Linking myofibroblast generation and microvascular alteration: The role of CD248 from pathogenesis to therapeutic target (Review)

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Abstract. Fibrosis is characterized by excessive extracellular matrix (ECM) deposition, and is the pathological outcome of tissue injury in a number of disorders. Accumulation of the ECM may disrupt the structure and function of native tissues and organs, including the lungs, heart, liver and skin, resulting in significant morbidity and mortality. On this basis, multiple lines of evidence have focused on the molecular pathways and cellular mechanisms involved in fibrosis, which has led to the development of novel antifibrotic therapies. CD248 is one of several proteins identified to be localized to the stromal compartment in cancers and fibroproliferative disease, and may serve a key role in myofibroblast generation and accumulation. Numerous studies have supported the contribution of CD248 to tumour growth and fibrosis, stimulating interest in this molecule as a therapeutic target. In addition, it has been revealed that CD248 may be involved in pathological angiogenesis. The present review describes the current understanding of the structure and function of CD248 during angiogenesis and fibrosis, supporting the hypothesis that blocking CD248 signalling may prevent both myofibroblast generation and microvascular alterations during tissue fibrosis.

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1. Introduction

Fibrosis is defined by the overgrowth, hardening and/or scarring of various tissues, and is responsible for the excessive deposition of extracellular matrix (ECM) proteins, including collagen (Col), fibronectin (FN) and hyaluronan, in the surrounding tissues (1,2). Fibrosis affects a number of different organ systems and is the hallmark of fibroproliferative diseases (FPDs) such as systemic sclerosis (SSc), liver cirrhosis, kidney fibrosis and interstitial lung disease (2). Given that various diseases are associated with the same fibrotic changes in different organ systems, the involvement of common pathogenic pathways may be speculated (3). Furthermore, it is well documented that fibrosis may cause progressive organ dysfunction and lead to significant morbidity and mortality (2-6), highlighting the requirement for new therapeutic targets. However in the past decade, the results of several promising early-phase studies of anti-fibrotic therapies have not been confirmed in large randomised clinical trials, in terms of both expected efficacy and unexpected side-effects; this has revealed a knowledge gap, particularly in the field of immune-associated tissue fibrosis (3).

The key pathogenic mediator in FPDs is the myofibroblast (7-12), which is responsible for ECM synthesis and remodelling. Notably, myofibroblasts may originate from different cellular sources; endothelial cells (ECs) and pericytes originate from the microvasculature and epithelium of organs such as the lung, and may lose their tissue-specific markers and trans-differentiate into myofibroblasts (2,13). The process of trans-differentiation is believed to be initiated by transforming

growth factor-β (TGFβ), and the recruitment of ECs into injured tissue may be ECM protein-dependent (14-16). In addition, a strong correlation has been revealed between microvascular dysregulation and fibrosis (2); in fact, abnormal microvasculature structure is a characteristic of FPDs that is associated with endothelial swelling, necrosis, pericyte detachment and thickening of the vascular basement membrane (17-19).

CD248 (also known as endosialin and TEM-1) is considered to be a specific marker of fibrosis. It is localized to fibroblasts and pericytes in FPDs and cancer, serving a pivotal role in tissue remodelling and repair (20,21). Given its high expression level in different types of tumours, numerous studies have evaluated its role in angiogenesis despite the hypothesis that CD248, by interaction with the ECM, may enhance the invasiveness of cancer cells (22-24). The role of CD248 has also been investigated in FPDs; a number of *in vitro* studies have illustrated the interplay between CD248 and several profibrotic molecules, including TGFβ, platelet-derived growth factor subunit B (PDGF-BB) and ECM proteins, which were the basis for further *in vivo* investigations of fibrosis (25-28). Additionally, CD248 was over-expressed in the lungs of patients with pulmonary (29) and hepatic fibrosis (30,31); in this context, the genetic loss of CD248 significantly reduced both microvascular rarefaction and fibrosis via the modulation of pericyte and stromal cell function in experimental models of kidney (32) and hepatic fibrosis (30,31,33). Therefore, it has been suggested that CD248 over-expression may serve an important role in fibrosis, and that it may be considered as a possible therapeutic target (21,32-34).

In the present review, current understanding of the structure and function of CD248 has been described, and the role of CD248-associated pathological angiogenesis assessed. Additionally, the role of CD248 in the pathogenesis of FPDs has been highlighted, indicating that CD248 inhibition may potentially modulate both microvascular alterations and myofibroblast generation during tissue fibrosis.

2. CD248 structure

CD248 is a heavily glycosylated, single-pass transmembrane protein that was initially identified as an overexpressed cell surface marker in the cancer vasculature (35,36). It has subsequently been revealed that CD248 is expressed by pericytes and not by the underlying ECs (37). CD248 is regarded as a mesenchymal marker involved in the regulation of cellular proliferation (33,37), and is expressed on mesenchymal stem cells (MSCs), fibroblasts, pericytes, smooth muscle cells and osteoblasts (21,26,38). The 2,274 bp human CD248 gene does not contain introns and is localized to the long arm of chromosome 11 (11q13); it has a 2,274 bp-long open reading frame which encodes a 757 amino acid type I transmembrane protein (39). CD248 is also a C-type lectin-like protein with a signal leader peptide, 5 globular extracellular domains consisting of a C-lectin domain, one domain similar to the Sushi/ccp/scr pattern and three EGF repeats, followed by a mucin-like region, a transmembrane segment and a short cytoplasmic tail (40). Its extracellular N-terminal domain (360 amino acids in length) shares structural and sequence homology with thrombomodulin (CD141) and C1qRp (CD93) proteins (39,41,42). The cytoplasmic tail of CD248 contains a putative PSD-95/Discs-large/ZO-1 (PDZ) binding domain (42,43), which is involved in protein-protein interactions and acts as an adaptor molecule, holding receptor and signalling molecule in large protein complexes (40). Notably, the cytoplasmatic domain of CD248 is highly conserved among different vertebrate species (Fig. 1). Structural homologies to receptor proteins suggest that CD248 may be a cell surface receptor (44), and previous molecular and cellular studies have demonstrated that CD248 selectively binds the ECM proteins FN, ColI and ColIV; in fact, engineered cells expressing CD248 exhibit enhanced adhesion to FN and migration through an FN-enriched cancer matrix (40), which are suppressed by anti-CD248 antibodies (44). FN, ColI and IV bind to the ectodomain of CD248, promoting cell attachment and migration during cancer invasion by stimulating the release of active matrix metalloproteinase (MMP)-9 (40,42). Furthermore, CD248 may bind the endothelial-specific ECM protein multimerin-2 (MMRN2) (45), which is typically deposited along blood vessels and serves anti-(46-48) and pro-(49) angiogenic roles, depending on the specific step of vessel development involved. Moreover, CD248-MMRN2 complexes may bind to CD93 expressed on the surface of ECs, where MMRN2 acts as an 'extracellular glue' between ECs and pericytes/fibroblasts.

3. Physiologic role of CD248

A primary feature of CD248 is its temporal pattern of expression during development, which is high in the embryo and progressively diminished postnatally (40). In line with this, CD248 may be functionally involved not only pathologically, but also in physiological angiogenesis (50). In the embryonic mouse, CD248 is predominantly expressed on stromal fibroblasts and cells of the developing vasculature (51-53). It is also expressed to greater degree on fibroblasts closely associated with epithelial structures, such as those adjacent to immature alveoli in the embryonic lung and the dermal condensate of developing skin (51). Furthermore, it has been shown that gene knockout (KO) mice may develop with a lack of CD248 expression altogether (23), thus CD248 may be redundant in certain physiological mechanisms. Rupp *et al* (52) showed that, at embryonic stage E10.0, CD248 was expressed in the ECs of the dorsal aorta. During stages E10.5 and E12.0, a prominent CD248+ perineural vascular plexus may develop in the head region, and angiogenic sprouts may be generated from the perineural plexus to invade the proliferating neuroectoderm. At stage E12.0, the vascular network is significantly developed throughout entire embryo; during stages E13.5-E14.5, CD248 may be expressed in clusters of mesenchymal cells in the head region and in the developing genitourinary system. Furthermore, CD248 expression is observed in the lung and salivary glands, where CD248+ fibroblast-like cells have been reported at the epithelial-mesenchymal interface. By late-gestation, clusters of CD248+ mesenchymal cells are present in the mucosa of the gastric cavity, in the dermis and in the area separating the skeletal muscle fibres (41,52,53). In healthy adult mice, CD248 is undetectable in all blood vessel types of the organs and tissues examined, with the exception of scattered stromal fibroblasts of the uterus and ovary, specialized cells of the kidney glomeruli and bone marrow

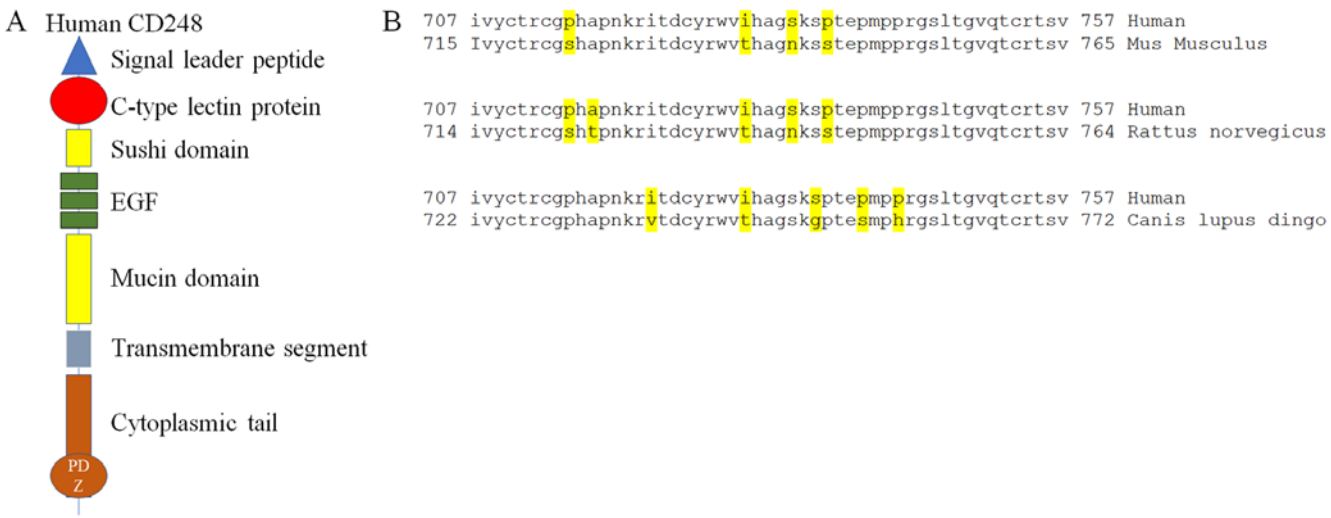

Figure 1. Schematic of the structure of CD248 and the amino acid sequence of the cytoplasmic domain. (A) The human CD248 protein comprises a signal-leader peptide (blue triangle), 5 globular extracellular domains [a C-lectin domain (red), a Sushi domain (yellow) and 3 EGF repeats (green)], a mucin-like region (yellow), a transmembrane segment (grey) and a short cytoplasmic tail (brown); the cytoplasmic tail contains a PDZ binding domain. (B) Sequence of the human CD248 protein reported in the National Centre for Biotechnology Information database, compared with that of *Mus Musculus*, *Rattus norvegicus* and *Canis lupus* dingo. Amino acid differences between sequences are indicated in yellow. EGF, epidermal growth factor; PDZ, PSD-95/Discs-large/ZO-1.

fibroblasts (41,52) (Table I). This may reflect the dynamic remodelling of the uterus during this period, which closely resembles that in embryonic tissues (52-54).

4. CD248 molecular function

As aforementioned, CD248 is involved in the fibro-proliferative process by modulating the PDGF-BB (24) and TGFβ pathways, and promoting alpha smooth muscle actin (αSMA) expression (25,27). On this basis, CD248 may induce the proliferation of both pericytes and fibroblasts following tissue injury, resulting in myofibroblast accumulation. CD248 could potentially alter PDGF-BB signalling, acting downstream of its receptor (PDGFR) to induce the proliferation of pericytes following fibrotic tissue damage. Furthermore, a high expression level of CD248 may induce TGFβ signalling. Recent studies have highlighted the importance of the TGFβ-CD248 signalling pathway as a potential therapeutic target for cancer and FPDs such as SSc. These studies revealed that the expression of CD248 by non-cancerous cells of mesenchymal origin was downregulated at both the transcriptional and protein level. On the contrary, in a pathological setting characterized by higher CD248 expression levels, TGFβ failed to downregulate the expression level of CD248 (25,28). Suresh Babu *et al* (28) speculated that CD248 may be one of the TGFβ-effector molecules that undergoes context-dependent switching (55-58). Moreover, it has been reported that the increased expression of CD248 may upregulate the expression of various other genes, including IL6, CCL2, TGFβ1 and TGFβR1 (22,59), which may stimulate αSMA and ColI-associated myofibroblast generation. In addition, TGFβ was unable to induce αSMA expression in CD248-silenced pericytes (25,27).

Conflicting results have been reported concerning the expression of different smooth muscle-associated genes during fibroblast proliferation and activation. Transcript expression levels of transgelin were shown to be elevated in fibroblasts derived from mice lacking the cytoplasmatic domain of CD248 (40). In addition, CD248 knock-out did not affect the *in vitro* expression of αSMA in normal human lung fibroblasts (29). Furthermore, in a model of renal fibrosis, no increase in αSMA expression level was observed in CD248-deficient mice (32), and in an experimental model of liver fibrosis, the total hepatic mRNA levels of Col and αSMA were reduced in CD248-deficient, compared with wild-type (WT) mice (33). These discrepancies may be partially explained by the use of different experimental models, and the reported differences in cell manipulation.

As far as its angiogenic role is concerned, CD248 may promote the interaction between ECs, pericytes and fibroblasts via MMRN2. In fact, EC-expressed CD93 may bind to MMRN2 present in the ECM, which in turn binds CD248 expressed by fibroblasts or vasculature-associated pericytes. CD93 also serves a pivotal role in endothelial migration and tube formation; CD93-deficient mice displayed defects in angiogenesis (60) and the CD93-binding fragment of MMRN2 exhibited anti-angiogenic effects, presumably by disrupting its normal function (47). In this context, pericyte-expressed CD248 may promote EC apoptosis via MMRN2. Simonavicius *et al* (61) speculated that CD248 was able to bind to the vascular basement membrane, directly disrupting EC adhesion to the matrix. Alternatively, matrix-bound CD248 may impair cross-talk between endothelial integrins and vascular endothelial growth factor receptor 2 (VEGFR2), resulting in the attenuation of vascular endothelial growth factor (VEGF) signalling and subsequent EC apoptosis. These findings are summarized in Fig. 2.

5. CD248 and cancer angiogenesis

The Analysis of CD248 expression in 250 clinical specimens (158 carcinomas and 92 sarcomas), revealed that 19 out of the 20 cancer subtypes in question had CD248-positive specimens (62). Regarding its expression pattern in cancer tissues, where the formation and reorganisation of blood vessels

Table I. Murin CD248 expression pattern from pre-natal stage (E) until adult stage.

Author, year	Pre-natal stage (E)	Expression pattern	(Refs.)
Rupp *et al*, 2006	E10.0	CD248 expression could be observed in the endothelial cells of the dorsal aorta.	(52)
Valdez *et al*, 2012	E10.5-E12.0	CD248 expression could be observed in the perineural vascular plexus and in the angiogenic sprouts, invading the proliferating neuroectoderm	(41)
Valdez *et al*, 2012; Rupp *et al*, 2006; MacFadyen *et al*, 2007	E13.5-E14.5	CD248 could be expressed in clusters of mesenchymal cells, in the head region, in the genitourinary system, in the lungs and in the salivary gland. By late-gestation, CD248 could be expressed in the mucosa of the gastric cavity, in the dermis and in the area separating skeletal muscle fibres	(41,52,53)
Valdez *et al*, 2012; Rupp *et al*, 2006	Adult	CD248 was undetectable in all the blood vessels of the organs and tissues examined, except for scattered stromal fibroblasts of the uterus and ovary, specialized cells of the kidney glomeruli and bone marrow fibroblast	(41,52)

Where E refers to the number of days post-conception.

occurs at a high rate, CD248 has been proposed to be a potential target of antiangiogenic cancer therapy (21,22,61). *In vitro,* Brett *et al* (63) demonstrated that the CD248+ subpopulation within the lipoaspirate stromal vascular fraction (SVF) showed an increased expression of angiogenic genes. The authors speculated that CD248+ pro-angiogenic cells obtained from SVF could represent a suitable strategy in wound healing by promoting increased vessel growth in the wound. In line with this, CD248-deficient mice displayed a specific defect in the early stage of angiogenesis during muscle remodelling (64). Facciponte *et al* (65) developed a DNA vaccine that expressed full-length mouse Tem1 cDNA fused to a TT adjuvant; immunization with the Tem1-TT vaccine reduced tumour vasculature compared with the control vaccine, as determined by microvasculature density, functional imaging (ultrasound imaging of blood perfusion and blood flux) and haematocrit levels. However, conflicting results have been reported; Nanda *et al* (23) illustrated that abdominally-implanted cancers proliferated significantly more slowly in CD248-KO mice than in their WT counterparts, but that the number of small blood vessels was increased in the KO mice. Similarly, murine brain tumours from the CD248-KO animals possessed ~40% more vessels than tumours extracted from WT mice (41). The authors speculated that CD248 served a critical role in determining cancer progression, and that the increase in small blood vessels observed in the stroma of CD248-KO mice may represent the pro-angiogenic response to an abnormal microenvironment (23,28,66,67). Furthermore, CD248 is expressed predominantly on cells which line the blood vessels, but is also detectable in fibroblast-like stromal cells (23); the authors did not elucidate whether the effects of CD248 disruption were due its absence in blood vessels or fibroblast-like stromal cells. It is possible to speculate that the effects of CD248 expression on cancer progression may also be associated with its absence in the fibroblastic stroma. The interaction between the stroma and cancer cells may induce the expression of ECM proteins, MMPs and growth factors (68), thus facilitating invasiveness by stimulating the growth of irregular and tortuous new vessels (69-71). Viski *et al* (24) revealed that in 3 different preclinical models, upregulated CD248 expression levels may have promoted cancer cell intravasation into the circulation, facilitating interactions with perivascular cells and promoting transmigration across the endothelium. In this study, impaired cancer cell intravasation in CD248-KO mice was not associated with vascular alterations, suggesting that CD248 inhibition in cancer has minimal impact on vascular integrity. Concurrently, treatment of syngeneic cancer-bearing human-CD248 knock-in mice with the anti-human endosialin antibody MORAb-004 did not lead to a reduction in vessel number or destabilization of the vasculature, but significantly impaired cancer cell proliferation following subcutaneous or intravenous inoculation (24,27).

Another consideration is to clarify the phase of angiogenesis that CD248 is associated with. Physiological angiogenesis is initiated in response to the local production of pro-angiogenic factors (particularly VEGF-A), which promotes vascular sprout formation by the induction and migration of leading tip cells, and by stimulating the proliferation of neighbouring stalk cells. After sprouting, the initial vascular plexus is extensively remodelled. A pivotal feature of this remodelling is the pruning of unwanted capillaries through selective branch regression (61). CD248 may serve a primary role in vascular pruning, promoting vessel regression and apoptosis of redundant ECs (61). The capillary regression resulting from the apoptosis of ECs marks the end of vessel plasticity and reflects the quiescent, mature state of the new vascular network (61,72). In pathological conditions in which CD248 is overexpressed, it is possible that the remodelling and pruning of new vessels may be increased, promoting the formation of irregular, tortuous and leaky blood vessels. Of note, retarding

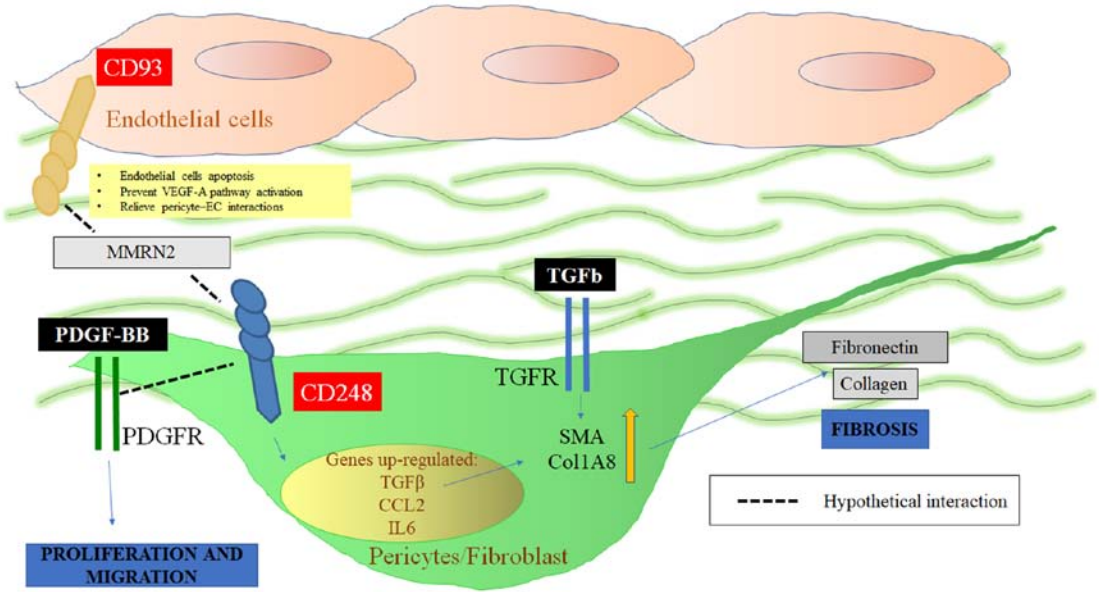

Figure 2. Hypothetical model of CD248 functions. CD248 expressed by fibroblasts or vasculature-associated pericytes may bind MMRN2, which may in turn bind CD93 expressed by ECs. This presents a scenario where MMRN2 may allow for the connection between ECs and pericytes during vessel formation and maturation. In a pathological setting in which it's expression levels may be increased, CD248 may exacerbate vessel pruning, resulting in increased EC apoptosis, preventing VEGF-A pathway activation and inhibiting pericyte-EC interactions. In perivascular cells and fibroblasts, the upregulation of CD248 may also promote the expression of genes stimulating the TGFβ pathway, leading to αSMA expression and Col release into tissues, resulting in ECM accumulation and fibrosis. Furthermore, CD248 may establish a hypothetical interaction with PDGFR which may be responsible for cellular proliferation. MMRN2, endothelial-specific ECM protein multimerin-2; ECM, extracellular matrix; EC, endothelial cells; VEGF-A, vascular endothelial growth factor-A; TGFβ, transforming growth factor-β; αSMA, α-smooth muscle actin; PDGFR, platelet-derived growth factor receptor; PDGF-BB, platelet-derived growth factor subunit B; TGFR, transforming growth factor-β receptor.

CD248 function may prevent vasculature remodelling during cancer (72), resulting in more stable vessels. This would be advantageous in the treatment of cancer, allowing efficient delivery of therapeutics and increasing the responsiveness to VEGF inhibition (61,71). Furthermore, during FPDs characterized by microvascular alteration, blocking CD248 may prevent endothelial damage, inhibiting vessel remodelling towards myofibroblasts generation (32). In addition, hypoxia (a primary promoter of angiogenesis) regulates CD248 gene transcription via the HIF-2 transcription factor (73). It is possible to speculate that in fibrotic tissues with impaired angiogenesis, hypoxia may further stimulate CD248 over-expression, resulting in the exacerbation of microvascular damage from excessive CD248-mediated pruning (61,74,75).

6. CD248 and microvascular damage

Although CD248 has been proposed as a potential target of antiangiogenic cancer therapy, blocking CD248 did not lead to a reduction in vessel number, though did prevent cancer stromal cell interaction with vessels (23,24). Notably, in pathological fibrosis, CD248 inhibition may even reverse microvascular damage (30-33). In fact, it has been shown that CD248+ pericytes may promote EC apoptosis, resulting in the attenuation of VEGF signalling (61). In a previous study (61), the authors investigated the potential mechanism by which CD248+ pericytes promote vessel regression, via the generation of a soluble CD248-Fc construct cultured with ECs; ECs exhibited attenuated VEGF-mediated signalling with reduced VEGFR2 Tyr1175 and ERK1/2 phosphorylation. Furthermore, flow cytometric analysis highlighted an increase in apoptotic ECs compared with cells cultured with a control Fc construct, suggesting a potential role for CD248. In another experimental model, HeLa cells were transfected with the phCMV1-CD248 plasmid; as a consequence, the genes involved in cell-cell communication, adhesion and motility (Ang-2, Angiopoietin-like 3 and 4, IL-1β, EGF, TGF-β receptor, Ephrin-A3, Neuropilin 1) were upregulated, while VEGF-A expression levels were significantly decreased (22).

Using an animal model of renal fibrosis, following unilateral ureteral obstruction (UUO)-associated kidney injury, Smith *et al* (32) showed that the genetic deletion of CD248 protected mice from microvascular rarefaction. Prior to UUO injury, there was no difference in the size of the vessel area between WT and KO mice. However, following kidney injury in WT mice, there was an initial increase in vessel density 3 days post-injury, followed by progressive vascular regression up to 14 days post-injury. These results suggested an early phase of reparative angiogenesis followed by vascular regression (microvascular rarefaction). Conversely, CD248-KO mice did not exhibit an early phase of reparative angiogenesis, and the kidneys of these animals were protected from vessels loss in response to UUO injury (32).

Despite the fact that the exact mechanism by which CD248-expressing cells contribute to microvascular rarefaction is not fully elucidated, CD248 may bind the ECM proteins FN, ColI, ColIV, and MMRN2 (26,46) in fibrotic tissues. Furthermore, the absence of CD248 may reduce the interaction between pericytes, ECM proteins and stromal fibroblasts, preventing migration from the vasculature into the tissue stroma, and subsequent myofibroblast generation. In turn, this would result in reduced stromal fibrosis and microvascular rarefaction (32).

Previously, Khan *et al* (46) demonstrated that ECs expressing CD93 may bind MMRN2 in the ECM, which in turn is bound by CD248 expressed by fibroblasts or vasculature-associated pericytes. CD248 binds MMRN2 in the 133-486 region, whereas CD93 binds in the 530-624 region. Binding both CD93 and CD248, MMRN2 may promote the interaction between pericytes, ECs and ECM protein, potentially retarding angiogenic sprouting by sequestering VEGF-A and disrupting the VEGF/VEGFR2 signalling axis (46,48,49,76). On the contrary, Khan *et al* (46) and Zanivan *et al* (77), described MMRN2 as a pro-angiogenic molecule, though these conflicting roles may be associated with different experimental conditions.

7. CD248 and fibrosis

FPDs are able to cause significant morbidity and mortality in affected patients (1,2,78), thus the development of novel therapies are still required (79-81). For this reason, gaining a greater understanding of the origin and differentiation pathways of myofibroblasts *in vivo* is a priority. In this context, recent studies (82-87) have suggested a role for mesenchymal perivascular cells (88); these cells have held various names including mural cells and pericytes, and several of their functions remain largely unknown. Dulauroy *et al* (89) used genetic studies in mice to map the fate of neural crest cell-derived embryonic mesenchymal cells that expressed A disintegrin and metalloprotease isoform 12 (ADAM12). It was observed that fetal ADAM12+ cells contributed to the generation of perivascular cells in adult skeletal muscle. In fact, a subset of these cells derived from the ADAM12+ lineage expressed pericyte markers and lined the capillaries. Following fibrotic injury, the reactivation of ADAM12+ cells stimulated ontogenetic signalling to restore vascular integrity. ADAM12+ cells were shown to be significantly increased in the perivascular skin cells of SSc, a model of chronic FPDs, suggesting that myofibroblasts may originate from perivascular cells (83). Therefore, the investigation of novel targets involved in pericyte-to-myofibroblast transition may reveal therapeutic possibilities for FDPs. It has also been shown that CD248+ perivascular cells may serve a role in myofibroblast generation. In fact, CD248 expression is required for TGFβ-induced αSMA expression on normal human pericytes, in addition to that on the perivascular MSCs of patients with SSc (25,27). Furthermore, in injured fibrotic kidney tissue exhibits increased CD248 expression within a CD248+/αSMA+ subpopulation of myofibroblasts, in addition to a population of CD248+/αSMA-stromal fibroblasts. Notably, a subset of CD248-/αSMA+ myofibroblasts was also identified, emphasizing the heterogeneity of these cells in fibrotic kidney tissues (32). Of note, Smith *et al* (32) demonstrated that CD248-deficient mice were protected from renal fibrosis, illustrating a pathogenic role for CD248 in the development of renal fibrosis. In this context the loss of CD248 may modulate the response of renal pericytes and stromal fibroblasts to UUO injury. Although the precise mechanism has not been fully elucidated, it has been revealed that the genetic loss of CD248 significantly reduced the formation of matrix-depositing myofibroblasts in response to renal injury by UUO, with a subsequent decrease in tissue fibrosis. However, it should be clarified whether the reduction in stromal myofibroblasts and pericytes in CD248-deficient mice was the result of impaired migration or a specific proliferative defect *in vivo*. PDGF-BB/PDGFR signalling between ECs and pericytes has been shown to be important for vascular stabilization following kidney injury (26,90-92), indicating that CD248 may exert its effects by modulating the PDGF-BB pathway. Furthermore, PDGF-induced autophosphorylation of extracellular signal-regulated kinase (ERK; but not PDGFR itself) was markedly diminished in CD248-deficient pericytes (26). These findings suggest that CD248 may perturb PDGF signalling downstream of PDGFR and upstream of ERK1/2, by an as yet unidentified mechanism. CD248 may therefore promote the proliferation of pericytes after injury, as they differentiate into Col-secreting myofibroblasts (26,93).

Studies of hepatic fibrosis revealed that CD248 promoted fibrosis by enhancing a PDGF pro-proliferative signal to hepatic stellate cells (33). Combined with the established roles of the PDGF signalling axis in renal fibrosis (93), the CD248-associated enhancement of PDGF signalling may be speculated in pericytes during fibrosis.

8. Myofibroblast origin in cancer and the role of CD248

In order to identify the source(s) of αSMA-positive myofibroblasts in different pathological conditions, fibrosis and cancer development, a number of cell tracing studies were performed (94). In response to both injury and dysregulated tissue homeostasis, local fibroblasts were able to undergo trans-differentiation to myofibroblasts, and activation in the skin, liver, lung, heart and kidney, and in the stromal reaction to epithelial tumours (95,96). Epithelial mesenchymal transition is another mechanism of myofibroblast generation from local epithelial layers during cancer development (97,98), mirroring what observed in kidney (99) and lung fibrosis (100,101). In addition, the de-differentiation of perivascular cells into ECM-producing cells may contribute to myofibroblast development and function (102). Furthermore, a number of studies have reported that bone marrow MSCs and hematopoietic stem cells may be the precursors of myofibroblasts (103,104). Independent of this speculation, few essential factors are required for the activation of myofibroblasts. TGFβ is the most potent myofibrogenic growth factor, and the inhibition of cancer cell responsiveness to TGFβ has metastasis-suppressing effects (105). Another important factor in myofibroblast activation is the mechanical stress resulting from remodelling activities in the stroma, and the mechanical properties of the ECM (106,107). The activation and differentiation of stromal cells into cancer-associated myofibroblasts has consequences for tumour development, progression and metastasis (108). Fibrosis and cancer are known to be intimately linked and myofibroblast activation may serve a pivotal role in cancer chemoresistance (109,110). Also, a dense fibrotic stroma correlated with a poor response to neoadjuvant treatments, including 5-fluorouracil epirubicin and cyclophosphamide (FEC) in breast cancer, and gemcitabine in pancreatic ductal adenocarcinoma (PDAC) (111,112). Furthermore, cancer-associated fibroblasts may secrete hyaluronan, which is responsible for the regulation of interstitial pressure within the tumour, and results in blood vessel collapse and impaired drug delivery (113,114). It has also been shown that CD248 was

expressed by these cancer-associated fibroblasts and mural cells, but not ECs (114), and that its expression correlated with poor prognosis in patients with gastric cancer (115). These findings suggest that the inhibition of CD248 expression may represent a novel strategy for perturbing the differentiation of stromal cells into cancer-associated fibroblasts.

9. Therapies targeting CD248

The results of a first-in-human, open-label, phase I study have recently been published, enrolling patients with refractory solid cancers treated with a humanized monoclonal antibody against CD248 (MORAb-004). Following the administration of MORAb-004, a prolonged, stable disease state of >106 days was observed in patients with cancer subtypes believed to be of epithelial origin, including colorectal carcinoma (116). Although the mechanism of action of MORAb-004 is not completely understood, preclinical models have suggested that CD248 is removed from the cell surface upon MORAb-004-mediated internalization, while MORAb-004 exhibits no antibody-dependent cellular cytotoxicity (45). Furthermore, it is possible that MORAb-004 may disrupt protein-protein interactions that serve to signal between tumour and stromal cells within the cancer microenvironment (116). Another phase I study enrolling children with relapsed or refractory solid cancer, demonstrated that MORAb-004 was well tolerated and that its pharmacokinetics did not significantly differ in children compared with adults (117). Concurrently, a multicentre, open-label, phase II study did not produce the same encouraging results; this study evaluated the 24-week progression-free survival, pharmacokinetics and tolerability of 2 doses of MORAb-004 in patients with metastatic melanoma. However, the efficacy of single-agent MORAb-004 treatment in melanoma was low. The principal limitations of this study were the small sample size and population heterogeneity of previously treated melanoma patients (118). Furthermore, a randomized, double-blind, placebo-controlled phase II study of patients with chemo-refractory metastatic colorectal cancer confirmed that MORAb-004 was well tolerated, despite the fact that no improvement in overall survival and/or response rate was demonstrated (119). However, it should be noted that the results of this trial may be skewed by the enrolment of patients at advanced cancer stages; indeed, it was speculated that MORAb-004 may be more effective at treating patients affected by early-onset cancer and with a short duration of disease.

Future studies with more stringent inclusion criteria are necessary to fully evaluate the efficacy of MORAb-004, and an alternative approach may be considered to improve efficacy, including antibody-drug conjugates to selectively deliver cytotoxic agents to tumour sites. Anti-CD248 drug conjugates delivering monomethyl auristatin E, a synthetic antineoplastic agent, were tested for their activity in 4 human cancer cell lines. The study demonstrated that CD248-positive tumours may be specifically and effectively targeted by a monoclonal anti-CD248 antibody conjugated to an anti-neoplastic agent, and the response was complete and sustained (120). Collectively, the results of these studies suggest that additional pre-clinical investigations are required to better understand the mechanism of action of anti-CD248 monoclonal antibodies, and to determine their most suitable clinical application (116). However, despite the CD248 expression pattern and encouraging *in vivo* results suggesting a potential anti-cancer target, the exact role of CD248 in cancer is not fully understood. Current research is hindered by inadequate knowledge of the factors and pathways that control CD248 expression and distribution. It is also unclear whether the effects of CD248 on tumour growth are due to its expression in fibroblastic stromal cells or vascular cells (23).

Although the pathogenic role of CD248 in fibrosis has been strongly indicated, there are currently no published studies assessing its potential as a therapeutic target in patients affected by FPDs. Future studies are required to completely elucidate the possible therapeutic applications of targeting CD248.

10. Conclusions

Fibrosis is the hallmark of pathologic remodelling in a number of tissues, a contributor to clinical disease and one of the leading causes of mortality in the developed world (2). Therefore, there is a great deal of interest in identifying a means of inhibiting, or even reversing the progression of tissue fibrosis. Notably, FPDs are characterized by common pathogenic pathways preserved between different organs, thus an understanding of the mediators and pathways activated in tissue fibrosis may help to establish potential therapeutic targets across different diseases and organs systems (108). Another unifying characteristic of FPDs is microvascular alteration and its common pathogenic pathways, the constituents of which may also be an attractive therapeutic target. At present, an improved understanding of the mechanisms involved in vascular remodelling are essential for the implementation of therapies stimulating vascular network stabilization during FPDs. Interestingly, this could also be required for cancers vascularised by immature, disorganised and leaky blood vessels, with structural abnormalities which contribute to blood flow disturbances and cancer cell extravasation. Indeed, for more effective anti-angiogenic therapies, it has been speculated that in addition to targeting excessive angiogenesis, the cancer vasculature should also be normalized (121).

CD248 is a glycosylated transmembrane protein that is overexpressed in the perivascular cells and fibroblasts of several diseases characterized by abnormal vascular remodelling, including cancer and FPDs. The exact role of CD248 is not fully understood; however, *in vitro* and *in vivo* studies have revealed that inhibiting CD248 signalling may prevent myofibroblast accumulation and promote vessel stabilisation. In line with these findings, anti-CD248 therapy may be considered a promising therapeutic strategy in both cancer and FPDs. Indeed, interfering with CD248 may prevent the myofibroblast proliferation responsible for ECM stiffening, which in turn contributes to the persistence and progression of both cancer and fibrosis. Furthermore, anti-CD248 therapy may also prevent pericyte trans-differentiation into mature αSMA+ cells, which may subsequently limit myofibroblast generation (25,82,87,121). In future, further studies are required to clarify the role of CD248 in FPDs, securing this molecule as a potential therapeutic target in a clinical setting, in which an effective therapeutic approach to prevent fibrosis has yet to be developed.

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Authors' contributions

PDB, PR, FDG, RG and PC conceived and designed the review, performed the literature search, and wrote and revised the manuscript. VL performed the literature search and revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare that they have no competing interests.

References

1. Wynn TA: Cellular and molecular mechanisms of fibrosis. J Pathol 214: 199-210, 2008.
2. Pu KM, Sava P and Gonzalez AL: Microvascular targets for anti-fibrotic therapeutics. Yale J Biol Med 86: 537-554, 2013.
3. Wynn TA: Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. J Clin Invest 117: 524-529, 2007.
4. Sziksz E, Pap D, Lippai R, Béres NJ, Fekete A, Szabó AJ and Vannay A: Fibrosis related inflammatory mediators: Role of the IL-10 cytokine family. Mediators Inflamm 2015: 764641, 2015.
5. Giacomelli R, Afeltra A, Alunno A, Baldini C, Bartoloni-Bocci E, Berardicurti O, Carubbi F, Cauli A, Cervera R, Ciccia F, *et al*: International consensus: What else can we do to improve diagnosis and therapeutic strategies in patients affected by autoimmune rheumatic diseases (rheumatoid arthritis, spondyloarthritides, systemic sclerosis, systemic lupus erythematosus, antiphospholipid syndrome and Sjogren's syndrome)? The unmet needs and the clinical grey zone in autoimmune disease management. Autoimmun Rev 16: 911-924, 2017.
6. Cox TR and Erler JT: Remodeling and homeostasis of the extracellular matrix: Implications for fibrotic diseases and cance. Dis Models Mech 4: 165-178, 2011.
7. Gabbiani G: The myofibroblast in wound healing and fibrocontractive diseases. J Pathol 200: 500-503, 2003.
8. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML and Gabbiani G: The myofibroblast: One function, multiple origins. Am J Pathol 170: 1807-1816, 2007.
9. Cipriani P, Marrelli A, Liakouli V, Di Benedetto P and Giacomelli R: Cellular players in angiogenesis during the course of systemic sclerosis. Autoimmun Rev 10: 641-646, 2011.
10. Desmouliere A, Darby IA and Gabbiani G: Normal and pathologic soft tissue remodeling: Role of the myofibroblast, with special emphasis on liver and kidney fibrosis. Lab Invest 83: 1689-1707, 2003.
11. Virag JI and Murry CE: Myofibroblast and endothelial cell proliferation during murine myocardial infarct repair. Am J Pathol 163: 2433-2440, 2003.
12. Phan SH: The myofibroblast in pulmonary fibrosis. Chest 122: 286S-289S, 2002.
13. Cipriani P, Di Benedetto P, Ruscitti P, Capece D, Zazzeroni F, Liakouli V, Pantano I, Berardicurti O, Carubbi F, Pecetti G, *et al*: The Endothelial-mesenchymal transition in systemic sclerosis is induced by endothelin-1 and transforming growth factor-β and May Be blocked by macitentan, a dual endothelin-1 receptor antagonist. J Rheumatol 42: 1808-1816, 2015.
14. Liakouli V, Cipriani P, Di Benedetto P, Ruscitti P, Carubbi F, Berardicurti O, Panzera N and Giacomelli R: The role of extracellular matrix components in angiogenesis and fibrosis: Possible implication for systemic sclerosis. Mod Rheumatol 15: 1-11, 2018.
15. Sacchetti C, Bai Y, Stanford SM, Di Benedetto P, Cipriani P, Santelli E, Piera-Velazquez S, Chernitskiy V, Kiosses WB, Ceponis A, *et al*: PTP4A1 promotes TGFβ signaling and fibrosis in systemic sclerosis. Nat Commun 8: 1060, 2017.
16. Abe R, Donnelly SC, Peng T, Bucala R and Metz CN: Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. J Immunol 166: 7556-7562, 2001.
17. Leaf IA and Duffield JS: What can target kidney fibrosis? Nephrol Dial Transplant 32 (Suppl 1): i89-i97, 2017.
18. Magro CM, Ross P, Marsh CB, Allen JN, Liff D, Knight DA, Waldman WJ and Cowden DJ: The role of anti-endothelial cell antibody-mediated microvascular injury in the evolution of pulmonary fibrosis in the setting of collagen vascular disease. Am J Clin Pathol 127: 237-247, 2007.
19. Ruart M, Chavarria L, Campreciós G, Suárez-Herrera N, Montironi C, Guixé-Muntet S, Bosch J, Friedman SL, Garcia-Pagán JC and Hernández-Gea V: Impaired endothelial autophagy promotes liver fibrosis by aggravating the oxidative stress response during acute liver injury. J Hepatol 70: 458-469, 2019.
20. Lax S, Hardie D, Wilson A, Douglas M, Anderson G, Huso D, Isacke CM and Buckley CD: The pericyte and stromal marker CD248 (endosialin) is required for efficient lymph node expansion. Eur J Immunol 40: 1884-1889, 2010.
21. Bagley RG, Honma N, Weber W, Boutin P, Rouleau C, Shankara S, Kataoka S, Ishida I, Roberts BL and Teicher BA: Endosialin/TEM1/CD248 is a pericyte marker of embryonic and tumour neovascularisation. Microvasc Res 76: 180-188, 2008.
22. Kontsekova S, Polcicova K, Takacova M and Pastorekova S: Endosialin: Molecular and functional links to tumour angiogenesis. Neoplasma 63: 183-192, 2016.
23. Nanda A, Karim B, Peng Z, Liu G, Qiu W, Gan C, Vogelstein B, St Croix B, Kinzler KW and Huso DL: Tumor endothelial marker 1 (TEM1) functions in the growth and progression of abdominal tumours. Proc Natl Acad Sci USA 103: 3351-3356, 2006.
24. Viski C, König C, Kijewska M, Mogler C, Isacke CM and Augustin HG: Endosialin-expressing pericytes promote metastatic dissemination. Cancer Res 76: 5313-5325, 2016.
25. Di Benedetto P, Liakouli V, Ruscitti P, Berardicurti O, Carubbi F, Panzera N, Di Bartolomeo S, Guggino G, Ciccia F, Triolo G, *et al*: Blocking CD248 molecules in perivascular stromal cells of patients with systemic sclerosis strongly inhibits their differentiation toward myofibroblasts and proliferation: A new potential target for antifibrotic therapy. Arthritis Res Ther 20: 223, 2018.
26. Tomkowicz B, Rybinski K, Nicolaides NC, Grasso L and Zhou Y: Endosialin/TEM-1/CD248 regulates pericyte proliferation through PDGF receptor signaling. Cancer Biol Ther 9: 908-915, 2010.
27. Rybinski K, Imtiyaz HZ, Mittica B, Drozdowski B, Fulmer J, Furuuchi K, Fernando S, Henry M, Chao Q, Kline B, *et al*: Targeting endosialin/CD248 through antibody-mediated internalization results in impaired pericyte maturation and dysfunctional tumour microvasculature. Oncotarget 22: 25429-25440, 2015.
28. Suresh Babu S, Valdez Y, Xu A, O'Byrne AM, Calvo F, Lei V and Conway EM: TGFβ-mediated suppression of CD248 in non-cancer cells via canonical Smad-dependent signaling pathways is uncoupled in cancer cells. BMC Cancer 14: 113, 2014.
29. Bartis D, Crowley LE, D'Souza VK, Borthwick L, Fisher AJ, Croft AP, Pongrácz JE, Thompson R, Langman G, Buckley CD and Thickett DR: Role of CD248 as a potential severity marker in idiopathic pulmonary fibrosis. BMC Pulm Med 16: 51, 2016.

30. Mogler C, Wieland M, König C, Hu J, Runge A, Korn C, Besemfelder E, Breitkopf-Heinlein K, Komljenovic D, Dooley S, *et al*: Hepatic stellate cell-expressed endosialin balances fibrogenesis and hepatocyte proliferation during liver damage. EMBO Mol Med 7: 332-338, 2015.
31. Mogler C, König C, Wieland M, Runge A, Besemfelder E, Komljenovic D, Longerich T, Schirmacher P and Augustin HG: Hepatic stellate cells limit hepatocellular carcinoma progression through the orphan receptor endosialin. EMBO Mol Med 9: 741-749, 2017.
32. Smith SW, Croft AP, Morris HL, Naylor AJ, Huso DL, Isacke CM, Savage CO and Buckley CD: Genetic deletion of the stromal cell marker CD248 (Endosialin) protects against the development of renal fibrosis. Nephron 131: 265-277, 2015.
33. Wilhelm A, Aldridge V, Haldar D, Naylor AJ, Weston CJ, Hedegaard D, Garg A, Fear J, Reynolds GM, Croft AP, *et al*: CD248/endosialin critically regulates hepatic stellate cell proliferation during chronic liver injury via a PDGF-regulated mechanism. Gut 65: 1175-1185, 2016.
34. Smith SW, Eardley KS, Croft AP, Nwosu J, Howie AJ, Cockwell P, Isacke CM, Buckley CD and Savage CO: CD248+ stromal cells are associated with progressive chronic kidney disease. Kidney Int 80: 199-207, 2011.
35. Rettig WJ, Garin-Chesa P, Healey JH, Su SL, Jaffe EA and Old LJ: Identification of endosialin, a cell surface glycoprotein of vascular endothelial cells in human cancer. Proc Natl Acad Sci USA 89: 10832-10836, 1992.
36. Brady J, Neal J, Sadakar N and Gasque P: Human endosialin (tumor endothelial marker 1) is abundantly expressed in highly malignant and invasive brain tumors. J Neuropathol Exp Neurol 63: 1274-1283, 2004.
37. St Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B and Kinzler KW: Genes expressed in human tumor endothelium. Science 289: 1197-1202, 2000.
38. MacFadyen JR, Haworth O, Roberston D, Hardie D, Webster MT, Morris HR, Panico M, Sutton-Smith M, Dell A, van der Geer P, *et al*: Endosialin (TEM1, CD248) is a marker of stromal fibroblasts and is not selectively expressed on tumour endothelium. FEBS Lett 579: 2569-2575, 2005.
39. Naylor AJ, Azzam E, Smith S, Croft A, Poyser C, Duffield JS, Huso DL, Gay S, Ospelt C, Cooper MS, *et al*: The mesenchymal stem cell marker CD248 (Endosialin) is a negative regulator of bone formation in mice. Arthritis Rheum 64: 3334-3343, 2012.
40. Christian S, Ahorn H, Koehler A, Eisenhaber F, Rodi HP, Garin-Chesa P, Park JE, Rettig WJ and Lenter MC: Molecular cloning and characterization of endosialin, a C-type lectin-like cell surface receptor of tumour endothelium. J Biol Chem 276: 7408-7414, 2001.
41. Valdez Y, Maia M and Conway EM: CD248: Reviewing its role in health and disease. Curr Drug Targets 13: 432-439, 2012.
42. Carson-Walter EB, Watkins DN, Nanda A, Vogelstein B, Kinzler KW and St Croix B: Cell surface tumour endothelial markers are conserved in mice and humans. Cancer Res 61: 6649-6655, 2001.
43. Maia M, de Vriese A, Janssens T, Moons M, van Landuyt K, Tavernier J, Lories RJ and Conway EM: CD248 and its cytoplasmic domain: A therapeutic target for arthritis. Arthritis Rheum 62: 3595-3606, 2010.
44. Gardiol D: PDZ-containing proteins as targets in human pathologies. FEBS J 279: 3529, 2012.
45. O'Shannessy DJ, Smith MF, Somers EB, Jackson SM, Albone E, Tomkowicz B, Cheng X, Park Y, Fernando D, Milinichik A, *et al*: Novel antibody probes for the characterization of endosialin/TEM-1. Oncotarget 7: 69420-69435, 2016.
46. Khan KA, Naylor AJ, Khan A, Noy PJ, Mambretti M, Lodhia P, Athwal J, Korzystka A, Buckley CD and Willcox BE: Multimerin-2 is a ligand for group 14 family C-type lectins CLEC14A, CD93 and CD248 spanning the endothelial pericyte interface. Oncogene 36: 6097-7008, 2017.
47. Andreuzzi E, Colladel R, Pellicani R, Tarticchio G, Cannizzaro R, Spessotto P, Bussolati B, Brossa A, De Paoli P, Canzonieri V, *et al*: The angiostatic molecule Multimerin 2 is processed by MMP-9 to allow sprouting angiogenesis. Matrix Biol 64: 40-53, 2017.
48. Colladel R, Pellicani R, Andreuzzi E, Paulitti A, Tarticchio G, Todaro F, Colombatti A and Mongiat M: MULTIMERIN2 binds VEGF-A primarily via the carbohydrate chains exerting an angiostatic function and impairing tumor growth. Oncotarget 7: 2022-2037, 2016.
49. Lorenzon E, Colladel R, Andreuzzi E, Marastoni S, Todaro F, Schiappacassi M, Ligresti G, Colombatti A and Mongiat M: MULTIMERIN2 impairs tumor angiogenesis and growth by interfering with VEGF-A/VEGFR2 pathway. Oncogene 31: 3136-3147, 2012.
50. Galvagni F, Nardi F, Spiga O, Trezza A, Tarticchio G, Pellicani R, Andreuzzi E, Caldi E, Toti P and Tosi GM: Dissecting the CD93-Multimerin 2 interaction involved in cell adhesion and migration of the activated endothelium. Matrix Biol 64: 112-127, 2017.
51. Opavsky R, Haviernik P, Jurkovicova D, Garin MT, Copeland NG, Gilbert DJ, Jenkins NA, Bies J, Garfield S and Pastorekova S: Molecular characterization of the mouse Tem1/endosialin gene regulated by cell density in vitro and expressed in normal tissues in vivo. Biol Chem 276: 38795-38807, 2001.
52. Rupp C, Dolznig H, Puri C, Sommergruber W, Kerjaschki D, Rettig WJ and Garin-Chesa P: Mouse endosialin, a C-type lectin-like cell surface receptor: Expression during embryonic development and induction in experimental cancer neoangiogenesis. Cancer Immun 6: 10, 2006.
53. MacFadyen J, Savage K, Wienke D and Isacke CM: Endosialin is expressed on stromal fibroblasts and CNS pericytes in mouse embryos and is downregulated during development. Gene Expr Patterns 7: 363-369, 2007.
54. Lax S, Hou TZ, Jenkinson E, Salmon M, MacFadyen JR, Isacke CM, Anderson G, Cunningham AF and Buckley CD: CD248/Endosialin is dynamically expressed on a subset of stromal cells during lymphoid tissue development, splenic remodeling and repair. FEBS Lett 581: 3550-3556, 2007.
55. Croft AP, Naylor AJ, Marshall JL, Hardie DL, Zimmermann B, Turner J, Desanti G, Adams H, Yemm AI, Müller-Ladner U, *et al*: Rheumatoid synovial fibroblasts differentiate into distinct subsets in the presence of cytokines and cartilage. Arthritis Res Ther 18: 270, 2016.
56. Rahimi RA and Leof EB: TGF-beta signaling: A tale of two responses. J Cell Biochem 102: 593-608, 2007.
57. Schiemann WP: Targeted TGF-beta chemotherapies: Friend or foe in treating human malignancies? Exp Rev Anticancer Ther 7: 609-611, 2007.
58. Tian M and Schiemann WP: The TGF-beta paradox in human cancer: An update. Future Oncol 5: 259-271, 2009.
59. Murray LA, Argentieri RL, Farrell FX, Bracht M, Sheng H, Whitaker B, Beck H, Tsui P, Cochlin K, Evanoff HL, *et al*: Hyper-responsiveness of IPF/UIP fibroblasts: Interplay between TGFbeta1, IL-13 and CCL2. Int J Biochem Cell Biol 40: 2174-2182, 2008.
60. Langenkamp E, Zhang L, Lugano R, Huang H, Elhassan TE, Georganaki M, Bazzar W, Lööf J, Trendelenburg G, Essand M, *et al*: Elevated expression of the C-type lectin CD93 in the glioblastoma vasculature regulates cytoskeletal rearrangements that enhance vessel function and reduce host survival. Cancer Res 75: 4504-4516, 2015.
61. Simonavicius N, Ashenden M, van Weverwijk A, Lax S, Huso DL, Buckley CD, Huijbers IJ, Yarwood H and Isacke CM: Pericytes promote selective vessel regression to regulate vascular patterning. Blood 120: 1516-1527, 2012.
62. Rouleau C, Curiel M, Weber W, Smale R, Kurtzberg L, Mascarello J, Berger C, Wallar G, Bagley R, Honma N, *et al*: Endosialin protein expression and therapeutic target potential in human solid tumors: Sarcoma versus carcinoma. Clin Cancer Res 14: 7223-7236, 2008.
63. Brett E, Zielins ER, Chin M, Januszyk M, Blackshear CP, Findlay M, Momeni A, Gurtner GC, Longaker MT and Wan DC: Isolation of CD248-expressing stromal vascular fraction for targeted improvement of wound healing. Wound Repair Regen 25: 414-422, 2017.
64. Naylor AJ, McGettrick HM, Maynard WD, May P, Barone F, Croft AP, Egginton S and Buckley CD: A differential role for CD248 (Endosialin) in PDGF-mediated skeletal muscle angiogenesis. PLoS One 22: e107146, 2014.
65. Facciponte JG, Ugel S, De Sanctis F, Li C, Wang L, Nair G, Sehgal S, Raj A, Matthaiou E, Coukos G and Facciabene A: Tumor endothelial marker 1-specific DNA vaccination targets tumor vasculature. J Clin Invest 124: 1497-1511, 2014.
66. Tlsty TD and Hein PW: Know thy neighbor: Stromal cells can contribute oncogenic signals. Curr Opin Genet Dev 11: 54-59, 2001.
67. Bissell MJ, Kenny PA and Radisky DC: Microenvironmental regulators of tissue structure and function also regulate tumor induction and progression: The role of extracellular matrix and its degrading enzymes. Cold Spring Harb Symp Quant Biol 70: 343-356, 2005.

68. Bhowmick NA, Neilson EG and Moses HL: Stromal fibroblasts in cancer initiation and progression. Nature 432: 332-337, 2004.
69. Yang F, Tuxhorn JA, Ressler SJ, McAlhany SJ, Dang TD and Rowley DR: Stromal expression of connective tissue growth factor promotes angiogenesis and prostate cancer tumorigenesis. Cancer Res 65: 8887-8895, 2005.
70. Kucerova L, Zmajkovic J, Toro L, Skolekova S, Demkova L and Matuskova M: Tumor-driven molecular changes in human mesenchymal stromal cells. Cancer Microenviron 8: 1-14, 2015.
71. Nagy JA, Chang SH, Dvorak AM and Dvorak HF: Why are tumour blood vessels abnormal and why is it important to know? Br J Cancer 100: 865-869, 2009.
72. Kiyohara E, Donovan N, Takeshima L, Huang S, Wilmott JS, Scolyer RA, Jones P, Somers EB, O'Shannessy DJ and Hoon DS: Endosialin expression in metastatic melanoma tumor microenvironment vasculature: Potential therapeutic implications. Cancer Microenviron 8: 111-118, 2015.
73. Ohradanova A, Gradin K, Barathova M, Zatovicova M, Holotnakova T, Kopacek J, Parkkila S, Poellinger L, Pastorekova S and Pastorek J: Hypoxia upregulates expression of human endosialin gene via hypoxia-inducible factor 2. Br J Cancer 99: 1348-1356, 2008.
74. Jain RK: Molecular regulation of vessel maturation. Nat Med 9: 685-693, 2003.
75. Tian Y, Deng H, Han L, Hu S and Qi X: Hypoxia-inducible factor may induce the development of liver fibrosis in budd-chiari syndrome by regulating CD248/endosialin Expression: A Hypothesis. J Transl Int Med 6: 66-69, 2018.
76. Lin SL, Chang FC, Schrimpf C, Chen YT, Wu CF, Wu VC, Chiang WC, Kuhnert F, Kuo CJ, Chen YM, *et al*: Targeting endothelium-pericyte cross talk by inhibiting VEGF receptor signaling attenuates kidney microvascular rarefaction and fibrosis. Am J Pathol 178: 911-923, 2011.
77. Zanivan S, Maione F, Hein MY, Hernandez-Fernaud JR, Ostasiewicz P, Giraudo E and Mann M: SILAC-based proteomics of human primary endothelial cell morphogenesis unveils tumor angiogenic markers. Mol Cell Proteomics 12: 3599-3611, 2013.
78. Wynn TA: Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 4: 583-594, 2004.
79. Fraticelli P, Gabrielli B, Pomponio G, Valentini G, Bosello S, Riboldi P, Gerosa M, Faggioli P, Giacomelli R, Del Papa N, *et al*: Imatinib in Scleroderma Italian Study Group. Low-dose oral imatinib in the treatment of systemic sclerosis interstitial lung disease unresponsive to cyclophosphamide: A phase II pilot study. Arthritis Res Ther 16: R144, 2014.
80. Rosenbloom J, Macarak E, Piera-Velazquez S and Jimenez SA: Human fibrotic diseases: Current challenges in fibrosis research. Methods Mol Biol 1627: 1-23, 2017.
81. Giacomelli R, Afeltra A, Alunno A, Bartoloni-Bocci E, Berardicurti O, Bombardieri M, Bortoluzzi A, Caporali R, Caso F, Cervera R, *et al*: Guidelines for biomarkers in autoimmune rheumatic diseases-evidence based analysis. Autoimmun Rev 18: 93-106, 2019.
82. Krieg T, Abraham D and Lafyatis R: Fibrosis in connective tissue disease: The role of the myofibroblast and fibroblast-epithelial cell interactions. Arthritis Res Ther 9 (Suppl 2): S4, 2007.
83. Cipriani P, Di Benedetto P, Ruscitti P, Liakouli V, Berardicurti O, Carubbi F, Ciccia F, Guggino G, Zazzeroni F, Alesse E, *et al*: Perivascular cells in diffuse cutaneous systemic sclerosis overexpress activated ADAM12 and are involved in myofibroblast transdifferentiation and development of fibrosis. J Rheumatol 43: 1340-1349, 2016.
84. Cipriani P, Di Benedetto P, Ruscitti P, Verzella D, Fischietti M, Zazzeroni F, Liakouli V, Carubbi F, Berardicurti O, Alesse E and Giacomelli R: Macitentan inhibits the transforming growth factor-β profibrotic action, blocking the signaling mediated by the ETR/TβRI complex in systemic sclerosis dermal fibroblasts. Arthritis Res Ther 17: 247, 2015.
85. Sato M, Suzuki S and Senoo H: Hepatic stellate cells: Unique characteristics in cell biology and phenotype. Cell Struct Funct 28: 105-112, 2003.
86. Lin SL, Kisseleva T, Brenner DA and Duffield JS: Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. Am J Pathol 173: 1617-1627, 2008.
87. Cipriani P, Di Benedetto P, Ruscitti P, Campese AF, Liakouli V, Carubbi F, Pantano I, Berardicurt O, Screpanti I and Giacomelli R: Impaired endothelium-mesenchymal stem cells cross-talk in systemic sclerosis: A link between vascular and fibrotic features. Arthritis Res Ther 16: 442, 2014.
88. Cipriani P, Marrelli A, Di Benedetto P, Liakouli V, Carubbi F, Ruscitti P, Alvaro S, Pantano I, Campese AF, Grazioli P, *et al*: Scleroderma mesenchymal stem cells display a different phenotype from healthy controls; implications for regenerative medicine. Angiogenesis 16: 595-607, 2013.
89. Dulauroy S, Di Carlo SE, Langa F, Eberl G and Peduto L: Lineage tracing and genetic ablation of ADAM12(+) perivascular cells identify a major source of profibrotic cells during acute tissue injury. Nat Med 18: 1262-1270, 2012.
90. Chang-Panesso M and Humphreys BD: CD248/Endosialin: A novel pericyte target in renal fibrosis. Nephron 131: 262-264, 2015.
91. Chen YT, Chang FC, Wu CF, Chou YH, Hsu HL, Chiang WC, Shen J, Chen YM, Wu KD, Tsai TJ, *et al*: Platelet-derived growth factor receptor signalling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. Kidney Int 80: 1170-1181, 2011.
92. Cipriani P, Di Benedetto P, Dietrich H, Ruscitti P, Liakouli V, Carubbi F, Pantano I, Berardicurti O, Sgonc R and Giacomelli R: Searching for a good model for systemic sclerosis: The molecular profile and vascular changes occurring in UCD-200 chickens strongly resemble the early phase of human systemic sclerosis. Arch Med Sci 12: 828-843, 2016.
93. Boor P, Ostendorf T and Floege J: PDGF and the progression of renal disease. Nephrol Dial Transplant 29 (Suppl 1): i45-i54, 2014.
94. Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmoulière A, Varga J, De Wever O, Mareel M and Gabbiani G: Recent developments in myofibroblast biology: Paradigms for connective tissue remodeling. Am J Pathol 180: 1340-1355, 2012.
95. De Wever O, Demetter P, Mareel M and Bracke M: Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer 123: 2229-2238, 2008.
96. Cirri P and Chiarugi P: Cancer-associated-fibroblasts and tumour cells: A diabolic liaison driving cancer progression. Cancer Metastasis Rev 31: 195-208, 2012.
97. Kalluri R and Zeisberg M: Fibroblasts in cancer. Nat Rev Cancer 6: 392-401, 2006.
98. Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2: 442-454, 2002.
99. Cruz-Solbes AS and Youker K: Epithelial to mesenchymal transition (EMT) and endothelial to mesenchymal transition (EndMT): Role and implications in kidney fibrosis. Results Probl Cell Differ 60: 345-372, 2017.
100. Chapman HA: Epithelial-mesenchymal interactions in pulmonary fibrosis. Annu Rev Physiol 73: 413-435, 2011.
101. Piera-Velazquez S, Li Z and Jimenez SA: Role of endothelial-mesenchymal transition (EndoMT) in the pathogenesis of fibrotic disorders. Am J Pathol 179: 1074-1080, 2011.
102. Coen M, Gabbiani G and Bochaton-Piallat ML: Myofibroblast-mediated adventitial remodeling: An underestimated player in arterial pathology. Arterioscler Thromb Vasc Biol 31: 2391-2396, 2011.
103. Lim H and Moon A: Inflammatory fibroblasts in cancer. Arch Pharm Res 39: 1021-1031, 2016.
104. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL and Weinberg RA: Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 121: 335-348, 2005.
105. Padua D, Zhang XH, Wang Q, Nadal C, Gerald WL, Gomis RR and Massagué J: TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. Cell 133: 66-77, 2008.
106. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C and Brown RA: Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3: 349-363, 2002.
107. Hinz B: The myofibroblast: Paradigm for a mechanically active cell. J Biomech 43: 146-155, 2010.
108. Otranto M, Sarrazy V, Bonté F, Hinz B, Gabbiani G and Desmoulière A: The role of the myofibroblast in tumor stroma remodeling. Cell Adh Migr 6: 203-219, 2012.
109. Liao Z, Tan ZW, Zhu P and Tan NS: Cancer-associated fibroblasts in tumor microenvironment-Accomplices in tumor malignancy. Cell Immunol, 2018 (Epub ahead of print).
110. Ireland LV and Mielgo A: Macrophages and fibroblasts, key players in cancer chemoresistance. Front Cell Dev Biol 6: 131, 2018.

111. Farmer P, Bonnefoi H, Anderle P, Cameron D, Wirapati P, Becette V, André S, Piccart M, Campone M, Brain E, *et al*: A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer. Nat Med 15: 68-74, 2009.
112. Pandol S, Edderkaoui M, Gukovsky I, Lugea A and Gukovskaya A: Desmoplasia of pancreatic ductal adenocarcinoma. Clin Gastroenterol Hepatol 7 (11 Suppl): S44-S47, 2009.
113. DuFort CC, Delgiorno KE and Hingorani SR: Mounting pressure in the microenvironment: Fluids, solids, and cells in pancreatic ductal adenocarcinoma. Gastroenterology 150: 1545-1557.e2, 2016.
114. Christian S, Winkler R, Helfrich I, Boos AM, Besemfelder E, Schadendorf D and Augustin HG: Endosialin (Tem1) is a marker of tumor-associated myofibroblasts and tumor vessel-associated mural cells. Am J Pathol 172: 486-494, 2008.
115. Fujii S, Fujihara A, Natori K, Abe A, Kuboki Y, Higuchi Y, Aizawa M, Kuwata T, Kinoshita T, Yasui W and Ochiai A: TEM1 expression in cancer-associated fibroblasts is correlated with a poor prognosis in patients with gastric cancer. Cancer Med 4: 1667-1678, 2015.
116. Diaz LA Jr, Coughlin CM, Weil SC, Fishel J, Gounder MM, Lawrence S, Azad N, O'Shannessy DJ, Grasso L, Wustner J, *et al*: A first-in-human phase I study of MORAb-004, a monoclonal antibody to endosialin in patients with advanced solid tumors. Clin Cancer Res 21: 1281-1288, 2015.
117. Norris RE, Fox E, Reid JM, Ralya A, Liu XW, Minard C and Weigel BJ: Phase 1 trial of ontuxizumab (MORAb-004) in children with relapsed or refractory solid tumors: A report from the Children's Oncology Group Phase 1 Pilot Consortium (ADVL1213). Pediatr Blood Cancer 65: e26944, 2018.
118. D'Angelo SP, Hamid OA, Tarhini A, Schadendorf D, Chmielowski B, Collichio FA, Pavlick AC, Lewis KD, Weil SC, Heyburn J, *et al*: A phase 2 study of ontuxizumab, a monoclonal antibody targeting endosialin, in metastatic melanoma. Invest New Drugs 36: 103-113, 2018.
119. Grothey A, Strosberg JR, Renfro LA, Hurwitz HI, Marshall JL, Safran H, Guarino MJ, Kim GP, Hecht JR, Weil SC, *et al*: A randomized, double-blind, placebo-controlled phase II study of the efficacy and safety of monotherapy ontuxizumab (MORAb-004) plus best supportive care in patients with chemo-refractory metastatic colorectal cancer. Clin Cancer Res 24: 316-325, 2018.
120. Rouleau C, Gianolio DA, Smale R, Roth SD, Krumbholz R, Harper J, Munroe KJ, Green TL, Horten BC, Schmid SM and Teicher BA: Anti-endosialin antibody-drug conjugate: Potential in sarcoma and other malignancies. Mol Cancer Ther 14: 2081-2089, 2015.
121. Lee S: What tumor vessels can tell us. Pigment Cell Melanoma Res 23: 309-311, 2010.