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REVIEW ARTICLE

Podoplanin expression and clinicopathological correlation in oral squamous cell carcinoma - A review

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Abstract

Podoplanin is a mucin-like transmembrane glycoprotein that been highly and specifically expressed in the lymphatic endothelial cells but not in the endothelium of blood vessels. Studies have shown the expression of podoplanin in squamous cell carcinomas and a relationship with the clinicopathological features. This raises a possibility that podoplanin may have a biological function in tumor cells and its expression could be used as a biomarker for diagnosis and prognosis. A review was undertaken to summarize the results of the published studies on the expression of podoplanin in oral squamous cell carcinoma (OSCC) in relation to clinicopathological parameters from the year 2006 to 2016. This review showed that podoplanin was highly expressed in OSCC, and podoplanin expression was associated with certain clinicopathological characteristics. Since OSCC spreads through the lymphatic route, podoplanin could be used as a potential biomarker in assessing the lymph node metastasis and prognosis. The expression of podoplanin by the tumor cells can also help in assessing the tumor progression.

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies involving the head and neck region. The development of this is a multistep process involving various genetic, epigenetic, and metabolic alterations, resulting from exposure to carcinogens.[1] The use of tobacco, alcohol, and betel constitutes the main risk factors. The high-risk groups include older adult males who consume tobacco and alcohol. A significant proportion of OSCC develops from pre-malignant lesions.[2]

Despite various advances in diagnosis and treatment, the survival rates have not significantly improved, with the 5-year survival rate after diagnosis remaining around 15–50%. The prognosis remains poor due to late-stage diagnosis, high rates of primary-site recurrence, and metastasis to regional lymph node. Early diagnosis of OSCC can improve the prognosis and reduce the mortality. Metastasis of primary tumors to secondary sites within the body is the main cause for deaths associated with cancer.[2]

OSCC in its advanced stages spreads loco-regionally, where the tumor cells travel through the lymphatics to the cervical lymph nodes. The presence of lymph node metastasis is an indicator for the poor prognosis of OSCC. Histopathology has remained the gold standard in the diagnosis of OSCC for many years.[2] However, due to the high frequency of recurrence, this may be inadequate. Therefore, it is important to further understand the pathogenesis and characteristics of OSCC and improve the diagnostic capabilities.[3]

It has been noted that changes at molecular level occur before the occurrence of any clinical change. It is, therefore, important to identify biological markers that may augment the clinical staging system.[4]

Podoplanin

Podoplanin is a mucin-like transmembrane glycoprotein that is highly and specifically expressed in lymphatic endothelial cells (LECs) but not in the endothelium of the blood vessels.[4-6]

Podoplanin was identified for the first time in 1990 when its mRNA was found in the murine osteoblastic cell line.[7,8] Podoplanin was first described on lymphatic endothelial cells (LECs) as the E11 antigen.[7,9] Podoplanin has been described on fibroblastic reticular cells of lymphoid organs and thymic epithelial cells as gp38. It is also homologous to T1a/rTI40, one of the first molecular markers of alveolar type I epithelial cells;

PA2.26, which is upregulated in skin keratinocytes on injury; OTS-8, a molecule induced in osteoblasts upon phorbol ester treatment; and Aggrus, a platelet-aggregating factor. The name Podoplanin was given to this molecule due to its expression on kidney podocytes and possible involvement in the flattening of podocyte foot processes.[10]

Podoplanin is expressed in normal cells such as the mesothelial cells, choroid plexus, osteocytes, follicular dendritic cells, and squamous cells in the skin, esophagus, and uterine cervix. In the oral cavity, it is expressed only in LECs and myoepithelial cells of normal salivary glands.[1]

Studies have also shown that podoplanin may be expressed in certain tumor cells, including squamous cell carcinoma, raising a possibility that podoplanin may have biologic functions in tumor cells.[4] Podoplanin expression is seen in various neoplasms such as lymphangioma, angiosarcoma, ovarian carcinoma, testicular carcinoma, mesothelioma, astrocytoma, and SCCs of the skin, lung, uterine cervix, esophagus, and larynx.[1] Recent studies have shown that podoplanin is also expressed in oral dysplastic and hyperplastic lesions with a risk of cancer development raising the question whether podoplanin may have further biological functions in premalignant oral lesions.[6]

In the past, podoplanin has been frequently used to assess intra- and peri-tumoral lymphatic vessel density in OSCCs, which are correlated with metastatic spread to lymph nodes and a poor prognosis.[1]

It has been seen that podoplanin-null mice died at birth as a result of lethal respiratory failure. Podoplanin deficiency resulted in defects in the development of lymphatic vasculature, leading to dilation of cutaneous and intestinal lymphatic vessels and congenital lymphedema, indicating that podoplanin plays an important role in regulating peripheral lung cell proliferation and lymphatic vascular development.[4,5,11] Therefore, it is well established as one of the lymphatic markers.[5]

Although the biologic functions of podoplanin are not fully understood, numerous studies have suggested that overexpression of the protein can promote cancer cell migration, invasion, metastasis, and malignant progression.[4,6,7,11] It has been seen that the podoplanin homolog PA2.26 was found to be upregulated during epidermal remodeling and carcinogenesis in a mouse model. Induction of PA2.26 in mouse epidermal cells and tumor cells resulted in an increased cell migration and malignant transformation, suggesting a role of the class molecules in epithelial tumor progression.[4,6]

The demonstration of podoplanin expression is mainly based on immunohistochemistry.7 A recent study has found that monoclonal antibody D2-40 specifically recognizes human podoplanin, which has biochemical characteristics similar to the M2A antigen. D2-40 antibody was initially developed to recognize the M2A antigen, which is an oncofetal glycoprotein expressed by testicular germ cell neoplasm. Since this antigen is found in LECs and not in the blood endothelial cells, D2- 40 has been used to detect lymphatic vessels in recent studies. This makes D2-40 a valuable reagent to study podoplanin and also to determine the biologic roles of podoplanin in tumorigenesis.[4]

Objective

This literature review was performed to appraise the literature on the expression of podoplanin in OSCC in relation to clinicopathological parameters.

Materials and Methods

Study design

The review was undertaken to summarize the results of the published studies on the expression of podoplanin in OSCC in relation to clinicopathological parameters from the year 2006 to 2016.

Search strategy

Articles were searched and identified using the PubMed, EBSCO, Science Direct, and Medline databases. The search included articles published from 2006 to 2016. The keywords such as podoplanin, OSCC, D2-40, and immunohistochemistry were used to maximize the search for relevant articles.

Selection criteria

Article titles and abstracts were reviewed, and irrelevant papers were excluded from the study. If the abstract was deemed relevant, then the full paper was reviewed. Articles that focused on the expression of podoplanin in OSCC were selected. Studies including oral premalignant lesions, such as leukoplakia and erythroplakia, were excluded unless data on dysplasia were reported separately and could be extracted from published tables. Additional articles on squamous cell carcinoma of the oropharynx, esophagus, and larynx were also reviewed. Only studies in English language were considered. Studies where the information was insufficient for the review and the full copy of the article was not available were excluded. While tabulating the results, only data related to OSCC were considered.

Data collection

Once the final selection of articles for inclusion was done, the required information was collected and the extracted data were tabulated using a standardized data table. For all of the included studies, the information regarding the expression of podoplanin in relation to the following characteristics was recorded: Population characteristics (sample size), patient characteristics (age and gender), site, clinical and pathological staging, lymph node metastasis, and prognosis.

Discussion

The purpose of this literature review was to appraise the literature on the expression of podoplanin in OSCC in relation to clinicopathological parameters.

Recent studies have shown correlations between the level of podoplanin expression and lymph node metastasis, clinical and

Table 1: Podoplanin expression

Year	Authors/year	Total number of cases of OSCC	No expression	Podoplanin expression	Weak	Moderate	High
2006	Yuan *et al.*						
	Group 1	35*	2	33	13 (weak+moderate)		20
	Group 2	60	2	58	22		36
2010	Kreppel *et al.*	80	13	67	18	30	19
2011	Kreppel *et al.*	63	10	53	15	17	21
2012	Bartuli *et al.*	20	11	9	-	-	-
2013	Almeida *et al.*	33	-	13	-	-	-
2014	Logeshwari *et al.*	50	9	41	-	-	-
2015	Prasad *et al.*	30	3	27	7	15	5
2015	Ciurea *et al.*	25	-	94%	-	-	-
2015	de Vincente *et al.*	92	4	-	34 (low)	33	21
2016	Sgaramella *et al.*	129	8	121	55 (low)	-	66 (high)

OSCC: Oral squamous cell carcinoma

pathological staging, and survival in oropharyngeal, esophageal, and laryngeal squamous cell carcinoma, suggesting that podoplanin may have a role in OSCC.[12-15] Dumoff *et al.* reported a strong correlation between low expression of podoplanin and lymphatic invasion and nodal metastasis in uterine cervical cancer. This finding indicates that the biological function of podoplanin may vary among different types of cancer.[4,6]

Studies showed that podoplanin was expressed in most of the cases of OSCC. Yuan *et al.* considered both cytoplasm and membrane immunoreactivity to indicate podoplanin expression. In their study, quantity scores of 0–5 were given if 0%, 1–10%, 11–30%, 31–50%, 51–80%, and 81–100% of the tumor cells were podoplanin positive, respectively. The staining intensity was rated on a scale of 0–3, with 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. The raw data were then converted to a German Immunoreactive Score (IRS) by multiplying the quantity and staining intensity scores. The scores could range from 0 to 15. An IRS score of 8 or higher was considered high reactivity, 4–7 moderate, and 0–3 weak.[4] Some studies have considered the IRS as only low and high. Furthermore, there is no uniformity in the studies regarding what range of IRS was considered as weak/low, moderate, and high [Table 1].

In a study by Kawaguchi *et al.*, cell membrane immunoreactivity was considered to indicate podoplanin expression. The expression was scored as: 0 if no expression was observed in any part of the epithelium, 1 if expression was restricted to the basal layer of the epithelium, 2 if expression was observed in the basal and suprabasal layers at one area, 3 if the suprabasal layer expression was observed at two or three areas, and 4 if the suprabasal layer expression was observed at more than three areas.[11]

Podoplanin was highly expressed in the endothelial cells of the lymphatics but not in those of the blood vessels. Podoplanin expression was not detectable in the normal epithelial cells adjacent to the tumors. However, some of the dysplastic and hyperplastic cells adjacent to the tumors have shown podoplanin expression in the basal cell layers. Podoplanin expression was displayed in two patterns: Diffuse expression in most of the tumor cells and a focal expression at the periphery of the tumor cell nests with no expression in the central areas.[4]

Table 2: Association between podoplanin expression and age/gender

Year	Authors	Findings
2010	Kreppel *et al.*	No association of podoplanin expression with age and gender
2012	Lee *et al.*	No significant correlation of expression of podoplanin age and gender
2013	Almeida *et al.*	Strong podoplanin expression was associated with male gender
2015	Prasad *et al.*	Correlation between podoplanin expression and sex was not statistically significant
2015	Ciurea *et al.*	No statistically significant association was found between podoplanin expression and age and gender
2016	Sgaramella *et al.*	Patients aged <40 were more likely to express high levels of podoplanin. No significant association between podoplanin expression and gender

The podoplanin-positive tumor cells appear to be more localized to the periphery of the tumor nests, whereas the central area appeared negative. The expression of the marker at the periphery of the tumor cells is suggestive of a higher proliferative capacity, whereas the central cells are suggestive of terminal differentiation of tumor cells, resulting from maturation and/or degenerative changes.[16]

A study conducted by Sgaramella *et al.* showed that SCCs arising in young patients (<40 years of age) are more likely to express high levels of podoplanin than those in the elderly [Table 2].[17] Earlier oral carcinoma was primarily considered to be a disease of the older patients and cases occurring in the younger age group were uncommon. However, due to lifestyle changes and increased exposure to deleterious habits, there are a greater number of younger individuals with OSCC.

Table 3: Association between podoplanin expression and degree of differentiation

Year	Authors	Total number of cases	WDSCC	MDSCC	PDSCC	Findings
2015	Prasad *et al.*	30	9 (10)	10 (10)	8 (10)	-
2015	de Vicente *et al*	-	-	-	-	High podoplanin expression in highly differentiated tumors.
2015	Ciurea *et al.*	25	(11)	(8)	(6)	Higher intensity of podoplanin expression in poorly and MDSCC compared to WDSCC

WDSCC: Well-differentiated squamous cell carcinoma, MDSCC: Moderately differentiated squamous cell carcinoma, PDSCC: Poorly differentiated squamous cell carcinoma

Most of the studies did not show any association between the expression of podoplanin and the gender of the patient.[6,16-19] However, a study done by Almeida *et al.* has shown that a strong podoplanin expression is associated with the male gender [Table 2].[20]

In relation to the tumor location, no significant correlation was found between the tumor site and podoplanin expression.[19] Only one study by de Vincente *et al.* showed a higher staining intensity of podoplanin in the carcinomas of the tongue and floor of the mouth. They also found that the tumors of the tongue and floor of the mouth frequently developed lymph node metastasis when the primary tumor showed high podoplanin expression.[21]

Studies showed podoplanin expression in well-differentiated SCC, moderately differentiated SCC, and poorly differentiated SCC. However, there was variability in studies regarding the association of podoplanin expression and degree of differentiation [Table 3].

Podoplanin expression was found not only in advanced stages but also in the early stages of OSCC, suggesting an important role of this marker in the early stages of oral carcinogenesis. High expression in early stages compared to advanced stages may indicate that podoplanin overexpression occurs early in tumorigenesis [Table 4].

Podoplanin expression has been described as an early event in oral carcinogenesis and as a predictor of oral malignant transformation in oral potentially malignant disorders. In oral leukoplakia, high podoplanin expression has been associated with an increased risk of progression to invasive cancer, suggesting that podoplanin could serve as a powerful biomarker to predict the risk for oral cancer development in patients with oral leukoplakia.[11] This finding supports the importance of podoplanin in oral tumorigenesis and malignant transformation.

A prominent feature of malignant behavior is the capability of the tumor cells to metastasize to various organs. Metastases of human carcinomas, including that in OSCC, occur primarily through the lymphatic system.[1] The presence of lymph node metastasis is one of the most important factors in prognosis of the patients with OSCC.[21] Studies have shown a strong association of podoplanin expression with lymph node metastasis [Table 5].[4,6,19,21-24] This finding supports the importance of podoplanin in oral tumorigenesis. However, Sgarmella *et al.* and Lee *et al.* did not find any significant association between podoplanin expression and lymph node metastasis.[17,18]

Table 4: Association between podoplanin expression and T-stage

Year	Authors	Findings
2006	Yuan *et al.*	High podoplanin expression was associated with a higher pathological stage†
2010	Kreppel *et al.*	Strong association between podoplanin expression and higher pT stage (UICC stage)†
2012	Lee *et al.*	Expression of podoplain was greater in the early stages (cT1 and cT2) compared to the advanced stages (cT3 and cT4)†
2013	Almeida *et al.*	Podoplanin expression was associated with early clinical stage (T1 and T2) (UICC stage)†
2015	Ciurea *et al.*	pT3 and pT4 carcinomas had higher intensity of podoplanin immunoreactivity compared to T1 and T2 lesions†
2016	Sgaramella *et al.*	No significant association between podoplanin expression and cT-stage

†Not of statistical significance

Table 5: Association between podoplanin expression and lymph node metastasis

Year	Authors	Findings
2006	Yuan *et al.*	Tumors expressing high levels of podoplanin had a higher rate of lymph node metastasis
2010	Kreppel *et al.*	Strong association of podoplanin expression with lymph node metastasis
2011	Kreppel *et al.*	Presence of lymph node metastasis after neoadjuvant RCT had a very strong correlation with podoplanin expression
2012	Lee *et al.*	No significant correlation between expression of podoplanin and lymph node metastasis
2012	Bartuli *et al.*	Tumors which expressed high levels of podoplanin had higher rate of lymphnode metastasis
2015	de Vicente *et al.*	Lymph node metastasis was more frequently found in tumors with high podoplanin expression†
2015	Ciurea *et al.*	More podplanin-positive scores were seen in N+lesions compared to N0 lesions†
2016	Monteiro *et al.*	Association of high podoplanin expression with lymph node metastasis†
2016	Sgaramella *et al.*	No significant association between podoplanin expression and N-status

†Not of statistical significance

Table 6: Association between podoplanin expression and prognosis

Year	Authors	Findings
2006	Yuan *et al.*	Patients with lymph node metastasis and high levels of podoplanin showed a shorter disease-specific survival
2010	Kreppel *et al.*	5-year overall survival for patients with high levels of podoplanin expression was significantly lower than for patients with low and moderate expression
2011	Kreppel *et al.*	High podoplanin expression was associated with non-regression of the tumor, risk of locoregional recurrence, and poor overall survival
2012	Bartuli *et al.*	Tumors which expressed high levels of podoplanin had a shorter survival rate†
2013	Almeida *et al.*	Podoplanin expression by malignant cells did not have any prognostic value for OSCC
2015	de Vicente *et al.*	Podoplanin expression was not associated with tumor recurrence
2016	Sgaramella *et al.*	Podoplanin expression was related to poor survival in patients with OSCC†
2016	Monteiro *et al.*	Podoplanin expression was predictive of lower cancer specific survival and recurrence free survival

†Not of statistical significance, OSCC: Oral squamous cell carcinoma

In OSCC, high levels of podoplanin were associated with a higher recurrence rate and poor prognosis. This finding was contradictory to studies by Almeida *et al.* and de Vincente *et al.* [Table 6].[20,21]

Conclusion

This review showed that podoplanin was highly expressed in OSCC and podoplanin expression was associated with certain clinicopathological characteristics. Since OSCC spreads through the lymphatic route, podoplanin could be used as a potent biomarker in assessing the lymph node metastasis and prognosis. The expression of podoplanin by the tumor cells can also help in assessing the tumor progression.

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