Oral Oncology 78 (2018) 126–136

Contents lists available at ScienceDirect

Oral Oncology

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

Review

Podoplanin emerges as a functionally relevant oral cancer biomarker and therapeutic target

Edward P. Retzbach[a], Stephanie A. Sheehan[a], Evan M. Nevel[a], Amber Batra[a], Tran Phi[a], Angels T.P. Nguyen[a], Yukinari Kato[b], Soly Baredes[c], Mahnaz Fatahzadeh[d], Alan J. Shienbaum[e], Gary S. Goldberg[a,*]

[a] Department of Molecular Biology and Graduate School of Biomedical Sciences, School of Osteopathic Medicine, Rowan University, Stratford, NJ 08084, USA
[b] New Industry Creation Hatchery Center, Tohoku University; Department of Antibody Drug Development, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
[c] Department of Otolaryngology-Head and Neck Surgery, Rutgers New Jersey Medical School, Newark, NJ 07103, USA
[d] Department of Diagnostic Sciences, New Jersey School of Dental Medicine, Rutgers University, Newark, NJ 07103 USA
[e] Department of Pathology, School of Osteopathic Medicine, Rowan University, Stratford, NJ 08084, USA

ARTICLE INFO

Keywords:
Oral cancer
Oral squamous cell carcinoma
Podoplanin
Signal transduction
Cancer therapy
Biomarkers
Contact normalization
Chemotherapy
CLEC-2

ABSTRACT

Oral cancer has become one of the most aggressive types of cancer, killing 140,000 people worldwide every year. Current treatments for oral cancer include surgery and radiation therapies. These procedures can be very effective; however, they can also drastically decrease the quality of life for survivors. New chemotherapeutic treatments are needed to more effectively combat oral cancer. The transmembrane receptor podoplanin (PDPN) has emerged as a functionally relevant oral cancer biomarker and chemotherapeutic target. PDPN expression promotes tumor cell migration leading to oral cancer invasion and metastasis. Here, we describe the role of PDPN in oral squamous cell carcinoma progression, and how it may be exploited to prevent and treat oral cancer.

The oral cancer burden

Over 14 million new cancer cases are diagnosed each year, which kill a person about every 5 seconds around the world [4]. These statistics indicate that many cancers are not treated successfully. Oral cancer has earned its place among the world's most vicious malignancies. Oral cancer kills over 8,000 people in the USA and 140,000 people worldwide every year, and these numbers are rising [5,6].

Sites for oral cavity cancer include the lips, tongue, floor of the mouth, upper and lower alveolar ridge, retromolar trigone, buccal mucosa, and hard palate. Precancerous and early presentations are often found as white or red mucosal lesions defined as oral leukoplakia (OLP) or erythroplakia, respectively. Precancerous lesions proceed from hyperplasia to dysplasia and carcinoma in situ before developing into invasive malignancies [7,8].

Oral cancer is categorized into four groups according to their tumor, node, and metastasis (TNM) stages. Tumor stages T0, T1, T2, and T3 cases are classified as having no evidence of tumor, tumors less than or equal to 2 cm, greater than 2 cm, or greater than 4 cm, respectively. Tumors are categorized as T4 if they have invaded another portion of the mouth or jaw, including but not limited to the cortical bone, floor of the mouth, tongue, and the skull base. In addition, numbers of lymph nodes (N) and distant sites (M) invaded by the tumors are included in the TNM classification [9].

The development of oral cancer is a complex process involving genetic and environmental factors. Major risk factors include tobacco and alcohol use. Indeed, tobacco and alcohol have synergistic effects that may increase the risk of oral cancer up to 30-fold [10]. The human papillomavirus (HPV) has also been implicated in the development of oral cancer, although not as often as more proximal sites such as the pharynx, tonsils, and base of tongue [6].

Over 90% of oral cancers are oral squamous cell carcinoma (OSCC) [5], and these tumors are notoriously resistant to chemotherapeutic agents. Decades of research with a variety of compounds including alkylating agents, tubulin disruptors, and anthracyclines have failed to significantly increase patient survival or quality of life [11]. Therefore, surgery and radiation therapy are primarily used to treat oral cancer patients. These procedures can extend survival rates; however, they also

* Corresponding author.
E-mail address: gary.goldberg@rowan.edu (G.S. Goldberg).

https://doi.org/10.1016/j.oraloncology.2018.01.011
Received 17 November 2017; Received in revised form 14 December 2017; Accepted 18 January 2018

Table 1
Examples of tumor suppressors and promoters altered in oral cancer.

Suppressor	Function	References
Bax	Apoptotic activator	[172]
E-Cadherin	Homotypic adherins junction	[16]
p16	CDK4/6 inhibition	[23]
p21	CDK1/2 inhibition	[20]
p27	Cyclin E – CDK2 inhibition	[172,173],
p53	Cell cycle arrest, genome protection, and apoptosis activation	[20,22]
P120/catenin	Cell adhesion signaling	[17,18]
Rb	Cell cycle arrest	[23,174,175]
Promoter	**Function**	**References**
Bcl-2	Apoptotic modulator	[172,176,177]
CD133	Hematopoietic stem cell marker	[25]
Cyclin D1	CDK activator	[21,23,178–180]
EGFR	Receptor tyrosine kinase	[19,22,26,181]
Ki-67	Cell proliferation marker	[182]
MMP-2/9	Extracellular matrix degradation	[183]
PDPN	Cell motility	[27]
Src	Nonreceptor tyrosine kinase	[25,27]
Wnt	Migration, differentiation	[24]

cause disfigurations and sequelae that drastically decrease the quality of life for survivors. These patients often experience difficulty in airway management, speech, and mastication [12,13]. A better understanding of mechanisms leading to OSCC is needed to develop more effective methods to detect and treat oral cancer.

A number of tumor promoters and suppressors have been identified in OSCC progression, some of which are shown in Table 1. Activities of tumor suppressors including p53 [14,15], E-cadherin [16], and p120-catenin [17,18] are often disrupted in OSCC cells, while the activities of tumor promoters such cyclin D1 [19–23], Wnt signaling pathways [24], the EGFR and Src tyrosine kinases [25,26], and transmembrane receptor podoplanin (PDPN) [27] are often increased. In particular, PDPN has emerged as a functionally relevant biomarker and potential chemotherapeutic target to prevent and treat oral cancer.

The emergence of PDPN

Aggregate analysis of over 1,000 samples indicate that PDPN is rarely expressed in normal oral epithelial cells, but is found in over 50% of oral cancers [2,3,27–31]. Moreover, PDPN expression in oral cancers is likely to be much higher than actually reported. This is because PDPN expression is found mainly in the invasive fronts of oral cancers. For example, PDPN is clearly seen at the invasive front of OSCC as shown in Fig. 1a. Indeed, we [1] and others [32] found PDPN expression in 100% of OSCC examined in focused studies.

PDPN expression is induced very early in the OSCC transformation process, and can be used to identify premalignant lesions that are bound to develop into oral cancer. Analysis of over 300 premalignant oral lesions pooled from several retrospective studies [7,33–36] is summarized in Fig. 2a. These data indicate that over 46% of lesions with notable PDPN expression progressed to OSCC. In contrast, less than 14% of lesions without notable PDPN expression progressed to OSCC. Thus, PDPN expression increases the probability of OSCC formation from histologically benign lesions by over 3-fold. However, this number could be an underestimate of PDPN involvement since more suspicious lesions are most likely to be slated for biopsy by health care professionals. For example, data from some studies indicate that over 80% of oral leukoplakias that express high levels of PDPN convert to oral cancer [7].

In addition to malignant progression, PDPN expression also correlates with OSCC mortality. As shown in Fig. 2b, 5 year overall and disease free survival drops from 86% and 100% for patients with tumors with undetectable PDPN expression levels to 23% and 37% for patients with tumors exhibiting high PDPN expression [2,3]. These data indicate that PDPN expression leads to a 3 to 4-fold decrease in 5 year survival for oral cancer patients.

PDPN is expressed in many forms of cancer. As with OSCC, PDPN expression has been shown to promote mammary carcinoma [37,38], glioma [39], other types of squamous cell carcinoma (SCC) [7,8], melanoma [40–42], ovarian cancer [43], and pulmonary adenocarcinoma [44,45]. Indeed, PDPN appears to play a key role in fundamental mechanisms leading to tumor invasion and metastasis.

PDPN plays a unique role in the tumor microenvironment. In addition to tumor cells, PDPN is expressed in infiltrating cancer associated fibroblasts (CAFs). Results from several reports indicate that CAFs utilize PDPN to increase motility and survival of neighboring tumor cells, particularly lung adenocarcinoma and melanoma cells [41,44,45]. Xenograft human OSCC cells induce PDPN expression in mouse host infiltrating CAFs in experimental models as shown in Fig. 3. Lymph node stromal cells that express PDPN have also been shown to inhibit the proliferation of T helper cells in order to promote melanoma progression [46,47].

PDPN in development and disease

As with many tumor promotors, PDPN plays critical roles in ontogeny [48]. PDPN is expressed early in development, starting with the proepicardial organ in the embryonic foregut [49]. Studies with PDPN knockout mice demonstrate that it goes on to play key roles in the development of the heart, lungs, and lymphatic system [50].

PDPN associates with the C-type lectin-like 2 (CLEC2) receptor on platelets as described below (see Fig. 4) [51]. This association plays a critical role in the genesis of the lymphatic system. Presumptive lymphatic vessels sprout from the cardinal vein during mammalian development. Platelets that express CLEC2 interact with PDPN on these

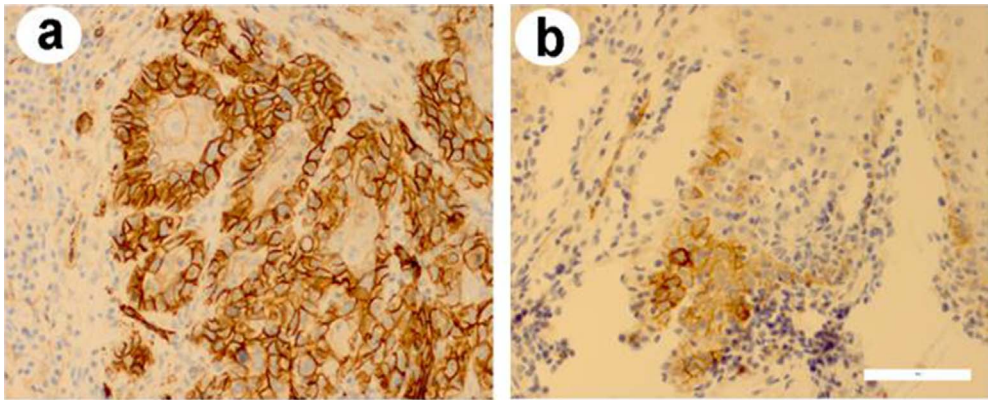

Fig. 1. PDPN expression in human oral cancer. (a) Stage 1 OSCC from the tongue of a 61 year old male. (b) Oral leukoplakia from the tongue of a 47 year old male. Bar = 40 μm.