bodies has been identified. The *CRKL* (V-crk avian sarcoma virus CT10 oncogene homolog-like, present at the locus 22q11) is a novel putative oncogene that is differentially expressed in various human cancers, including ovarian carcinoma, synovial sarcoma, glioblastoma, and head and neck squamous cell carcinoma (HNSCC). Gupta et al. proposed that these Crkl-containing microvesicles can promote cell proliferation upon contact with each other as a part of the compensatory signaling mechanism. The inactivation of Crkl by a bacterial toxin known as Exo T or mutagenesis disrupted the biogenesis of microvesicles, with a concomitant decline in the compensatory proliferating signaling (CPS) process.[17] **A similar study proved an abundance of expression of *CRKL* in laryngeal squamous cell carcinoma tumors and cell lines. Silencing the expression of *CRKL* was found to reduce the proliferation and migration rates in vitro markedly.[18]**

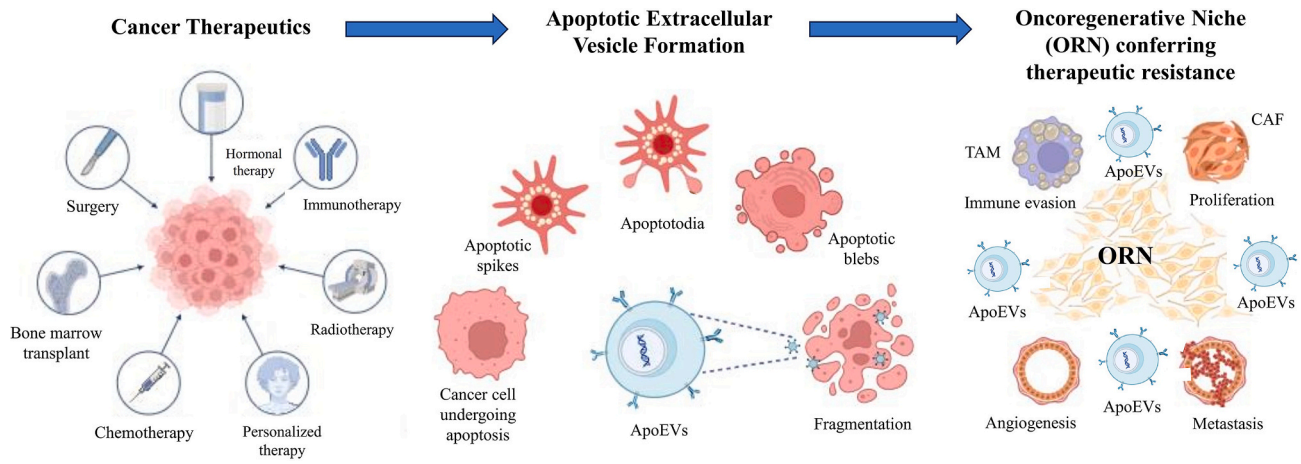


**Fig. 1.** Apoptotic Extracellular Vesicles (ApoEVs) are microvesicles released from apoptotic cells as a consequence of pre-apoptosis stress signals or post-apoptotic necrosis. They contain macromolecules (DNA, RNA, miRNA) and enable the apoptotic cells to transduce signals to the tumor microenvironment. Oncoregenerative niche (ORN) is represented by ApoEVs communicating with non-tumor cellular elements of the niche such as tumor-associated macrophages (TAM), cancer associated fibroblast (CAF), endothelial cells and viable tumor cells. ApoEVs transfer cargoes, including surface receptors and nucleic acids, to recipient cells, triggering the pro-oncogenic activities of the ORN such as the cancer cell stemness, angiogenesis, immune evasion and metastasis.

- **Carcinogens and ApoEVs – Role of ApoEVs in OPMDs and OSCC:** Considering the literature on the production of microvesicles during apoptosis, a hypothesis can be formulated to address how chronic habitual practices such as tobacco and areca nut chewing can induce AiP in oral potentially malignant disorders (OPMDs) and OSCC. Oral leukoplakia (OL) is the most common premalignant lesion with a prevalence of 1.5% and 2.6% and malignant transformation up to 5–36%. OL is significantly associated with tobacco addiction. Cigarette smoke contains around $10^{15-17}$ oxidants or free radicals and a complex mixture of 4500–4700 compounds, including nicotine, nitrosamines, reactive aldehydes, and quinones. Recent evidence suggests that oxidative stress promotes the release of extracellular vesicles (EVs). They manifest great cellular toxicity and act as mutagens and carcinogens by regulating cellular survival, apoptosis, ferroptosis, autophagy, cell proliferation, and angiogenesis.[19] Areca nut, the major causative factor for oral submucous fibrosis, has a higher concentration of copper liberated in saliva.[20] Elevated levels of copper have been demonstrated to induce apoptosis through caspase-dependent and independent pathways. A cascade of signaling processes that are activated thereafter produces microvesicles, harboring proliferative signals that initiate tumor transformation processes.[21] Mohammed et al. investigated the ability of cancer cell lines, viz., primary and metastatic **OSCC**, to produce microvesicles when exposed to normal and apoptotic conditions. The process, when replicated using primary tumors from patients with **OSCC**, showed that cancer cell lines and tumor samples from patients produced enormous amounts of ApoEVs under apoptotic conditions.[22] Wang et al. elucidated the mechanism by which ApoEVs derived from mesenchymal stem cells induce Fas-mediated apoptosis of multiple myeloma cells. ApoEVs stimulate calcium influx in the cytosol and transport Fas ligands in the cytoplasm to the cell membrane. Fas activation initiates apoptosis in multiple myeloma cells and demonstrates the therapeutic value of ApoEVs.[23]

## 3. Conclusion

Detecting these unique apoptotic signatures might provide new avenues for designing and developing biomarkers for early oral cancer diagnosis. Cell proliferation is an important hallmark of any cancer. Considering the various signaling pathways coupled with cell proliferation and cell cycle checkpoints, mitogenic factors play a pivotal role in the uncontrolled proliferation of cells. Further studies on tumor cell-derived ApoEVs can broaden our understanding of the compensatory apoptotic signaling process. Identifying these unique microvesicles in circulation can be the next milestone in the liquid biopsy approach intended for the early diagnosis of oral cancer. The prognostication can be made possible by determining the frequency of ApoEVs in the circulation of OSCC patients. A reduction in ApoEVs can indicate a positive prognosis and better survival outcomes. The synergistic action of drugs combined with inhibitors directed towards the arrest of ApoEVs biogenesis might render the cancer cells vulnerable to treatment and open new avenues in cancer therapeutics.

## Patient/guardian consent

Not Applicable.

## Ethics approval and consent to participate

Not applicable.

## Funding

None.

## Author contribution

KL - Manuscript writing, preparation of figure.
VPJ - Conceptualisation, manuscript preparation, editing.
AP - Manuscript writing.
PA - Manuscript drafting and editing.

## Acknowledgments

None.

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