

Significance of podoplanin expression in cancer-associated fibroblasts: A comprehensive review

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Received January 17, 2013; Accepted March 14, 2013

DOI: 10.3892/ijo.2013.1887

Abstract. Cancer-associated fibroblasts (CAFs) are well-known to be part of the tumor microenvironment. This heterogeneous population of cells of the tumor microenvironment via secretion of various growth factors and cytokines was shown to contribute to increased cancer cell proliferation rate, migration, invasiveness and other key processes such as angiogenesis and lymphangiogenesis. Recent studies identified podoplanin as a marker of CAFs in various malignancies and its expression in these cells was shown to influence cancer progression. In some studies it yielded a prognostic impact on patient survival which was strongly dependent on the entity of the tumor. This review summarizes recent findings concerning the biology of podoplanin in cancer progression with particular emphasis on its expression in CAFs.

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1. Introduction

As early as 1863 Rudolf Virchow hypothesised that cancer cell growth may be stimulated by surrounding stromal cells

and that the neoplastic process begins at the site of chronic inflammation, since leukocytes were seen in the stroma of neoplastic tissues (1). This hypothesis is actually somewhat true since recent research has shown that inflammatory cells may contribute to the development and progression of various malignancies (2,3). Besides inflammatory cells, in the majority of various tumors fibroblasts are seen (4). Under normal conditions, fibroblasts synthesize the components and regulate the deposition of the extracellular matrix (ECM), epithelial differentiation and inflammation (5-8). Fibroblasts are also key in the process of wound healing and fibrosis during which they exert different properties than those of normal fibroblasts (6,9). Their phenotype also changes, resulting in increased proliferation, motility and synthesis of various growth factors, referred to as 'activation' (4).

Cancers are often called 'wounds that do not heal'. Recent research has shown that fibroblasts of cancerous stroma, the so-called cancer-associated fibroblasts (CAFs), exert an 'activated phenotype' in the majority of the studied malignancies (4,7,10,11). There is evidence which suggests that CAFs are characterized by a distinct gene expression profile, and that their properties differ from those of normal fibroblasts (12). Furthermore, CAFs may be incorporated into the tumor environment via different pathological mechanisms (4,7,13). Transformation of normal fibroblasts under the regulation of cancer cells has been proposed. However, it is becoming apparent that the recruitment of bone marrow derived stem cells, trans-differentiation of cancer (endothelial-mesenchymal transition; EMT) or endothelial cells (endothelial-mesenchymal transition; EndMT) may also very significantly contribute to this process (4,7,13,14). CAFs of invasive breast cancers were shown to augment tumor growth and induce angiogenesis via recruitment of endothelial cells due to secretion of high levels of stromal-derived growth factor (SDF-1) (15). CAFs were also shown to promote tumor growth by secreting ECM-degrading proteases (MMPs), hepatocyte growth factor (HGF) or connective tissue growth factor (CTGF) (16-22). Moreover, it was found that CAFs contribute to tumor progression via recruitment of inflammatory cells into the tumor environment (23). CAFs may also modulate cancer stem cell phenotype, as was recently described in colorectal carcinoma (24,25).

Fibroblasts are a heterogeneous group of cells. Their phenotype is strongly dependent on the tissue origin and topography.

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Key words: podoplanin, cancer-associated fibroblasts, stroma

As a result, tissue specific markers of fibroblast activation are of particular importance (26). The α -smooth muscle actin (α SMA) is regarded as a marker of activated CAFs in most of the studies (4,6,27). Besides α SMA, vimentin, desmin, fibroblast-specific protein 1 (FSP1) or fibroblast-activation protein are also used to identify and characterize CAFs in human malignancies (27-33). There is also evidence that different subsets of CAFs are present in the tumor stroma and differ in their properties (34). Recent findings have identified podoplanin as a potentially new marker of CAFs in various malignancies, giving new insight into the biology of cancers and the tumor microenvironment (27,28,35-37).

2. Podoplanin discovery, structure and expression regulation

Podoplanin was first discovered in 1990, when its mRNA was found in the murine osteoblastic cell line (MC3T3-E1) and ras-transformed cells (38). In 1996, Wetterwald *et al* raised an antibody which could detect podoplanin (designated as E11 antigen due to the antibody clone) in rat osteoblasts, pre-osteocytes and osteocytes (39). Podoplanin (E11) positive cells were also identified as alveolar type 1 cells in lung, endothelial cells of choroid plexus and lymphatic endothelial cells (LECs) (39). Podoplanin was named for the above mentioned protein when Breiteneder-Geleff *et al*, discovered for the first time its expression on rat podocytes (40). In 1999 podoplanin was accepted as a novel marker of lymphatic endothelial cells (Fig. 1) (41,42).

The most popular synonym for podoplanin is D2-40, stemming from the name of the antibody clone which is widely used for its detection in paraffin-embedded tissues (27,43). Podoplanin, is also known as gp36, gp38, canine gp40, T1 α , PA2.26, Aggrus, OTS-8 or M2A oncofetal antigen (38,44-50).

Podoplanin is a 162-amino acid transmembrane sialoglycoprotein belonging to type-1 transmembrane sialomucin-like glycoproteins (51). Its mass varies from 38 to 50 kDa due to the extent of the sialylation of its extracellular domain with numerous serine and threonine amino acid residues (42,52,53). Podoplanin consists of a small transmembrane domain and an intracellular domain. The latter interacts with protein kinase C (PKC) and proteins of the ERM (ezrin, radixin, meosin) family, which were shown to influence cancer cell motility and invasive potential (51,54). The podoplanin gene consists of 34.4 kb and 8 exons. To date 2 isoforms of podoplanin have been discovered utilizing northern blotting. They probably resemble a product of alternative splicing, but the biological significance of this finding remains to be clarified (53,55). Its expression is regulated on the level of transcription (numerous initiation sites, alternative splicing, polyadenylation) and on the post-translational level due to podoplanin calpain-mediated proteolysis (53,56). Although the exact mechanism of its expression regulation remains unknown, numerous factors ranging from micro-RNA to signalling factors have been seen (42,57-60).

3. Podoplanin and lymphatic vessel development

As mentioned above, podoplanin is regarded as a marker of lymphatic vessel endothelium (41,42). Podoplanin-positive

cell structures were shown to be simultaneously positive for Prox1 and VEGFR-3 (61). It seems that VEGF-C upregulates podoplanin expression via *Prox1*, which is a master regulator gene of lymphatic vessel development facilitating LECs differentiation from lymphatic progenitor cells in embryonic veins (61,62). Podoplanin expression was also shown to be upregulated by IL-3 in dermal LECs (63).

The important role of podoplanin in lymphatic vessel development has been made clear by experiments performed with podoplanin-deficient mice which die at birth from respiratory failure caused by abnormal alveolar sac development due to the absence of podoplanin expression in alveolar type I cells (48,64). In these mice there were serious lymphatic abnormalities including abnormal lymphatic vessel patterning, lymph transport and lymphedema combined with lymphatic vessel dilatation (48). Although no connections between the blood and lymphatic vessels were reported in the above mentioned studies (the so-called 'separation phenotype'), Fu *et al* showed that mice deficient in endothelial O-glycans, from the targeting of T-synthase required for their production, possess a 'non-separation' phenotype with numerous misconnections between both vessel types (65). Mice lacking the T-synthase were also characterized by impaired podoplanin expression on endothelial cells. As a result, it was hypothesized that the abnormal connections between the blood and lymphatic vessels occurred due to the lack of its expression (65). Recently Uhrin *et al* showed that podoplanin platelet-aggregating properties are crucial for proper lymphatic vessel development. Thrombi are formed at sites of podoplanin-positive lymph sacs separating them from cardinal veins during mouse embryonic development, thereby enabling proper separation of blood and newly formed lymphatic vessels (66).

4. Podoplanin and tumor metastasis facilitated by thrombus formation

Recent findings have led to new discoveries which help clarify the functions of particular podoplanin domains. The extracellular domain of podoplanin consists of the EDxxVTPG segment (PLAG - platelet aggregating domain), which is responsible for platelet aggregation (51). C-type lectin-like receptor-2 (CLEC-2) was discovered as the first podoplanin receptor on human platelets responsible for platelet aggregating properties of podoplanin (67,68). Recombination of CLEC-2 or mutations in the threonine residues of PLAG result in abolished platelet aggregation by podoplanin (47,67). Podoplanin interaction with platelets was also shown to be inhibited by antibodies directed against CLEC-2, which may be significant in anti-cancer therapies (68,69). It was shown that tumor cells induce platelet aggregation which protects cancer cells from shear stress and host immunological defence (70). This phenomenon may result in increased tumor growth and enhanced metastatic potential of the tumors (47,71,72). Using animal models it was shown *in vivo* that antibodies targeting the interaction of the PLAG domain and CLEC-2 may suppress tumor metastasis (68,73). The blockade of CLEC-2 may be of potential importance since diminished functionality of this receptor results in decreased thrombus formation with a non-significant clinical rise in bleeding time (72,74-76). However, caution should be taken when using antibodies targeting podoplanin function

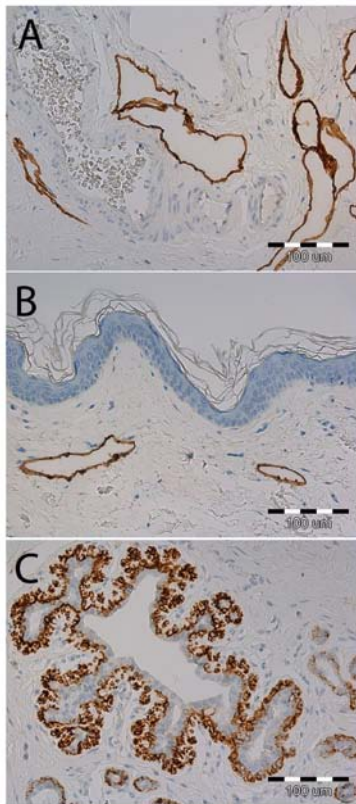


Figure 1. A membrane-cytoplasmic expression pattern of podoplanin in the lymphatic vessel endothelium was utilized to detect lymphatic vessels in various tissues, as none of its expression is noted in the blood vessel endothelium (A and B). Podoplanin expression in myoepithelial cells surrounding breast duct cells (C). None of its expression is noted in the normal, non-transformed stroma.

because podoplanin-Fc transgenically expressed in mouse skin resulted in lethal disseminated intravascular coagulation (77).

5. Podoplanin and cancer cell migration, invasion and progression

Several studies and reviews have already shown that podoplanin affects the migration of normal and cancer cells (42,52,59,78). Cancer cells may migrate in two distinctive patterns. The most often studied theory is based on the single cell migration model which is often connected to the EMT phenomenon. In this process, cancer cells lose their epithelial phenotype e.g., loss of E-cadherin, and acquire a mesenchymal phenotype which is characterized by expression increase of the mesenchymal markers N-cadherin, α SMA or FSP-1. This is often regarded as the cadherin switch (52). This process results in the increase of migratory and invasive potential, and cancer cell resistance towards apoptotic stimuli (79). Although this process is considered crucial in cancer cell dissemination, it is rarely observed in paraffin sections of embedded cancer tissues. On the contrary, large cancer cell bulks invading the neighboring tissues are most frequently observed in cancerous tissues. In this model, known as 'collective cell migration', cancer cells maintain the expression of epithelial markers (52). Experimentation has indicated that podoplanin may mediate cancer cell migration in both hypothetical invasion models.

Wicki *et al* in the Rip1Tag2 model of pancreatic β -cell carcinogenesis have shown that transgenic expression of podoplanin in pancreatic β -cells led to the formation of carcinomas in the absence of EMT, since the cancer cells

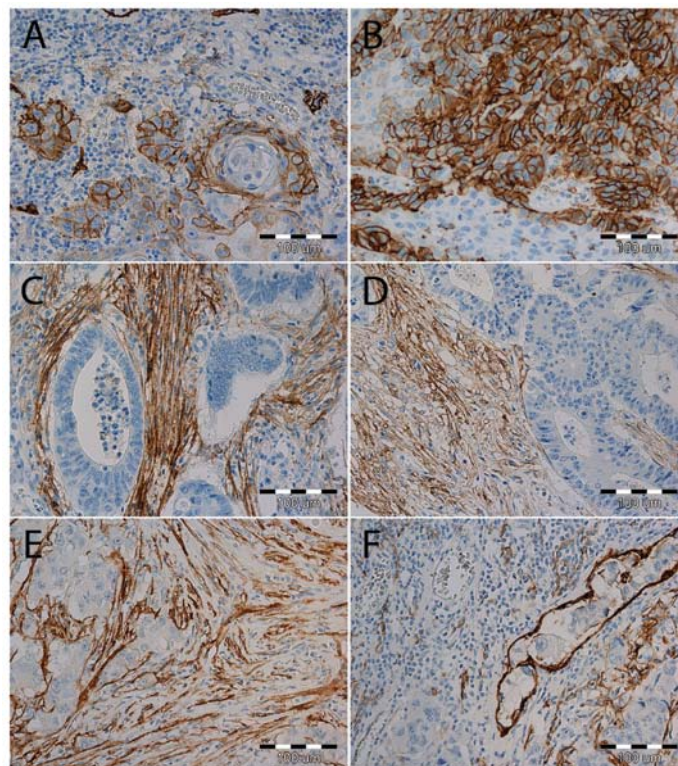


Figure 2. Podoplanin membrane staining of squamous cancer cells of skin (A) and lung (B) cancers. On the contrary no staining is visible in the adenocarcinoma of the lung (C), colon (D) and invasive ductal breast carcinoma (E and F). In these tumors, podoplanin expression is mainly noted in the CAFs of tumour stroma (C-E) and LECs (F).

invaded the surrounding tissues in a collective pattern (59). In this experimental model mice develop adenomas and carcinomas of β -cells in pancreatic Langerhans islets due to the expression of simian virus large T antigen under the control of rat insulin promoter (52,59). Moreover, podoplanin colocalized with E-cadherin on the invading tumor edge in various cancers of tumor origin (52,59). Neo-expression of podoplanin in MCF-7 (breast cancer cell line) supported this observation since no cadherin switch occurred in these cells. Nevertheless, the cells were characterized by increased migratory and invasive potential (59). Under the influence of transforming growth factor β (TGF β), epidermal growth factor (EGF) and hepatocyte growth factor (HGF), an increase in podoplanin expression could be noted in the podoplanin transfected MCF-7 cells (59). It is noteworthy that podoplanin is rarely expressed in breast cancer cell lines and tissues, not even in the most invasive and metastasizing cell lines showing expression of mesenchymal markers (MDA-MB-231 and MDA-MB-231. BO2) (27,36,53).

Podoplanin neo-expression in Madin-Darby canine kidney (MDCK) cells resulted in EMT. The cells acquired migratory features dependent on the interaction of podoplanin endodomain with ezrin and radixin, proteins of the ERM complex of cell membrane. Podoplanin binding to these proteins resulted in activation of RhoA small G protein (54). Moreover, induction of podoplanin expression in epidermal MCA3D keratinocytes (3D2.26) induced cell surface extensions, had increased motility, a loss of cortical actin and destabilization of adherens junctions. In addition, these cells also had a loss of keratin-14 and an increase of vimentin and keratin-14 expression. Upon injection into athymic mice, 3D2.26 cells formed undifferentiated tumors with aberrant E-cadherin expression and formation of tumor metastases in regional lymph nodes. This highlights the malignant transformation of these cells (80). Podoplanin was also shown to be upregulated in human epidermal keratinocytes by TGF β , IFN γ , IL-6 and IL-22, which partially confirms the findings in the MCF-7 cells (57).

Podoplanin was also identified in raft platforms, structures crucial for cell signaling (81). It seems that the short GXXXG motif of the podoplanin transmembrane domain is crucial for its association with detergent-resistant membranes (DRMs), ERM phosphorylation or induction of EMT and cell migration (81). MDCK cells with mutation in the GXXXG motif of podoplanin transmembrane domain were characterized by their impaired dimerization and lacked localization in the lipid raft platforms resulting in the absence of EMT (81). Apparently podoplanin inclusion in the raft platforms is crucial for its interaction with the actin cytoskeleton and activation of the RhoA/ROCK pathway. A recent study showed that podoplanin interacts with CD44 independently of ERM proteins in cell membrane protrusions which therefore ensures directional cell migration (82). CD44 is an ERM binding protein. Its interaction with actin cytoskeleton is also dependent on its inclusion in the lipid rafts (83). It therefore appears that podoplanin regulates the RhoA/ROCK activity via its inclusion (81-83).

Although it is clear that podoplanin mediates migration and invasiveness of cancer cells, the biology and interactions of this protein seem to be strongly dependent on the cell type. Martin-Villar *et al* and Fernandez-Munoz *et al* demonstrated

that podoplanin expression in MDCK cells led to induced activity of RhoA, whereas Wicki *et al*, demonstrated a decrease of RhoA, Rac1 and cdc42 activity upon podoplanin introduction into the MCF-7 cells (54,59,81).

It has also been shown, that the Src kinase stimulates podoplanin expression in the homozygous null gap junction Cx43 (connexin 43) knock-out brain cells by phosphorylating the focal adhesion adaptor protein Cas (Crk associated substrate). This led to induced cell migration (84). Interaction of the Src kinase with Cas was shown to promote anchorage-independent growth and migration of cancer cells and these effects may be mediated by induction of podoplanin expression (84). In addition, podoplanin expression was found to be decreased by contact normalization, a process that forces the cells to maintain normal non-transformed cell phenotype, therefore preventing their malignant transformation (84,85).

A recent study of Acton *et al* identified podoplanin as an inducer of dendritic cell (DC) migration via interaction with its receptor, the CLEC-2 protein (86). Binding of podoplanin expressed on fibroblastic reticular cells (FRCs) and LECs with CLEC-2 on DCs resulted in increased migratory potential of the latter. This mechanism might also be responsible for the migration of cancer cells along the lymphatic networks since the neoexpression of CLEC-2 in a human A375 melanoma cell line resulted in protrusion formation upon contact of the CLEC-2 overexpressing A375 melanoma cell line with podoplanin expressing FRCs (86).

Diversity of podoplanin biology is also apparent in different tumor types. Podoplanin expression in breast cancer cell lines augmented their metastatic potential and dissemination through villin-1 dependent induction of lymphangiogenesis (87). On the contrary, forced expression in the lung squamoid EBC-1 cancer cells attenuated pro-lymphangiogenic and metastatic potential by reducing the expression of VEGF-C in these cells via the downregulation of c-jun N-terminal kinase (JNK) (88). These results agree with the results obtained on human squamous non-small cell lung cancer and cervical cancer, where cases with high expression of podoplanin were characterized by a lower incidence of nodal metastasis (89-93). However, in head and neck cancers high podoplanin expression in cancer cells was found to be a negative prognostic factor (94-100). Interestingly, in some tumors cell lines derived from these tumor types e.g., invasive ductal breast carcinoma, lung, pancreatic and colorectal adenocarcinoma, podoplanin expression in cancer cells is rarely observed (27,28,36,37,53). There is abundant evidence pointing to elevated podoplanin expression in cancers. This shows squamous differentiation in comparison to those of the adenocarcinoma type (Fig. 2A and B) (42,52,53).

Podoplanin was also proposed to be a hallmark of tumor initiating cells in squamous cell cancers since podoplanin-positive cancer cells exerted augmented tumorigenic potential as compared to cells without podoplanin expression (101,102). In normal epidermis, podoplanin expression is observed in the basal layer and rises in the precancerous lesions (actinic keratosis) and skin squamous cell cancer (103,104). Moreover, the A431 squamous cancer cell line derived from the vulva and the TE-11 cell line derived from esophageal squamous cell cancer showed divergent

subpopulations with respect to podoplanin expression. Cells expressing podoplanin could give rise to both podoplanin expressing and non-expressing cells. Upon subcutaneous injection into SCID mice, tumors bearing podoplanin expressing cells were characterized by augmented growth as compared to tumors generated by cells without podoplanin expression (101,102). In addition, podoplanin-positive A431 cells exhibited high expression of CD44 and sonic hedgehog (SHH), which are both markers of tumor initiating cells (102).

6. Podoplanin expression in cells of normal and cancerous stroma

Skin cancer. Podoplanin expression was first studied as a potential marker of lymphangiogenesis. The assessment of this process in numerous tumors led to the discovery of podoplanin expression in cancer cells as well as 'activated fibroblasts' (42,52). At first, podoplanin expression, known as PA2.26, antigen was noted in the membrane of fibroblasts *in vivo* (NIH-3T3, Swiss-3T3 and 10T1/2) and in fibroblasts of tissue sections of skin exposed to wounding or stimulation with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (45). In this study, in normal, untreated skin podoplanin expression was not noted in the basal and suprabasal skin layers, suggesting that podoplanin may be a marker of cell activity in skin carcinogenesis (45).

Lung cancer. There is increasing evidence that podoplanin is possibly expressed in CAFs of cancerous stroma, which contribute to the progression of numerous tumors (Fig. 2C-F) (4,7). To date, podoplanin-positive CAFs are best characterized in lung adenocarcinoma. Kawase *et al* identified podoplanin-positive CAFs in tissue sections of 54 out of 177 cases of lung adenocarcinoma and confirmed its expression on the protein level in CAFs isolated from some of the tumors (28). It is noteworthy that podoplanin expression in CAFs was higher in comparison to non-cancerous fibroblasts isolated from surrounding tissues of the same patients (28). In this study cohort, podoplanin expression in CAFs was associated with a history of smoking, a primary tumor that was larger in size, the presence of lymph node metastasis, advanced pathological stage, poor grade of differentiation, as well as vascular and pleural invasion. Moreover, patients whose tumors were characterized by podoplanin expression in CAFs had significantly shorter overall survival in comparison to patients whose CAFs did not exhibit podoplanin expression (28). Podoplanin expressing CAFs were also identified as a marker of poor prognosis in other studies conducted on lung adenocarcinoma (35,105,106). Interestingly, podoplanin expression in CAFs had no prognostic significance in squamous lung cancer, which is characterized by elevated expression of podoplanin in cancer cells (35,107). As in cancer cells, tumor promoting effects of podoplanin-positive CAFs were found to be mediated by elevated RhoA activity, similar to observations in other cell types (54,80,81,108). Human fibroblasts isolated from vascular adventitia with ectopic expression of podoplanin exerted elevated RhoA activity. Injection of these cells was shown to augment tumor formation of human lung adenocarcinoma cell line A549 upon co-injection into SCID mice, as compared to A549 cells co-injected with control human fibroblasts (108).

As the A549 cell line does not express CLEC-2, the mechanism of enhanced tumor formation by podoplanin expressing fibroblasts remains to be elucidated in order to determine if this interaction is contact dependent or mediated via secretion of other stimulatory factors. In their previous study, the authors showed that fibroblasts derived from human vascular adventitia enhanced lung adenocarcinoma tumor formation *in vitro* and *in vivo* of lung adenocarcinoma cell lines (A549, PC-14, CRL-5807) (105). Moreover, the experiments revealed that the podoplanin expressing subset of these fibroblasts enhanced tumor formation and lymph node metastasis of A549 cells as compared to the subset lacking podoplanin expression (105). Interestingly, podoplanin expressing CAFs were also identified in nodal metastases of lung adenocarcinoma and were associated with poor overall survival of patients with pathological N2 stage III cancers. However, the presence of podoplanin expressing CAFs in the metastatic lesion did not correlate with any other clinicopathological factors of the patients (109). Recently, Ono *et al* showed that podoplanin assessment in CAFs of stage I human squamous cell carcinoma, in addition to other immunohistochemical markers, may identify patients with poor outcome risk (110). In this study, a combined survival analysis of patients with low E-cadherin expression in cancer cells and high podoplanin expression in CAFs revealed that only 7% of the patients achieved the 5-year overall survival time (110).

Breast cancer. Podoplanin expression in CAFs was also identified as a negative prognosis marker of invasive ductal breast carcinoma (IDC) (27,36). In normal breast and mastopathies, podoplanin expression was noted in myoepithelial cells surrounding the duct cells (27,36,104). Although, the percentage of cases showing podoplanin expression in the study of Pula *et al* (27) and Schoppmann *et al* (36) differed, podoplanin expressing CAFs were associated with nodal involvement, poor differentiation grade and negative estrogen receptor status. Of the analyzed invasive lobular breast cancers, only one out of 48 analyzed cases showed podoplanin immunoreactivity in CAFs (36). In both studies the authors analyzed the association between the podoplanin expression in CAFs and lymphatic vessel densities (LVD) in the intratumoral and peritumoral areas, but only in the study of Pula *et al* a significant rise of intratumoral LVD with increasing expression of podoplanin in CAFs was noted (27). This may indicate that podoplanin-positive CAFs can possibly be generated via EndEMT from LECs. This hypothesis remains to be clarified.

Intrahepatic cholangiocarcinoma. In a study conducted on 86 cases of intrahepatic cholangiocarcinoma, Aishima *et al* identified podoplanin-positive myofibroblasts in the tumor stroma of 33 cases. The presence of these cells was associated with lymph node metastasis and poor outcome of the patients (111).

Colorectal carcinoma. Although the majority of recently published studies identified podoplanin expression in CAFs as an unfavorable marker of prognosis, expression of podoplanin in the tumor stroma of colorectal cancer was shown to be associated with good outcome of the patients (37). Moreover, cases

characterized by podoplanin expression in the tumor stroma had shallower depths of tumor invasion, were localized more distally and had a lower incidence of liver metastasis (37). These contradictory results were supported with *in vitro* tumor invasion assays, which showed that colon adenocarcinoma cell lines (HCT116 and HCT15) exerted augmented invasive potential, when co-cultured with fibroblasts with podoplanin-siRNA mediated knock-down (37).

Uterine cervical carcinoma. The prognostic value of podoplanin expression in CAFs was also studied in uterine cervical carcinoma. Similar to the results obtained in colorectal carcinoma, cases characterized by podoplanin expression in CAFs had lower incidences of lymph node metastases (112). Although the proportion of fatal cases in this group was smaller than in cases without podoplanin in CAFs, this trend was not statistically significant (112). The presence of podoplanin expression in the squamous cell carcinomas of the uterine cervix was also noted by Dumoff *et al*, who showed that its levels in the stroma of invasive tumors were significantly elevated in comparison to normal stroma of the cervix. However, the prognostic significance of this finding was not assessed in this study (91).

Adenocarcinoma of the esophagus. Podoplanin expression in CAFs was recently shown in adenocarcinoma of the esophagus, whereas none of its expression was noted in the precursor lesions of this cancer (Barret's mucosa without dysplasia, with low grade and high grade dysplastic Barret's mucosa) (113). Twenty-two of the 200 (11%) studied adenocarcinomas were identified as podoplanin expressing and these cases were characterized by an advanced tumor stage, more frequent lymph node involvement and lymphatic vessel involvement. In addition, podoplanin expression in CAFs was identified as an independent marker of poor prognosis in the analyzed adenocarcinoma patients cohort supporting its role in the progression of this cancer (113). Interestingly, only 3% of the analyzed lymph node metastases revealed podoplanin expression.

Other cancers. A comprehensive study aimed at identifying podoplanin expression in the cancerous stroma of various cancers was undertaken by Kitano *et al* (35). The most abundant podoplanin expressing CAFs were found in colorectal, stomach and biliary tract and pancreatic cancer, whereas cancers of the bladder, lung, liver, uterine body, prostate and ovary were characterized by expression in less than half of the CAFs. In thyroid cancers no podoplanin expression in the cancerous stroma was noted (35). Although the authors reported that podoplanin expression in CAFs was associated with higher primary size, the presence of lymph node metastasis, advanced disease stage, higher LVD and lymphatic and blood vessel invasion, these results should be interpreted with caution since the statistical analysis was performed on a pooled cohort of the analyzed cancers (35).

7. Conclusion

Recent years have brought new insights into the biological role of podoplanin. It is becoming apparent, that this widely

expressed protein may become a key target for future anti-cancer therapies, regardless of its expression in cancer as well as CAFs. Nevertheless, the diversity of the biological roles of podoplanin in normal and cancerous transformed tissues requires further studies in order to better understand cancer-stromal interactions.

Acknowledgements

This study was part of the 'Wrovasc - Integrated Cardio-vascular Centre' project, co-financed by the European Regional Development Fund, within the Innovative Economy Operational Program, 2007-2013.

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