



Review

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



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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

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

Suppressor	Function	References
Bax	Apoptotic activator	[172]
E-Cadherin	Homotypic adhesins 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 disfigurements 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 promoters, 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

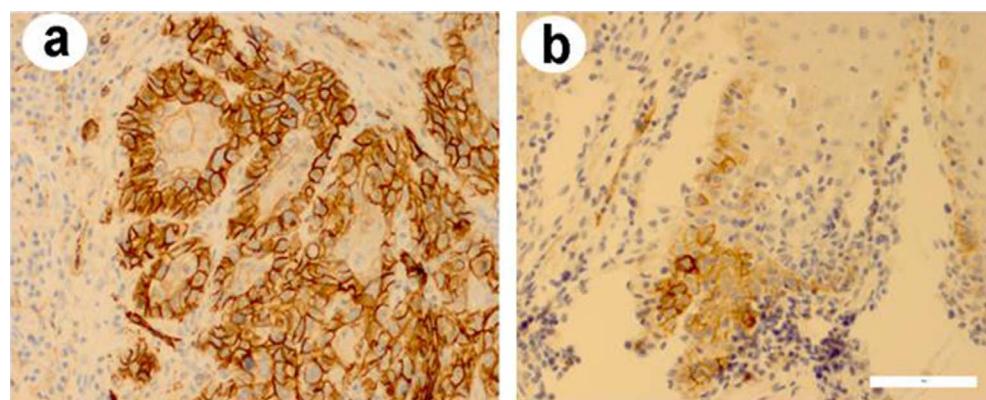


Fig. 1. PDPN expression in human oral cancer. (a) Stage 1 OSCC from the tongue of a 61 year old male. (b) Oral leukoplakia from the tongue of a 47 year old male. Bar = 40 μm.

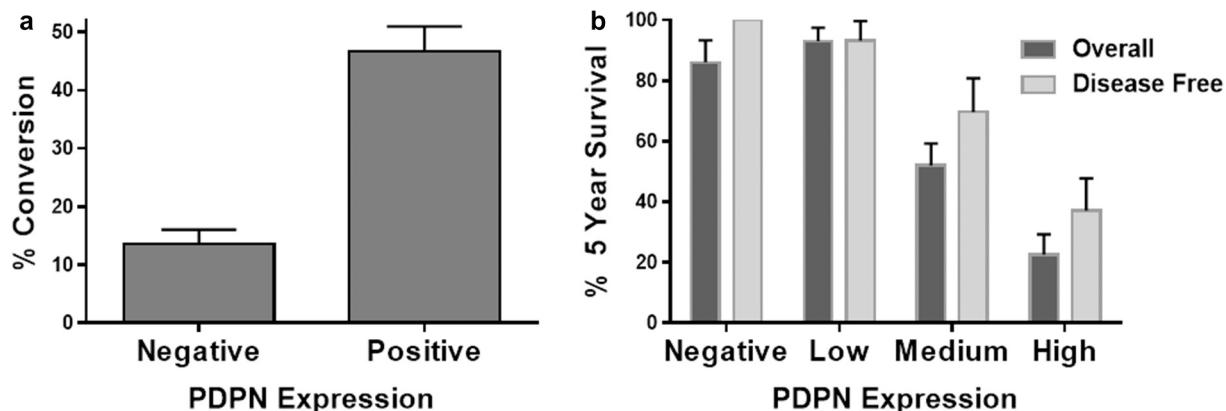


Fig. 2. PDPN expression and oral cancer mortality. (a) PDPN expression correlates with transformation of premalignant oral lesions to OSCC. Data are shown as mean + SEM ($n = 194$ and 135 for PDPN negative and positive lesions, respectively) [7,33–36]. (b) PDPN expression inversely correlates with overall and disease free 5 year patient survival. Data are shown as mean + SEM ($n = 143$ and 63 for overall and disease free survival, respectively) [2,3].

future LECs (lymphatic endothelial cells). This interaction causes lymphatic sacs to pinch off and separate from cardinal veins. This separation is crucial for the lymphatic system to arise from the embryonic cardiovascular system [49], which continues beyond development such that PDPN is used as an accepted marker for lymphatic endothelial cells (LECs) [52].

PDPN expression in type 1 alveolar cells is critical for proper lung development. This process is evidenced by abnormal differentiation and morphology of the distal lung in PDPN knockout mice. These mice display defective lymphatic systems, but usually die shortly after birth from respiratory failure [53–55]. PDPN null mice also suffer from heart deformation resulting from abnormal proepicardial organ morphology, inhibited migration of epicardium-derived cells, and abnormal pulmonary vein development [56,57]. PDPN is also expressed through the developing heart lymphatics, which allow for drainage of the sub-epicardium and the myocardial wall [58]. PDPN expression in heart development, as well as the vascular system, also plays a critical role in controlling venous thromboembolism and deep vein thrombosis, which may be another area where PDPN can become a clinically relevant target [59,60].

While PDPN knockout mice are considered to be embryonic lethal, a small percentage of these animals survive to adulthood without obvious phenotypic abnormalities. This phenomena may result from redundant receptor signaling systems. However, these PDPN null survivors provide a unique model to study PDPN functions in adult mice [61]. For example, recent studies indicate that PDPN expression in the dentate gyrus of the hippocampus aids in memory formation, spatial learning,

and long term synaptic potentiation. Data from these experiments also suggest that PDPN interacts with NGF (nerve growth factor) and may play a role in the development of Alzheimer's and other neurodegenerative diseases [62]. Interestingly, PDPN and amyloid precursor protein (APP) are both cleaved by presenilin-1/gamma-secretase [63].

PDPN is expressed in kidney podocytes that maintain selective glomerular filtration [64]. Decreased PDPN expression in kidney podocytes results in flattening of foot processes, causes proteinuria, and has been reported in nephrosis [64,65]. PDPN also appears to enable kidney podocytes to activate AKT kinase and avoid apoptosis in response to angiotensin signaling [66,67].

PDPN appears to play a key role in adaptive immunity and inflammatory disease. Dendritic cells express CLEC2, which associates with PDPN fibroblastic reticular cells to increase lymph node expansion and promote innate immunity [50,68]. Increased PDPN expression is found in chondrocytes and synovial fibroblasts associated with rheumatoid and osteoarthritis [69–75]. In addition, PDPN is expressed on inflammatory dermal fibroblasts that induce interleukin 17 production by neighboring T helper cells to promote psoriasis [76]. PDPN is also found in general lymphatic expansion related to inflammation. For example, PDPN is found in lymphatic vessel formation that occurs in the intestines of patients with ulcerative colitis and Crohn's disease [77].

Taken together, data indicate that PDPN plays key roles in development and homeostasis. In particular, PDPN promotes signaling events that promote cell motility and inflammation which are both fundamental hallmarks of cancer [48]. Indeed, the vast majority of oral

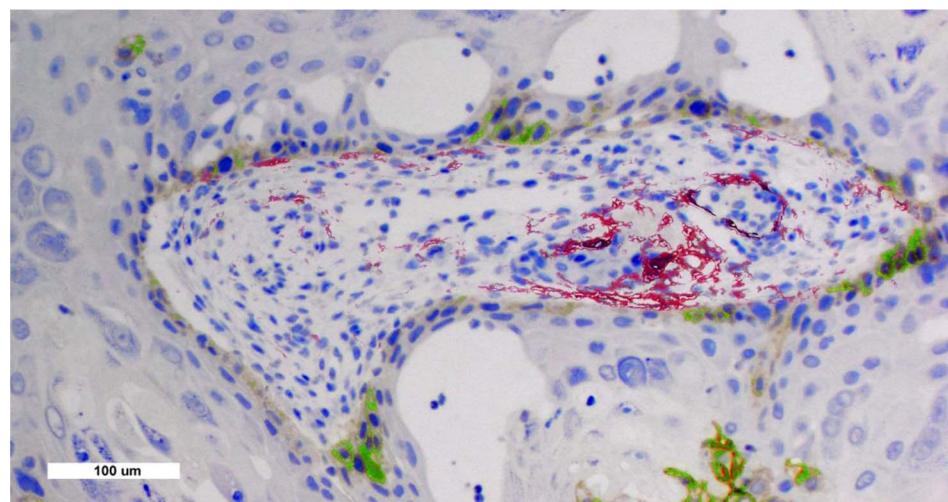


Fig. 3. OSCC cells induce PDPN expression in infiltrating host murine cancer associated fibroblasts in xenograft tumors. Tumors from human OSCC cells were examined with antisera specific for human (D2-40, green) and mouse (8.1.1, red) PDPN by immunohistochemistry as previously described [1].

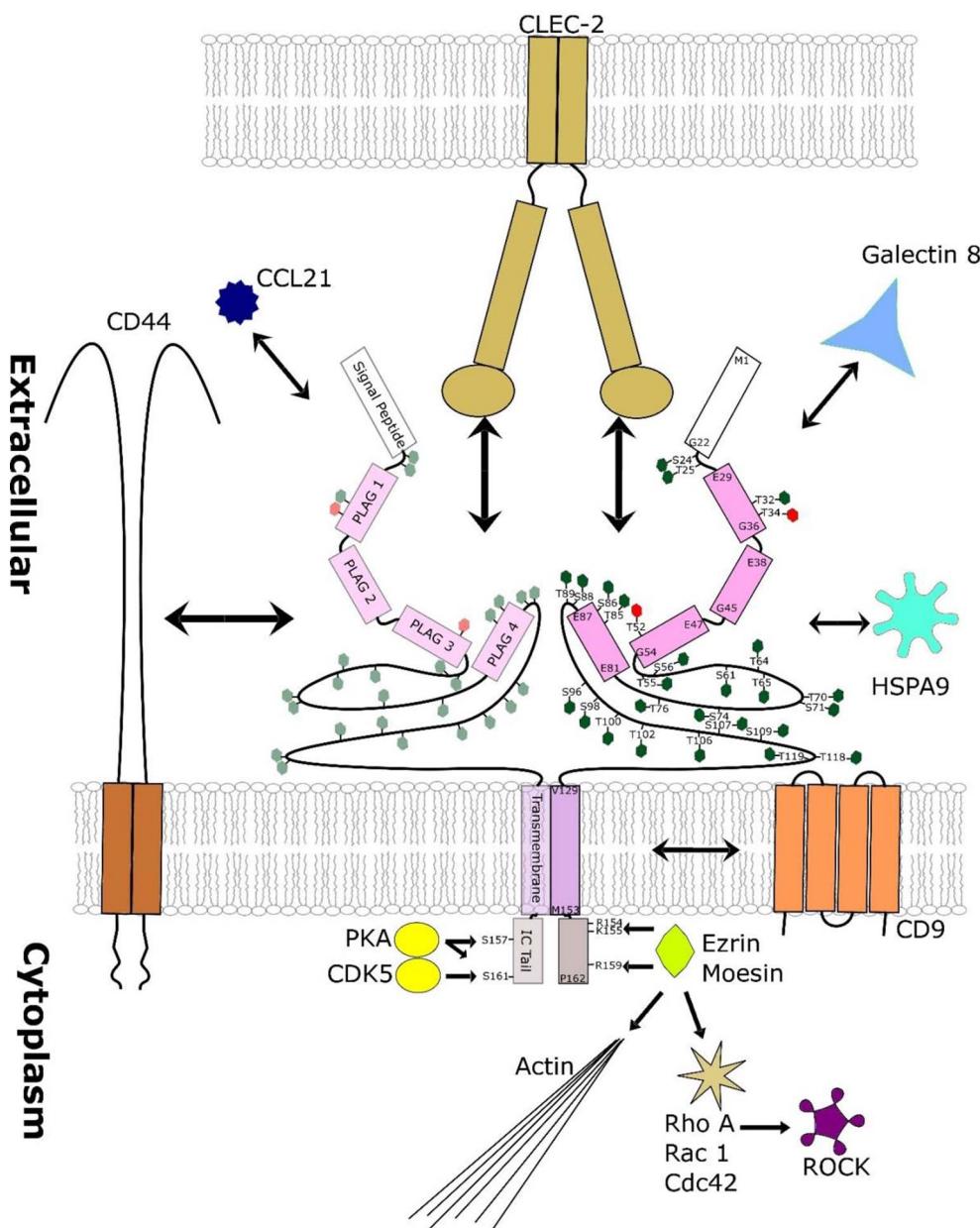


Fig. 4. PDPN topology and binding partners. Podoplanin contains an amino terminal signal peptide (white), 4 extracellular PLAG domains (pink), transmembrane region (lavender), and intracellular carboxyl tail (solid gray). Confirmed and potential glycosylation sites are indicated by red and green hexagons, respectively. A GxxxG motif in the transmembrane region may drive PDPN to form a homodimer and traffic to lipid rafts. CLEC2, HSPA9, CD44, Galectin 8, and CCL21 bind to extracellular PDPN domains. CLEC2 interaction sites have been localized to PDPN residues G45, D48, and D49, with the aid of glycosylation at T52, along with E81, D82, and T85 in a recently defined PLAG4 domain (E81-E87). The integral membrane protein tetraspanin CD9 interacts with the PDPN transmembrane domain. The ERM proteins ezrin and moesin interact with the PDPN intracellular tail to modulate pathways involving ROCK/LIMK activities, as well as actin polymerization by Rho GTPases. The PKA and CDK5 kinases can phosphorylate intracellular serines to inhibit cell motility. Figure is based on human PDPN with relevant amino acids indicated by number but not drawn to scale.

cancer treatment complications and deaths results from tumor invasion and metastasis [5,6]. PDPN structural and functional investigations can lead to a greater understanding of how these events can be treated as discussed below.

PDPN nomenclature, regulation, and structure

Podoplanin has been independently discovered and named by many groups over the past thirty years. A chronological list of these names is shown in Table 2. PDPN was first detected in human adenocarcinoma cells, fetal testis, seminomas, and dysgerminomas with the rabbit monoclonal antibody called “M2A” in 1986 [78]. PDPN was then discovered as a marker for type 1 alveolar cells and named “T1 α ” in 1988 [79]. PDPN was also found on mouse colon adenocarcinoma cells as a 44 kD glycoprotein called “gp44” in 1988 [80]. PDPN was then discovered again as a gene expressed by 12-O-tetradecanoylphorbol-13-acetate (TPA) and Ras transformed mouse osteoblastic cells and named “OTS-8” in 1990 [81]. The term “podoplanin” was first used upon discovering its role in promoting flattened foot processes in rat

Table 2
Podoplanin nomenclature.

Name	Source	Year	References
M2A	Human ovarian adenocarcinoma cells	1986	[78]
T1 α	Rat type 1 epithelial cells	1988	[79]
GP44	Mouse colon adenocarcinoma	1988	[80]
OTS-8	TPA and Ras transformed mouse osteoblastic cells	1990	[81]
GP38	Mouse lymphoid tissue	1992	[184]
E11	Rat osteosarcoma	1996	[185]
PA2.26	DMBA transformed mouse keratinocytes	1997	[186]
Podoplanin	Rat glomerular cells	1997	[64]
T1 α -2	Human lung cDNA expressed in <i>Xenopus</i> oocytes	1998	[187]
GP36	Human placenta	1998	[188]
M2A/D2-40	Human oncofetal and germ cell tumors	1999	[189]
RANDAM-2	Mouse embryonic carcinoma and central nervous system	2002	[190]
Aggrus	Human colorectal tumors	2003	[191]
rT140	Rat lungs exposed to inflammatory agents	2006	[192]

glomerular cells in 1997 [64]. “Podoplanin” and “PDPN” have become the officially accepted name and symbol as used throughout this review.

PDPN presents some complex regulatory considerations. The PDPN gene is located on chromosome 1 in humans (loci 1p36.21) and chromosome 4 in mice (loci 4E1). The gene is rather large at about 600,000 bases with 8 exons in humans and 200,000 bases with 6 exons in mice, compared to its mRNA transcript sizes of between 2,646 and 2,828 bases (4 variants) and about 1,800 bases (2 variants) in humans and mice, respectively [82,83]. PDPN is translated into a 162 amino acid (18 kD) primary translation product in humans and a 172 amino acid (19 kD) product in mice [84,85].

Several miRNAs (microRNAs), including miR-29b, miR-125a, and miR-363, target the 3' PDPN mRNA region to decrease PDPN expression. miR-29b decreases PDPN expression in kidney podocytes, which contributes to angiotensin II induced apoptosis [66]. Both miR-29b and miR-125a suppress PDPN expression to inhibit glioblastoma invasion [39], while miR-363 has been shown to inhibit head and neck squamous cell carcinoma metastasis [86].

Epigenetic regulation of PDPN expression is complex. For example, promoter methylation and histone hypoacetylation increased PDPN expression in osteosarcoma cells [87], while promoter methylation inhibited PDPN expression in glioblastoma cells [88]. In addition, histone deacetylase activity increased PDPN expression in OSCC cell lines (HSC-2, HSC-3, Scc-10, and Ca9-22), while there was no significant evidence of PDPN promoter methylation in these cells [31].

Several transcription factors have been shown to regulate PDPN expression. The PDPN promoter region contains Prox1 binding sites, which increases PDPN expression. This is particularly important for lymphangiogenesis during development. Binding sites for other transcription factors including AP-2, AP-4, C/EBP β , NF-1, and NF κ B sites are also been found in the PDPN promoter, and warrant further study [87,89]. For example, NF κ B expression correlates with lymphangiogenesis and podoplanin expression in cancers including esophageal SCC [90]. TPA and PI3K/Akt activity leads to AP-1 upregulation of PDPN expression in mouse skin cancer and human glioblastoma, respectively [88,91]. Sox2 can also increase PDPN expression in cancer stem cells [92]. Other factors have also been implicated in PDPN expression during cancer progression. These include Sp1 and Sp3 in osteosarcoma cells [87], as well as combined FGF2/BMP4 signaling in neural stem cells [93]. Most pertinent to this review, epidermal growth factor (EGFR) activation of STAT3 [94], TGF β [31,95], and Epb1 activity [96] can increase PDPN expression in cancers including OSCC.

PDPN contains an amino terminal signal peptide followed by extracellular platelet aggregation (PLAG) domains, a transmembrane region, and a short intercellular carboxyl tail as shown in Fig. 4. The transmembrane region contains glycine based motifs that may drive PDPN to form dimers in the cell membrane [97]. The transmembrane and intracellular PDPN domains consist of about 30 amino acids [64], which leave over 80% of the protein outside of the cell where it can interact with extracellular modulators.

PDPN contains 25 potential glycosylation sites along the extracellular region of the protein [98,99]. The primary PDPN translation product is modified from an 18–20 kD polypeptide into a mature protein with an apparent molecular mass in the 40 kD range. These modifications include galactose and N-acetylgalactosamine with α 2-3 and α 2-6 linked sialic acid glycosylation events which contribute to protein interactions and signaling events [84,100,101].

PDPN binding partners

PDPN is a unique protein without intrinsic functional domains or enzymatic activities. Thus, PDPN appears to interact with other proteins to affect cell behavior. These binding partners include CLEC2, heat shock protein A9 (HSPA9), CD9, CD44, Galectin 8, Chemokine (C-C motif) ligand 21 (CCL21), ezrin, moesin, protein kinase A (PKA), and

cyclin dependent kinase 5 (CDK5) as shown in Fig. 4.

CLEC2 interaction sites have been localized to PDPN residues G45, D48, and D49, with the aid of glycosylation at T52 [102,103], along with E81, D82, and T85 in a recently defined PLAG domain (E81-E87) [104]. This association activates Src dependent CLEC2 phosphorylation and directs platelet aggregation during lymphatic development and metastasis by tumor cells that express PDPN [105]. Indeed, antisera that block CLEC2 interactions with PDPN can significantly inhibit tumor growth and metastasis. In addition, dendritic cell migration can also be linked to PDPN-CLEC2 interactions [106].

The integral membrane protein tetraspanin CD9 interacts with the PDPN transmembrane domain. CD9 is a tetraspanin glycoprotein receptor that mediates a variety of events associated with cell adhesion, migration, and platelet aggregation. However, glycosylated tetraspanin CD9 appears to oppose the action of CLEC2, binding podoplanin to inhibit platelet aggregation and lung tumor metastasis [107].

The hyruaronan receptor CD44 is a widely expressed integral membrane protein that interacts with podoplanin. Like PDPN, CD44 is also a functionally relevant cancer biomarker that is found in many types of cancer including oral squamous cell carcinoma [108]. CD44 associates with PDPN to induce epithelial mesenchymal transition, lamellipodia formation, cell adhesion, and directional cell migration [109]. PDPN and CD44 also colocalize in microvesicles and exosomes that traffic between cells to induce epithelial mesenchymal transition (EMT) and lymphatic capillary morphogenesis [110].

PDPN interacts with soluble factors including Gal8 (galectin 8), CCL21 (C-C motif chemokine 21), and HSPA9 (heat shock protein A9) in addition to integral membrane proteins such as CLEC2 and CD9. This interaction is exemplified by Gal8. Gal8 is a mammalian lectin that is found on lymphatic and vascular endothelial cells. Gal8 interacts with glycosylated receptors on extracellular matrix proteins, integrins, and PDPN. In particular, Gal8 targets PDPN to promote lymphatic endothelial cell adhesion, migration, and lymphatic vessel formation [111]. The interaction between galectin 8 and PDPN also induces inflammatory lymphangiogenesis precipitated by immune responses [112].

CCL21 is secreted mainly by lymphatic endothelial cells, and by a number of other cell types to a lesser extent. CCL21 targets C-C chemokine receptor type 7 (CCR7) chemokine receptors to promote directional T-cell migration. However, CCL21 is also produced by, and targets PDPN on, fibroblast reticular cells to promote differentiation of regulatory T (Treg) cells in the thymus [113]. Interestingly, OSCC cells also produce CCL21 to promote lymph node invasion with the help of recruited macrophages [114].

HSPA9 is a cognate member of the heat shock family of proteins that is found in the extracellular matrix, mitochondria, and cytoplasm of variety of cells. HSPA9 associates with proteins including p53 and EGFR in addition to PDPN. HSP mutations lead to development of sideroblastic anemia and even-plus syndrome (characterized by epiphyseal and vertebral dysplasia). In addition, aberrant HSPA9 expression is associated with cancers including glioma and OSCC where it is colocalizes with PDPN at the tumor stromal interface [115].

The intracellular tail of PDPN contains two serine residues that can be phosphorylated by PKA and CDK5. These phosphorylation events effectively decrease PDPN mediated cell motility [41,116]. The PDPN intracellular tail also contains basic arginine and lysine residues that interact with the ezrin/radixin/moesin (ERM) protein family members ezrin and moesin [85]. This interaction activates small GTPases RhoA and Rac1, and proteins kinases including Rho associated protein kinase (ROCK) and LIM kinase (LIMK). These events induce actin polymerization and rearrangement to promote cell migration, invasion, and lymphatic capillary morphogenesis [117,118]. These relationships present clinical relevance since antibodies and lectins can target PDPN to inhibit Cdc42 GTPase activity, motility, and viability of OSCC cells [1]. In fact, PDPN is among the first proteins to be expressed in the transition from benign to malignant tumor progression as discussed

below.

Contact normalization

Nontransformed cells form junctions with adjacent cells that force them to assume a normal phenotype and morphology. This process is called contact normalization [116,119]. Clinical implications of contact normalization can be traced back as early as 1839 [120]. These studies report cancer recurrence after 15 years or more of disease-free survival, suggesting transformed cells can adopt a normal phenotype and reside dormant in the body for many years [119–121]. Contact normalization plays a chief role in the suppression of tumor cells during early stages of tumorigenesis. Tumor cells must escape contact normalization before they become malignant and metastasize [116].

Cadherin and connexin interactions promote contact normalization [116,122–124]. Cadherins are calcium dependent transmembrane glycoproteins that form adherens junctions to mediate intercellular adhesion [116,125]. “Classical” cadherins include E-cadherin (epithelial cadherin) and N-cadherin (neural cadherin). Both E-cadherin and N-cadherin have been identified as biomarkers in the progression of oral squamous cell carcinoma. Decreased E-cadherin expression is associated with increased OSCC malignancy and metastasis [126–129]. Conversely, inhibition of N-cadherin expression is associated with decreased OSCC malignancy and metastasis [127,130]. Replacement of E-cadherin with N-cadherin expression in tumor cells is known as the “cadherin switch” during the process of EMT [131]. Carcinoma cells often become more motile, invasive, and metastatic following this process [132,133].

As with cadherin interactions, gap junctions are also often disrupted in tumor cells. Connexin proteins form gap junctions to mediate direct communication between adjacent cells. Gap junctions enable neighboring cells to share intracellular signals including metabolites and ions in a controlled fashion. Connexin proteins interact with a number of partners including cadherins. Similar to cadherins, connexins can act as tumor suppressors. Repressed connexin expression has been found in cancers including lung, glioma, breast [134–137], and OSCC [138,139]. However, while gap junctions appear to enhance the ability of normal cells to control the growth of tumor cells, they are not required for contact normalization [122].

Approximately 0.1% of the transcriptome is affected by contact normalization. About 60% of these genes are considered tumor suppressors since they are suppressed in the transformed cells but increased by contact normalization, and 40% are considered tumor promoters with increased expression in transformed cells and decreased expression during contact normalization. These tumor suppressors include four and a half LIM domains (FHL1) and serum deprivation response protein (SDPR), while tumor promoters include transmembrane protein 163 (TMEM163), vascular endothelial growth factor receptor 2 (VEGFR2), and PDPN [116,140].

FHL1 belongs to a family of LIM only proteins that function to control multiple cellular processes including proliferation, differentiation, apoptosis, adhesion, migration, transcription, and signal transduction [116,141,142]. Decreased FHL1 protein and mRNA expression is observed in OSCC, which result from DNA methylation of its promoter region [143]. SDPR, a calcium-independent phospholipid binding protein, acts to induce caveolae formation jointly with caveolin, when phosphorylated by protein kinase C (PKC) and binding with polymerase I and transcript release factor (PTRF) [144,145]. SDPR expression may be regulated by FHL1, and decreased expression is observed in OSCC and many other cancers including breast, kidney, and prostate [146,147].

Tumor promoters involved in contact normalization are of particular interest since they may serve as drug targets. The receptor proteins TMEM163, VEGFR2, and PDPN rise to the top of this list [148]. TMEM163 is a transmembrane protein involved in glutamate transport. Increased TMEM163 expression has been found in cancers including

papillary thyroid carcinoma and nodular lymphocyte-predominant Hodgkin lymphoma [116,124,149]. VEGFR2 (also known as KDR) is expressed mainly by vascular endothelial cells, as well as many types of cancers including glioma, breast, and lung carcinoma. VEGFR2 is a tyrosine kinase receptor that promotes tumor cell proliferation, migration, and angiogenesis. VEGFR2 expression has become a prognostic tool to predict OSCC aggression and metastasis [150,151]. Chemotherapeutic drugs targeting VEGFR2 and its VEGF ligands have been developed to inhibit tumor progression and angiogenesis. These drugs include bevacizumab (Avastin), sunitinib (Sutent), and sorafenib (Nexavar). Effects of these drugs on oral cancer are currently being evaluated. Clinical trials are being conducted to study the efficacy of cytotoxic drugs in combination with Bevacizumab for head and neck SCC (HNSCC) patients [152,153]. Combination therapy utilizing sorafenib and radiation is also being evaluated as a treatment for OSCC patients [154–156].

In addition to VEGFR2, PDPN was identified as a tumor promoter that is upregulated during transformation and reduced by contact normalization. As discussed throughout this review, PDPN is highly expressed in many types of cancers including OSCC where it promotes tumor progression and metastasis. Studies indicate that antibodies and lectins can be used to target podoplanin in OSCC as described below.

Treatments targeting PDPN

PDPN has emerged as a clear chemotherapeutic target for oral cancer. Consequently, compounds that target PDPN are being developed as anticancer reagents. These include antibodies, cell based immunotherapies, and chemical biologics.

Antibodies against PDPN have shown promise as anticancer agents in preclinical studies. This work began with the NZ-1 monoclonal antibody which targets the PLAG domains of human podoplanin. Initially, NZ-1 was found to inhibit platelet aggregation to glioma cells mediated by PDPN-CLEC2 interactions [157]. Further experiments revealed that NZ-1 and its rat-human chimeric derivatives [158,159] can also inhibit the growth of glioma [160], mesothelioma [161], and, of interest for this review, OSCC cells [1]. For example, NZ-1 caused a 30-fold reduction in tumor weight compared with control tumor growth in a xenograft orthotopic mesothelioma model in SCID mice dosed at 100 µg i.p. twice a week for two weeks [159]. A mouse-human chimeric antibody against human PDPN, chLpMab-7, was also shown to inhibit metastasis in a pulmonary xenograft mouse model [162]. Other anti-PDPN monoclonal antibodies such as murine and humanized MS-1 also blocks PDPN-CLEC2 interactions and can effectively inhibit lung tumor growth by IV administration [103]. Genetically modified chimeric antigen receptor (CAR)-transduced T-cells that target PDPN have also been shown to inhibit glioblastoma progression in mouse models. For example, human T cells expressing modified NZ-1 injected into the tail vein 7 days after glioblastoma implantation caused a 3-fold reduction in tumor growth and 140% longer survival time than controls [163].

Intriguingly, the glycosylation pattern of PDPN appears to change when expressed by cancerous cells, leading to the development of cancer specific monoclonal antibodies (CasMabs) [164]. These highly specific reagents offer enhanced potential to target PDPN on cancer cells. For example, the CasMab chLpMab-2 reacts with glioblastoma, mesothelioma, and lung cancer cells [165]. This technology was also used to develop chLpMab-23, which can inhibit glioblastoma and OSCC growth [166]. For example, human OSCC cells that were subcutaneously injected into nude mice, followed by 100 µg of i.p. injected ChLpMab-23 six times over a thirty-five-day period showed a 3.5-fold decrease in tumor weight compared to controls. This precursor of this CasMab antibody, LpMab-23, also shows minimal binding to surrounding lymphatic endothelial cells which express podoplanin [167] as shown in Fig. 5. These cancer specific PDPN antibodies offer minimal offsite targeting to present a double-edged tool that can be used to exploit PDPN as a biomarker with reduced false positives due to

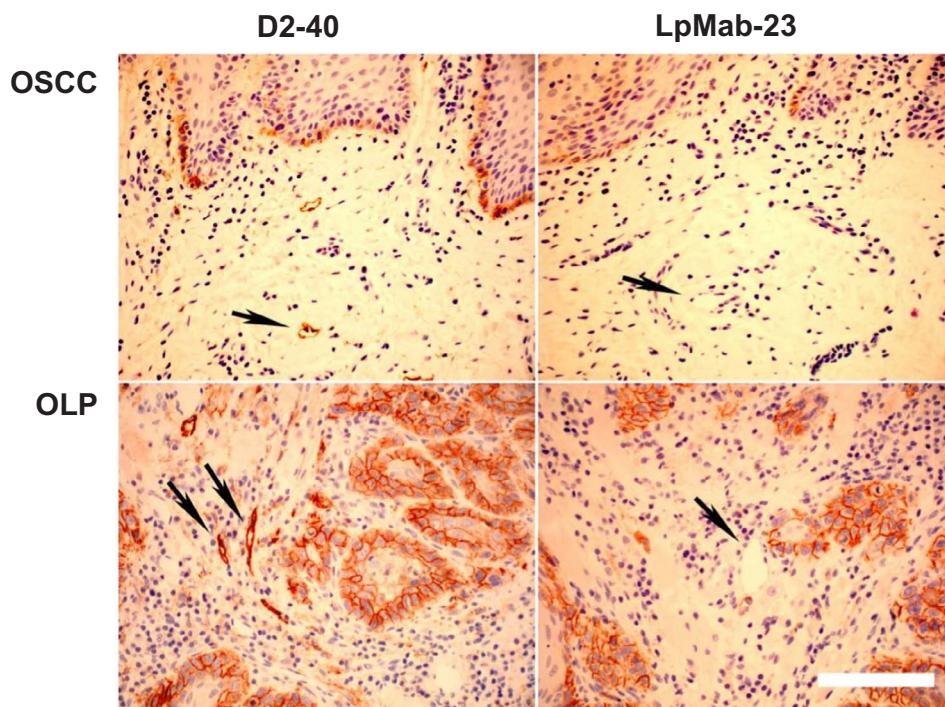


Fig. 5. Cancer specific monoclonal antibodies (CasMabs) can specifically detect PDPN on OSCC cells. Human clinical samples from a patient with OSCC and oral leukoplakia OLP were examined with antibodies directed against total PDPN (D2-40) and cancer specific PDPN (LpMab-23) as indicated. PDPN staining is evident in OSCC cells and OLP with presumptive OSCC potential by both antibodies, while only D2-40 stained PDPN on lymphatic endothelial cells (indicated by arrows). Bar = 80 μ m.

expression in normal tissues, and also as potential chemotherapeutic agents with minimal side effects. For example, as shown in Fig. 5, OSCC cells and oral leukoplakia cells that seem bound to evolve into OSCC are both recognized by LpMab-23. This reagent may provide a tool that can be used for early detection needed to formulate best care plans for best care treatment.

Podoplanin can also be targeted with other biological chemicals, exemplified by *Maackia amurensis* seed lectin (MASL) [1,40]. This compound is actually a coincidental component of traditional medicines used to treat cancer that have been prepared from the *Maackia amurensis* for centuries [168,169]. Comparison between MASL and NZ-1 indicate that both inhibit OSCC cell migration and viability at similar nM and μ M concentrations, respectively. However, MASL, which binds terminal sialic acid residues on PDPN [170], can be orally administered to treat cancer and dynamically targets OSCC cells within minutes of exposure [1,171]. This potential for topical and systemic treatments could be particularly useful for oral cancer and precancerous lesions that are destined to evolve into OSCC.

Conclusion

Oral cancer is a devastating malady that affects millions of people and their families. The lack of effective chemotherapeutics is a paramount problem facing this patient population. PDPN has emerged as a functionally relevant biomarker and chemotherapeutic target. Reagents are being developed to specifically target this extracellular receptor on cancer cells while minimizing off target chemotherapeutic side effects. The oral health community is now poised to take advantage of these technologies to better detect, treat, and prevent oral cancer.

Conflict of interest

Dr. Goldberg received funding from Sentrimed Inc which holds intellectual property for reagents that target PDPN to combat cancer. Other authors declare no conflict of interest.

Acknowledgments

This work was supported in part by funding from the Osteopathic Heritage Foundation, Northarvest Bean Growers Association, New Jersey Health Foundation, and Sentrimed to GSG, the Platform for Drug Discovery, Informatics, and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, by Regional Innovation Strategy Support Program from MEXT of Japan, by Grant-in-Aid for Scientific Research from MEXT of Japan to YK, and the New Jersey Health Foundation to AJS.

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