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REVIEW ARTICLE

Podoplanin: An Insight into its Role in Tumor Invasion and Metastasis

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ABSTRACT

Podoplanin is a mucin-like glycoprotein expressed by various cell types in the body. It was first identified in the murine osteoblastic cell lines and later discovered on the kidney podocytes. Podoplanin is known as a specific marker for lymphatic vessels and lymphangiogenesis. Podoplanin was shown to control the formation of lung, lymphatic vessels, heart, and kidney in embryonic development. Researchers have confirmed the role of podoplanin in carcinogenesis and metastasis by effecting lymphangiogenesis, epithelial mesenchymal transition, platelet aggregation, and directional cell migration. Recently, anti-podoplanin antibodies have been developed in an attempt to prevent podoplanin-induced hematogenous metastasis. This review discusses the structure and functional role of podoplanin, along with its mechanisms in carcinogenesis and invasion.

Keywords: Epithelial–mesenchymal transition, Metastasis, Podoplanin.

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INTRODUCTION

Podoplanin is a specific marker for lymphatic endothelial cells (LECs) and lymphangiogenesis in tumors. Podoplanin is expressed on lymphatic but not on blood vessel endothelium.[1] Higher podoplanin expression in the primary tumor was shown to be associated with advanced T stage, metastasis, and poor prognosis.[2] Podoplanin plays a role in tumor invasion and metastasis through its ability to remodel actin cytoskeleton of tumor cells.[1] Enhanced expression of podoplanin in oral potentially malignant disorders indicated a higher risk of malignant transformation.[3-6] In this review, the physiological and pathological roles of podoplanin will be discussed.

Human podoplanin is a type I transmembrane sialomucin-like glycoprotein consisting of 162 amino acids. In normal human tissue, podoplanin is expressed in kidney podocytes, skeletal muscle, placenta, lung, heart, myoepithelial cells of the breast and salivary glands, osteoblasts, and mesothelial cells. Podoplanin plays an important role in preventing cellular adhesion and is involved in the regulation of the shape of podocyte foot processes and in the maintenance of glomerular permeability. Podoplanin is involved in the formation of lymphatic vessels, but does not influence formation of blood vessels.[1,7-9]

DISCOVERY OF PODOPLANIN

Podoplanin messenger ribonucleic acid was identified first by Nose et al[10] in the murine osteoblastic cell line MC3Y3-E. Later, it was recognized in LECs and some other normal cells.[9] Podoplanin was identified on the podocytes of renal corpuscles, where a decrease in its expression resulted in extensive flattening of pdodocyte foot processes.[7] It is assumed that podoplanin plays a role in maintaining the unique shape of podocytes, and hence, the protein was named as podoplanin.[8] In humans, podoplanin, the oncofetal antigen M2A recognized by the D2-40 antibody, glycoprotein 36, and the type I alveolar cell marker hT1α-2 are found to be identical proteins.[11] It has homologues in mice (OTS-8, gp38, aggrus, antigen PA2.26, RANDAM-2 or retinoic acid-induced neuronal differentiated associated molecule-2), rats (T1α protein, E 11 antigen, podoplanin), canines (gp40, aggrus), and hamsters (aggrus).[7,9,10,12-20] The molecular structure of podoplanin seems to be well preserved among these species.[20]

STRUCTURE

Human podoplanin is a small (38 kDa) type-1 transmembrane glycoprotein containing 162 amino acids. It has an extracellular domain, a transmembrane domain, and a short intracellular domain consisting of only nine amino acids (Fig. 1).[21] The extracellular domain is rich in highly O-glycosylated Ser and Thr residues.[22,23] Within

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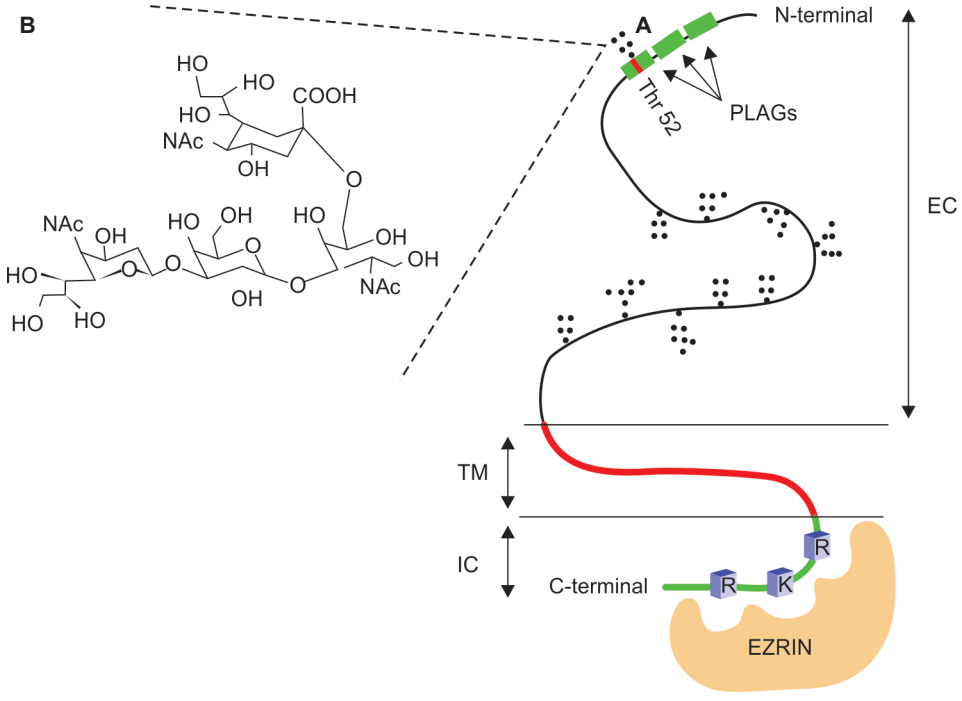

Figs 1A and B: (A) Schematic representation of podoplanin molecule. (B) Structure of O-glycan. EC: Extracellular domain; TM: Transmembrane domain; IC: Intracellular domain; PLAG: Platelet aggregation stimulating domain (Picture courtesy: Ugorski M et al)

the extracellular domain, three adjacent conservative amino acids have been found, first of which interacts directly with the podoplanin C-type lectin-like receptor-2 (CLEC-2) present on platelets called platelet aggregation stimulating domain (subdomain PLAG).[20,22] The short cytoplasmic domain shows a characteristic sequence of three basic amino acids, i.e., responsible for binding to ezrin, radixin, moesin (ERM) proteins.[24] The importance of CLEC-2 and ERM protein binding will be discussed later in the article.

OCCURRENCE OF PODOPLANIN

Podoplanin is expressed in various human tissues, such as LECs, renal podocytes, skeletal muscle, lung, heart, placenta, myoepithelial cells of breast, osteoblasts, osteocytes, prostate, myofibroblasts, mesothelial cells, follicular dendritic cells, Schwann cells, and occasionally on the basal layer of epidermis.[7,23,25-29] In oral tissues, podoplanin is expressed strongly in the myoepithelial cells of salivary glands.[30] Podoplanin expression in oral mucosa varied from negative to a few cluster of basal cells to majority of cells in the basal layer.[2,30-32] Positive podoplanin expression was also observed in the basal cells of inflamed gingiva.[33]

ROLE OF PODOPLANIN IN DEVELOPMENT

Various researches in knock-out mice have shown the role of podoplanin in the development of lungs, lymphatic vessels, and heart. Mice with podoplanin knock-out died immediately after birth due to respiratory failure. Podoplanin is required for the proliferation of lung cells and subsequent differentiation into type I pneumocytes in lung alveoli.[34]

Podoplanin is essential for the normal development of lymphatic system. Podoplanin knock-out mice exhibited malfunctioning lymphatic vessels that resulted in lymphedema. *In vitro* studies have shown that podoplanin increased the directional migration (haptotactic) and adhesion of endothelial cells to type I collagen and fibronectin. Podoplanin also promoted the formation of capillary vessels.[35] Other studies using podoplanin knock-out mice embryos have shown blood-filled lymphatic vessels and vessels incompletely separated from blood vessels.[36] Researchers also suggested that podoplanin in lymphatic endothelium stimulated the formation of platelet aggregates, which in turn closed the connection between lymphatic sac and cardinal vein or acted as a source of factors for vasoconstriction of blood vessels.[37] It is also required for the proper formation of lymph nodes and spleen.[38]

Podoplanin knock-out mice also exhibited abnormal heart development. Embryos exhibited hypoplasia and ventricular septal defects.[39] Decreased ability of cells for epithelial–mesenchymal transition (EMT) that enables migration is suggested to cause all the above-mentioned disorders.[21]

Podoplanin is also expressed by the inner and outer enamel epithelia of developing tooth in mouse at the

early bell stage and disappears afterward. Odontoblasts also express podoplanin during dentin formation period. Podoplanin might have a possible role in the odontoblast shape formation and the migration of epithelial cells into mesenchyme in tooth development.[40,41] Podoplanin expression was also observed in most of the epithelial and mesenchymal cells in human tooth germ. It was also expressed by odontoblasts and superficial pulp fibroblasts in human permanent teeth.[42]

PHYSIOLOGICAL ROLE OF PODOPLANIN

Podoplanin maintains the shape of foot processes of podocytes in kidney and has an important role in maintaining glomerular permeability.[7,43,44] It prevents cellular adhesion and promotes platelet aggregation.[21] Its expression in mouse keratinocytes during wound healing suggests a role in tissue regeneration.[16] Podoplanin may have a role in maintaining the shape of myoepithelial cells to press acinar cells in salivary glands.[41]

ROLE IN CANCER

As podoplanin is expressed in lymphatic but not in blood vessel endothelial cells, it has been used widely as a marker for lymphangiogenesis. Other specific markers for LECs include vascular endothelial growth factor receptor 3, lymphatic vascular endothelial hyaluronan receptor (LYVE-1), and prospero-related homeobox 1 (Prox-1).[11]

Podoplanin expression was found upregulated in several human cancers, including squamous cell carcinomas (SCCs) of skin, oral cavity, esophagus, larynx, lung, and uterine cervix.[27,45-51] Apart from SCC, podoplanin is also expressed in lymphangioma, angiosarcoma, ovarian carcinoma, testicular carcinoma, mesothelioma, and astrocytoma.[25,52-56] Data linking podoplanin expression with clinical prognosis in SCC have not been consistent and the biologic function of podoplanin may vary among different types of cancer.[4] In the SCC of the skin, oral cavity, esophagus, and early-stage laryngeal carcinoma, enhanced expression of podoplanin is associated with poor clinical outcome, whereas podoplanin-positive tumors show a better overall survival in SCC of lung.[45,47-51,57] In patients with SCC of the uterine cervix and advanced laryngeal carcinoma, low levels of podoplanin are associated with the presence of lymphatic invasion.[4,46,49] Lung and cervical SCCs are assumed to develop from metaplastic squamous epithelia; this is considered as the reason for their difference in characteristics from those of esophagus/oral squamous cell carcinoma (OSCC) cells.[58]

In the past, podoplanin had been used frequently to assess intratumoral and peritumoral lymphovascular density in OSCC, which was correlated with metastatic spread to the lymph nodes and a poor prognosis.[26,45,57] Many studies indicated that peritumoral lymphangiogenesis may be an indicator of the risk of lymph node metastasis in patients with head and neck squamous cell carcinoma (HNSCC).[59] Studies also showed that cancer cell-expressed podoplanin may be used as a predictive marker for sentinel lymph node metastasis in early HNSCC of the oral cavity and oropharynx.[60] Later, the level of podoplanin expression on the tumor cells was shown to be associated with advanced T stage, lymphatic spread to cervical region, and poor clinical outcome.[61,62] In human OSCC-derived cell lines, podoplanin was shown to be involved in a signaling pathway governing tumor growth and invasion in OSCC.[4]

Studies have shown that podoplanin is also expressed in oral dysplastic and hyperplastic lesions with a risk of cancer development.[3-6,31,32,62,63] Podoplanin expression increased with increased severity of dysplasia, and podoplanin-positive leukoplakia had a significantly higher incidence of oral cancer than the podoplanin-negative lesions. Podoplanin expression extending to suprabasal layers is thought to represent upward clonal expansion of stem cells during carcinogenesis; and oral premalignancy with such clonal expansion may imply significantly higher risk of malignant transformation. Podoplanin was suggested as a biomarker to assess oral cancer risk in patients with oral premalignancy.[3-5,7,32,63,64]

Increased expression of podoplanin was also shown in ameloblastoma and keratocystic odontogenic tumor, whereas expression was reduced in ameloblastic carcinoma. These findings suggest their role in local invasion than in malignant transformation of odontogenic tumors.[65-67] Enhanced podoplanin expression was also reported in human salivary gland pleomorphic adenoma.[68]

Proposed Mechanisms in Cancer

- EMT and single cell invasion
- Collective cell invasion
- Lymphangiogenesis
- Platelet aggregation
- Directional cell migration

Epithelial–mesenchymal Transition and Single-cell Invasion

Epithelial–mesenchymal transition is considered an important event in invasion of epithelial malignancies. During EMT, epithelia lose the epithelial adhesive proteins like E-cadherin and express mesenchymal markers, such as N-cadherin and vimentin (known as cadherin switch). These changes simulate those occurred during the embryonic period when the cells had the features of a

migratory mesenchymal cell.[1] As podoplanin is expressed in the invasive front of tumors, it is thought to have a role in EMT, invasion, and metastasis.[69,70]

Podoplanin increases cell motility by inducing actin cytoskeleton rearrangement via binding to ERM proteins.[70] Ezrin is a cytoplasmic linker protein expressed on epithelial cell surfaces. Ezrin-actin binding is required for the development of membrane-actin structures needed for cell shape and motility. Rho family guanosine triphosphate (GTP) binding proteins are expressed in cells that acquire the potential to migrate and invade.[71] Podoplanin-ezrin complex upregulates the RhoA GTP binding protein, leading to the activation of RhoA-associated kinase that phosphorylates ezrin.[72-74] As ezrin is a linker protein, it mediates the connection between membrane proteins and F-actin. The increased cell surface expression of phosphorylated ezrin induces rearrangement of actin cytoskeleton.[41,70] Podoplanin also binds to the cytoplasmic tail of ezrin to maintain a stable anchorage of F-actin to the plasma membrane. Ezrin was also shown to mediate the development of filopodia and promote metastasis.[75]

Collective Cell Invasion

Podoplanin was shown to mediate tumor invasion of single cells by EMT-dependent pathway; the same protein also promoted invasion of sheets of cells via a non-EMT mechanism.[1,69,76,77] Studies in human keratinocytes and MCF7 breast cancer cells have shown that podoplanin induces EMT in the presence of E-cadherin expression (i.e., no cadherin switch). The cells exhibited dramatic change in morphology, decrease in cellular stress fibers, and formation of filopodia-like structures.[69,78] Podoplanin was shown to mediate adhesion and spreading of cells on extracellular matrix protein fibronectin. Invasion of podoplanin-positive cells in sheets was thought to be mediated by matrix metalloproteinases.[69,76-78]

Lymphangiogenesis

Podoplanin was shown to produce more tumor lymphatic vessels and larger metastatic foci in lymph nodes.[79] Intra- and peritumoral lymphatics identified by podoplanin and LYVE-1 were shown to be involved in tumor cell adhesion, invasion, and migration.[80] The expression of podoplanin is controlled by the lymphatic specific homeobox gene Prox-1. It was shown to reprogram the vascular endothelial cells in culture to become podoplanin-positive LECs.[81]

Platelet Aggregation

Podoplanin can bind to the ligand CLEC-2 on the platelet surface and induce platelet aggregation via a mechanism similar to the platelet activating snake venom rhodocytin.[82,83] Platelet aggregates protect the tumor cells by surrounding them and enable tumor nest formation in blood vessels.[84,85] Platelet aggregates can facilitate the adhesion of these cell nests to vascular endothelium, whereas platelet-derived factors mediate their extravasation by retracting endothelial cells. Activated platelets can stimulate angiogenesis and tumor growth.[84]

Directional Cell Migration

In cell surface of SCC, podoplanin was shown to be colocalized with CD44, a major hyaluran receptor. Together, they stimulated the directional persistent motility of the cancer cells, which in turn promoted their directional migration.[86]

Podoplanin expressing cancer cells also exhibited stem cell-like properties, such as the ability to repopulate and produce a cancer cell population. They had a high colony-forming efficiency.[87] Podoplanin has been reported as a cancer stem cell marker in human SCC cell line A431.[49]

Podoplanin and Cancer-associated Fibroblasts

Apart from malignant keratinocytes, podoplanin was also shown to be expressed by the cancer-associated fibroblasts (CAFs) in mice. Positive podoplanin expression was found first in the CAF in lung adenocarcinoma. Later podoplanin-positive CAFs were also reported in cancers of breast, kidney, bile duct, thyroid, liver, colon, stomach, prostate, pancreas, urinary bladder, and uterus.[88] The role of podoplanin on the CAF was explained by its significantly higher expression in the lung vascular adventitial fibroblasts that came into contact with cancer cells during their intravasation. Knock-down of podoplanin in these cells decreased their tumor-promoting effects.[89] Enhanced RhoA activity was proposed to be the mechanism that promoted podoplanin-positive fibroblasts to favor cancer growth.[90]

The correlation between podoplanin expression by the CAF and the prognosis of the patients seems to be inconsistent. In most of the tumors, podoplanin expression by the myofibroblasts induced lymphangiogenesis and promoted metastasis. Contrary to this, podoplanin expression by the CAF indicated a favorable prognosis in uterine cancers and colorectal cancer.[91,92]

Development of Anti-podoplanin Antibodies

Anti-podoplanin antibodies were developed as an attempt to inhibit podoplanin-mediated metastasis. They include NZ-1, P2-0, hP2-0, and MS-1. NZ-1, a rat antibody, has shown to completely inhibit podoplanin-induced platelet aggregation and metastasis in experimental settings.[93] P2-0

was developed as an antihuman podoplanin antibody, which can attenuate the podoplanin–CLEC-2 binding and platelet aggregation. It also prevented experimental metastasis in a mouse model.[94] A murine/human chimeric antibody hP2-0 was also developed.[94] Recently, monoclonal anti-podoplanin antibodies P2-0 and MS-1 have shown to prevent hematogenous metastasis of podoplanin-positive bladder cancer in mice.[95] A major barrier for using anti-podoplanin antibodies for therapeutic purpose is that till date, no difference has been reported in podoplanin expression between normal and tumor lymphatics.[11]

CONCLUSION

Podoplanin is involved in embryological development, carcinogenesis, and metastasis. Expression of podoplanin in SCC cells is associated with lymph node metastasis, whereas in potentially malignant disorders it indicates a higher risk of malignant transformation. Various mechanisms underlying the podoplanin-induced metastasis have been unveiled. However, there is a lack of consistent results in studies regarding the exact role of podoplanin, especially in areas of EMT and the role of CAF. Further studies are warranted to elucidate the actual role of podoplanin as well as for the development and therapeutic use of anti-podoplanin antibodies to effectively control invasion and metastasis.

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