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THE ROLE OF D 240/PODOPLANIN IN ORAL SQUAMOUS CELL CARCINOMA- AN OVERVIEW

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ABSTRACT

The globe is seriously threatened by the aggressive malignancy known as head and neck squamous cell carcinoma. Intake of alcohol, pan chewing and smoking increases the risk factor for development of oral squamous cell carcinoma. It is a multi-factorial disease with genetic predisposition. There are six signs and of oral squamous cell carcinoma that predict the tumor's progression called as hallmark of cancer . Metastasis is one of the hallmark that prognosticate the tumoral behavior. The seeding of neoplastic cells occurs by hematogenous pathway, lymphatic pathway and transcoelomic pathway. The lymphatic spread is one of the oldest and uncomplicated route for metastasis. There are various markers for predicting the angiogenic spread but identification of markers that specifically demonstrate the lymph vessels plays a key role in understanding the phenomenon of lymphatic spread. D-240/podoplanin belonging to Prox-1 family of homeobox gene is an essential gene that regulates the growth of lymphatic progenitors is the Prox 1 family. The D-240 antibody reacts with lymphatic endothelial cells in the tumor and its expression is seen to increase with the grade of the tumor. Suggestive that D-240/podoplanin is a marker that may aid in predicting the malignancy transformation risk for development of oral cancer in patients with oral leukoplakia.

Key words: Oral Squamous cell carcinoma, metastasis, lymphatogenous spread, podoplanin.

INTRODUCTION

Oropharyngeal carcinoma positions seventh overall for disease related mortality[1]. 40 – 50 % of Oral squamous cell carcinoma (OSCC) cases are reported in tongue which is the most common site along with other sites such as floor of the mouth and buccal mucosa, retropharyngeal areas. In Asian mainland buccal mucosa is the most well-known site because of tobacco biting. Aggressiveness of the tumor is predicted by the characteristic hallmarks of cancer which includes metastasis and the most common and oldest pathway for metastasis is lymphangiogenesis. The dispersion of lymphatics in OSCC is as not yet satisfactory as its perception strategy is for the most part through

imaging procedures by means of infusion of colors that are taken up by the lymphatics.[2,3] Podoplanin (PA 2.26)has a place with the homeobox gene Prox 1 family, that regulate lymphatic progenitors from undeveloped veins. Elevated podoplanin results in increased cell migration and malignant transformation. [3] Also Podoplanin promotes cancer cell attack by causing cell migration, which is caused by the inhibition of a few signaling processes, small Rho family GTPases, a decrease in cell-cell adhesion, and an increase in the expression of E-cadherin. [4,5]

Clinically OSCC appears as a white patch / red patch with / without ulceration. Pain is not the initial clinical sign and symptom in the early stages. Only prolonged ulceration and induration fixed to underlying tissue make a person susceptible to malignant transformation. Destruction of bone is seen in cases of carcinomas, arising from alveolar mucosa.Clinically, an advanced or late lesion is one that has an ulcer with elevated, rolled inverted borders and appears as a mass with broad base that is exophytic and has a rough, nodular, warty, or hemorrhagic surface. [6]

Malignant transformation of a solid tumor results in metastasis consequently causing seeding of neoplastic cells to distant organs. The morphology of lymphatic vessels is different as compared to blood vessels. The head and neck region's lymphatic capillaries have the potential to spread to additional lymph nodes, as it has a considerably bigger lumen, lack a continuous basal membrane, and have poor resistance to serum toxicity. [3]

METASTASIS AND ITS SIGNIFICANCE IN DETERMINING PROGNOSIS IN OSCC

Metastasis in OSCC occurs through lymphatics (regarded as most precursor pathway) that results in tumor progression and is crucial for determination of cancer staging, treatment and prognosis. Regional metastasis is more invasive, difficult to treat and decreases survival role. The neoplastic cells release growth factors, mostly toxic VEGF-C is responsible for formation of growth of lymphatic vessels. In terms of cancer metastasis, the lymphatic system appears to have greater role than the circulatory system. Compare to blood capillaries, the lymphatic basement membrane have very aberrant morphology which in turn facilitates lymphatic spread of neoplastic cells. [7] As opposed to other cancers, OSCC selectively metastasis to 400 or so head- and neck-regional lymph nodes, making lymphatic dissemination more important in this tumor type. Cancer cells also release various growth factors such as lymphangiogenic growth factors viz VEGF- A, C and D that stimulates development of new lymphatic vessels in solid tumors. [8] In OSCC, the expression of these markers VEGF- A, C and D are seen in the entire tumoral tissue but its expression increases at the invasive front of the solid tumor. Consequently, several paths should be used to encourage the growth of lymphatic vessels in head and neck squamous cell cancer.For instance, via interacting with VEGFR-1 and VEGFR-T, VEGF-A controls endothelial cell function by influencing proliferation, migration, specialization, and survival. VEGFR-3 and VEGFR-2 are activated and bound to by VEGF-C and VEGF-D, but not by VEGFR-1.Additionally, compared to VEGFR3,

VEGF-C and VEGF-D have a lower affinity for VEGFR2. This forms a vicious cycle as the malignant cells aid in lymphangiogenesis and the newly formed aberrant lymphatic capillaries maintains the survival of these tumoral cells, altering the tumor microenvironment and enhancing the possibility of metastasis. [9,10]

RELEVANCE OF LYMPHANGIOGENESIS IN CANCER

The finest explanation of the lymphatic spread pathway came from Zhuang et al, using a Nathanson graphical model. Neoplastic cells emit lymphangiogenic cytokines as the tumor grows, which leads to lymphangiogenesis both around and inside the tumor.Malignant cells that develop later change phenotypically, they dissociate from the primary tumor and infect the extracellular matrix around it. The gradient of chemotaxis is established composed of chemokines that stimulates the malignant cell to proceed towards the lymphatic vessels. The cancerous cell attaches to the endothelium of the lymphatic system, allowing it to get through the reach the lymphatic outflow and cross the endothelial cell barrier. The lymphatic system then drains them into sentinel lymph nodes. These cancer cells' altered phenotype renders them resistant to hypoxic environments, promotes invasion, displays adhesion molecules, and produces lymphangiogenic factor to gain transport pathway and elude the host defense mechanism. [8]

The function of lymphatic endothelial cells (LEC) in metastasis that occurs through lymphatic channels is significant. The primary mechanism by which cancerous cells enter the lymphatic system is interaction with LEC. According to Ferreti et al., tissue fluid and tumor cells enter the lymphatic drainage tube together. In solid tumors interstitial fluid pressure (IFP) is higher in comparison to the normal tissue. This IFP increases with the enlargement in size of the tumor that encourages intravasation and metastasis.Increased interstitial fluid volume (IFV), which results from increased IFP, stretches anchoring filaments and opens the LEC, allowing cancer cells to access lymphatic channels. [11] Studies have shown that 42% of lymphatic vessels are composed of open junctions with an opening space of 0.3-5µm followed by 38% having overlapping junctions in the peritumoral tissues. Therefore, LEC surface interaction with the cancer cell is the significant process that have an effect on the lymphatic metastasis. [12].

The molecular mechanism involved in this interaction of cancer cells with the surface of LEC occur via many receptors, For example,the common lymphatic endothelial and vascular endothelial receptors (CLEVER-1), the mannose receptor (MR), the lymphatic vessel endothelial hyaluronan receptor (LYVE-1), and protease activated receptor-1 (PAR-1) control the movement of these cells. Many other authors have proposed that tumor cells release lymphangiogenisis growth elements like VEGF-C, D, and AThe phenotypic flexibility of LEC, which is evidenced by enhanced expression of endothelial-specific adhesion molecules, the angiogenesis-associated leptin receptor, and the transforming growth factor beta co receptor endoglin (CD105), provides strong evidence for its participation in metastasis. These growth factors are upregulated, which promotes

lymphatic vessel expansion as well as tumor cell invasion and metastasis. [13,14] The process of metastasis occurring through lymphangiogenisis is anvital element for the movement of cancer cells, and doesn't happen randomly. The chemotaxis of neoplastic cells occurs in an orderly manner by chemokine gradient of LEC aiding it to enter the blood and lymphatic circulation. [15]

LYMPHATIC MARKERS

The process of lymphangiogenisis was not very clear due to lack of specific lymphatic markers.By infusing essential dyes like Evans blue & trypan blue, lymphatic vessel visualization is primarily accomplished . These dyes are less toxic and are easily absorbed by the lymphatics and not by blood vessels. [16] Later IHC was introduced and staining was achieved using Pan endothelial markers of the basal lamina such as PECAM/CD-31. PECAM-1 antibodies stained the red blood cells and their lumen but lacked basement membrane staining. [17] VEGFR-3 stained the lymphatic endothelial cells normally and its expression is elevated on blood vessels of endothelial cells present in tumor and wound healing. Another specific marker of lymphatic vessel is the lymphatic receptor for hyaluronan (LYVE-1) that transports hyaluronan from tissue to the lymph and is expressed as a receptor both on lymphatic endothelium of normal and tumoral tissue. [18] Other markers that are reported are podoplanin and desmoplakin that shows positivity for endothelium of lymphatics of which podoplanin (D240) is a sensitive and specific marker. [19]

d2-40 / podoplanin

D2-40 is a 40-kDa sialoglycoprotein with a carbohydrate structure similar to that of a simple mucin, belonging to a new monoclonal antibody against the M2A antigen, an oncofetal antigen, to the podoplanin family.Vascular endothelial cells do not exhibit this particularly on their surface, only lymphatic endothelial cells (LECs) do. [19] Podoplanin proteins are necessary for the development and operation of the lymph vessel. But there is no clarity related to the exact mechanism in the formation of lymphatic endothelial vessel formation. A study was conducted on sixty patients with oral tongue cancer and was found that the expression of podoplanin was nil in normal oral mucousal epithelium and few in dysplastic epithelium but tumors with lymph node metastases displayed a very high expression of 57%. In another 60 set in oral tongue cancers showed 60% increased expression of podoplanin in the tumoral region. Thus, podoplanin might be involved in oral tumorigenesis and also affect the prognosis of the tumor resulting in poor prognosis and clinical outcome. [20] In another study conducted in 31 cases of HNSCC revealed strong podoplanin immunoreactivity in the intratumoral and peritumoral lymphatic vessels. The lymphatic endothelial cells in the intratumoral region showed a more tortuous and disorganized structure than in the peritumoral region. The positive staining of D240 of lymphatic vessels and not of blood vessels can be important in future strategies for therapy. [21] In another study of immunohistochemistry of D 240 done in 55 mesotheliomas, 8 synovial sarcomas, 16 sarcomatoid tumors, and 80 pulmonary adenocarcinomas carcinoma revealed an overall sensitivity and specificity of

84% and 99% suggestive that D240 expression is helpful in diagnosis of sarcomatoid mesothelioma. [22] To understand how podoplanin's protein expression can lead to malignancy, immunohistochemical analysis of 150 patients with oral leukoplakia was done. D 240 staining was conducted on Kaposi Sarcoma cases immunohistochemically. Intense expression of D 240 was seen in Kaposi sarcoma of lymphatic origin and differentiation of neoplastic cells resulting in tumor progression. [23]

Thirty-seven percentage exhibited positive D 240 expression in basal and suprabasal layers. The inference drawn from the study was patients with oral leukoplakia exhibiting positive D 240 expression have a oral cancer incidence is substantially higher than those patients of oral leukoplakia showing negative D 240 expression. Podoplanin is a useful biomarker for predicting the likelihood that oral cancer will develop in people with oral leukoplakia. [24]

Conclusion

Metastasis is one of the major factor that predicts the aggressiveness of the tumor, prognosis and also the treatment outcome. Metastasis occurs through various pathways the most historic pathway recognized is via lymphangiogenisis. Hence, it's a pre requisite to develop markers that differentiates between blood vessels and lymphatic vessels. Podoplanin/ D 240 antibody selectively recognizes the lymphatic endothelial cells in the tumoral region highlighting occurrence of lymphatic invasion. Thus, staining with D 240 might be helpful in determining the prognosis of the tumor.

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