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The role of CD26/dipeptidyl peptidase IV in cancer

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1. ABSTRACT

CD26/DPPIV is a multifunctional cell surface protein that is widely expressed in most cell types including T lymphocytes, on which it is a marker of activation. It is also present in serum and other body fluids in a truncated form (sCD26/DPPIV). It preferentially cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position, and in doing so, regulates the activities of a number of cytokines and chemokines. Due in part to this ability to regulate the activity of biopeptides, it can act as a tumor suppressor or activator. It can associate with several proteins, among them fibroblast activating protein-alpha (FAP-alpha), plasminogen, adenosine deaminase (ADA), the tyrosine phosphatase CD45, and the chemokine receptor CXCR4. It can also bind to the extracellular matrix (ECM) and depending on the presence of other ligands, this process can either lead to increased or decreased invasive activity of the cells on which it is expressed. As a result of these characteristics, CD26/DPPIV plays an important role in tumor biology, and is useful as a marker for various cancers, with its levels either on the cell surface or in the serum being increased in some neoplasms and decreased in others. Our group has shown that CD26/DPPIV can be manipulated by such agents as CD26 cDNA-carrying plasmids, siRNA and monoclonal antibodies, resulting in both *in vitro* and *in vivo* inhibition of cell growth, enhanced sensitivity to selected chemotherapeutic agents, and enhanced survival of mouse xenograft models. These studies have demonstrated the utility of these tools as potential targeted therapies for specific cancers expressing CD26/DPPIV.

2. INTRODUCTION

CD26 (DPPIV) is a multifunctional membrane-bound glycoprotein present on the surface of most cell types. It is a type II cell surface protein, as the bulk of the protein including the carboxy terminus faces the extracellular space. As a dipeptidyl peptidase, it preferentially cleaves N-terminal dipeptides from polypeptides with proline or alanine in the penultimate position. This activity places it in the prolyl peptidase family and is responsible for its best known functions—chemokine regulation and glucose homeostasis (1). Its enzymatic activity, which is responsible for many but not all of its activities, resides in the carboxy terminal extracellular domain and is involved in the activation/inactivation of a number of chemokines and cytokines. CD26/DPPIV has 3 domains: an extracellular domain, a transmembrane region, and a short cytoplasmic tail of 6 amino acids. Enzymatically active CD26/DPPIV is a homodimer, each subunit containing an alpha/beta hydrolase domain and a beta-propeller domain. A large cavity formed by the alpha/beta hydrolase and the eight-bladed beta-propeller domain acts as the substrate binding site (2). CD26/DPPIV was initially considered to cleave only after a proline or alanine residue, but its substrates now include hydroxyproline, serine, glycine, valine, threonine, and leucine. The discrimination between proline and alanine in the penultimate position is much greater for the dipeptide chromogenic and fluorogenic substrates than for its natural substrates (3). In addition to its exopeptidase activity, there is some evidence that it also has endopeptidase activity, which could play a role in extracellular matrix degradation and hence invasion (4). However, it is possible that this activity is mediated through CD26/DPPIV association with FAP-alpha (5).

A soluble form of the protein (sCD26/DPPIV) is present in the serum and other body fluids, presumably as a result of shedding or secretion from different cell types. The soluble form lacks the transmembrane region and cytoplasmic residues, as it begins at amino acid 39 (6). This form is also a dimer and has been detected in seminal fluid as a larger oligomer (>900 kDa) (7).

3. PROTEINS ASSOCIATED WITH CD26/DPPIV

CD26/DPPIV also exhibits biological functions that are unrelated to its dipeptidase activity. With its beta-propeller domain containing binding sites for several proteins, in addition to forming heterodimers with FAP-alpha, CD26/DPPIV has also been reported to associate with plasminogen 2, ADA, CD45, CXCR4, and mannose 6-phosphate/insulin-like growth factor II receptor (mannose-6-P/IGFIIR). Furthermore, it can bind to the ECM proteins fibronectin and collagen.

3.1. FAP-alpha (Seprase)

FAP-alpha cDNA also codes for a type II integral membrane protein with a large extracellular domain, a transmembrane segment, and a short cytoplasmic tail with 48% amino acid sequence identity with CD26/DPPIV. Although the active form of CD26/DPPIV is a homodimer, expression of both proteins in COS-1 cells resulted in the formation of heterodimeric complexes (8). The genes for both proteins are located in the 2q23 region, suggesting that they may have arisen by gene duplication. However, their expression patterns are different, with CD26/DPPIV being constitutively expressed in most tissues (except in the case of T and B cells, where its expression is regulated by activation), and FAP-alpha expression being more restricted. FAP-alpha has been found to be localized at the invasion front during invasion into the extracellular matrix by human melanoma (9), breast carcinoma (10), and endothelial cells (5). Formation of the CD26/DPPIV-FAP-alpha complex at invadopodia of migrating fibroblasts was required for cell invasion on a collagenous matrix. In addition, antibodies to the gelatin-binding domain of CD26/DPPIV reduced cell migration and degradation of collagen (11). Interestingly, CD26/DPPIV transfectants have been shown to induce FAP-alpha (12, 13).

3.2. Plasminogen 2

Plasminogen 2 (Pg 2) binding to CD26/DPPIV was first demonstrated in rheumatoid arthritis synovial fibroblasts (14). Pg 2 bound to CD26/DPPIV residues 313-319 (15) and was dependent on the sialic acid content of the plasminogen isoform. Pg 2-gamma, Pg 2-delta, and Pg 2-epsilon bound to CD26/DPPIV, whereas Pg 2-alpha and Pg2-beta did not bind. However, in the prostate tumor cell line 1-LN, only Pg 2-epsilon induced expression and secretion of metalloproteinase 9 (MMP-9) (16). Hence, the ability of CD26/DPPIV to associate with plasminogen may be a factor in the invasiveness of certain cancers.

3.3. ADA

ADA catalyzes the deamination of adenosine and deoxyadenosine to inosine and 2'-deoxyinosine, respectively. ADA is located in both the cytosol and on the surface of lymphocytes, where it is associated with CD26/DPPIV (17) and dependent on CD26/DPP IV expression. The function of ADA located on the cell surface is to regulate extracellular adenosine and deoxyadenosine, which are toxic to lymphocytes (18). Adenosine, which accumulates in the extracellular fluid of solid tumors, caused down-regulation of CD26/DPPIV in HT29 colorectal carcinoma cells, which resulted in the depletion of ADA on the cell surface and consequently, a further increase in adenosine. Since the presence of excess adenosine suppresses the immune system, this process may facilitate tumor survival (19). Meanwhile, a different response was reported in endothelial cells. In this case, although hypoxia led to an increase in adenosine level, ADA and CD26/DPPIV mRNA and protein expression were likewise upregulated. The differential effect of adenosine on ADA and CD26/DPPIV in these distinct experimental conditions may be partially due to differences in adenosine levels and also tissue types (20).

The crystal structure of the ADA-CD26/DPPIV complex has revealed binding of one ADA molecule to each beta-propeller domain of CD26/DPPIV (21). The crystal structure also indicates that tetramerization of CD26/DPPIV is a key mechanism for the regulation of its interaction with other components (22). It has been suggested that binding of ADA could regulate

CD26/DPPIV-mediated adhesion between cells by inhibiting tetramer formation between dimers. This is consistent with an earlier study which showed that addition of exogenous ADA inhibited adhesion between lymphocytes and epithelial cells promoted by CD26/DPPIV (23).

3.4. CD45

CD45 is a membrane tyrosine phosphatase that regulates Src-family kinases. It is highly expressed in hematopoietic cells where it chiefly regulates Lck activity. CD45 can regulate Lck activity (important for TCR signaling events) by dephosphorylation of Tyr 505, the negative regulatory site, or down-regulate Lck by dephosphorylation of Tyr 394 in the kinase domain (24). In lymphocytes, CD26/DPPIV and CD45 have been shown to be associated through the binding of CD26/DPPIV to the cytoplasmic domain of CD45 (25, 26). Furthermore, cross-linking of CD26 by a CD26-specific antibody leads to increased phosphorylation of several cell signaling proteins, including $p56^{lck}$, $p59^{fyn}$, ZAP70, and MAP kinase, most likely due to its association with CD45 (27). CD45 has also been implicated in the negative regulation of CD44-mediated cell spreading (28).

3.5. CXCR4

The consequences of the association between the chemokine receptor CXCR4 and CD26/DPPIV are less well understood. Stromal cell-derived factor-1-alpha (SDF-1-alpha)/CXCL12 and its receptor, CXCR4, have recently been shown to play a critical role in tumorigenesis in addition to their well-recognized role in mediating migration and activation of leukocytes during immune and inflammatory responses (29). CXCR4 is upregulated in hypoxic tissues (30) and is the main receptor for SDF-1-alpha, a chemokine which attracts cells expressing CXCR4 and which is cleaved by CD26/DPPIV. Binding of SDF-1-alpha triggers internalization of both CXCR4 and CD26/DPPIV in the T-cell line, Jurkat J32, the B-cell line, SKW6.4, and peripheral blood lymphocytes (31), suggesting that these two proteins may function together.

3.6. Mannose-6-P/IGFIIR

CD26/DPPIV binds to mannose-6-P/IGFIIR via mannose-6-phosphate residues in the carbohydrate moiety of CD26/DPPIV (32). This interaction is critical for CD26/DPPIV-mediated T cell activation and migration (32, 33). Endothelial cells expressing mannose-6-phosphate/IGFIIR on their surface bind to mannose-6-P on sCD26, indicating that sCD26/DPPIV is involved in T-cell migration via its interaction with mannose-6-P/IGFIIR. Moreover, enhanced migration was dependent on DPPIV enzyme activity. Although CD26/DPPIV has no known motif for endocytosis, upon T cell activation, mannose-6-phosphorylation increases, leading to increased binding to the mannose-6-P/IGFIIR and resulting in CD26/DPPIV internalization. Internalization of CD26/DPPIV can also occur following SDF-1-alpha binding to CXCR4, which causes cointernalization of CXCR4 and CD26/DPPIV (31). Of note is that internalization of both CXCR4 and CD26/DPPIV require phosphorylation; for CD26/DPPIV phosphorylation of sugar residues (32), and for CXCR4, phosphorylation of serine residues (34).

3.7. Collagen and Fibronectin

Both collagen and fibronectin are components of the extracellular matrix. The interaction between collagen and CD26/DPPIV is mediated by residues in the cysteine-rich region of CD26/DPPIV and not the catalytic domain (35). CD26/DPPIV also binds to fibronectin (36-38). A detailed study of the binding of CD26/DPPIV to fibronectin was carried out using fibronectin fragments, which found this process to be mediated by the consensus motif T (I/L)TGLX (P/R)G (T/V)X (37). CD26/DPPIV binding to fibronectin is important for adhesion to specific cell types. It is thought that cancer cells initially arrest in the microvasculature of the first organ they encounter with only a few cells forming metastases at a particular secondary site. Breast carcinomas most frequently metastasized to the lungs, for example, and were shown to bind to lung endothelia expressing CD26/DPPIV mediated by the fibronectin assembled on their surface (36).

4. DIFFERENCES BETWEEN CD26/DPPIV AND OTHER FAMILY MEMBERS

4.1. FAP-alpha (Seprase)

FAP-alpha was first identified in the malignant melanoma cell line LOX on the basis of its presence in invasive cell lines. It was also found in membrane vesicles obtained from conditioned media from this cell line. However, it was not detected in a control melanoma cell line or in 32 other tumor cell lines that were unable to degrade extracellular gelatin (39). Similar to CD26/DPPIV, FAP-alpha is a type II membrane protein and a member of the prolyl peptidase family, but unlike CD26/DPPIV, it is inhibited by cysteine protease inhibitors, such as N-ethylmaleimide (39). Also, dipeptides that are CD26/DPPIV substrates are cleaved with a substantially lower catalytic efficiency (100-fold) by FAP-alpha, due to the presence of different amino acids in its active site. In contrast to CD26/DPPIV, FAP-alpha has both gelatinase and collagenase activities (40). In addition, it is generally not expressed in normal tissue, but is present in epithelial cancers and reactive stromal fibroblasts of bladder, breast, colorectal, lung and ovarian carcinomas (41).

Although FAP-alpha is not expressed in normal adult tissues, it can be induced in fibroblasts in response to wounding and also in the reactive stroma of epithelial cancers and some sarcomas (41). Its expression in some malignant cells of epithelial origin, e.g. invasive ductal carcinoma cells from breast cancers, gastric carcinoma, and melanoma, has been demonstrated in several recent studies (10, 39, 42).

Human embryonic kidney cells (HEK293) transfected with murine FAP-alpha and injected into mice formed tumors that grew more rapidly than tumors from control vector-transfectants. Rapid growth depended on protease activity, and was negated by antibodies that inhibited dipeptide cleavage (43). Human breast cancer

cells transfected with FAP-alpha cDNA also formed tumors that grew more rapidly than tumors from transfectants not expressing FAP-alpha (44). In addition, the fast growing tumors exhibited a higher microvessel density. However, there was no difference in *in vitro* proliferation between parental and FAP-alpha-expressing cells. Data from this particular study would support the conclusion that FAP-alpha drives angiogenesis. Additional support for this idea was provided by another study showing that FAP-alpha mRNA upregulation by endothelial cells was involved in capillary morphogenesis (45). Interestingly, non-small-cell lung carcinoma (NSCLC) cells transfected with either a mutant lacking DPPIV activity (S630A) or wild-type CD26/DPPIV induced expression of FAP-alpha (13).

4.2. DPP8 and DPP9

It is likely that DPP8 and DPP9 contribute to biological functions previously attributed to CD26/DPPIV. Both DPP8 and DPP9 have been expressed in baculovirus and shown to function as dimers with similar kinetic and substrate profiles (46). When transfected into 293T cells, a cell line that does not express FAPα and expresses CD26/DPPIV intracellularly at low levels, both DPP8 and DPP9 localized to the cytoplasm. Furthermore, cell migration and monolayer wound healing were impaired by overexpression of either DPP8 or DPP9 (47). Using selective inhibitors, it has been shown recently that DPPIV activity attributable to DPP8/9 is present in human peripheral blood mononuclear cells (PBMC). Most of the DPP8/9 activity was localized to the cytosol, whereas CD26/DPPIV activity was concentrated in the membrane (48).

4.3. DPP10 (DPL2)

Although this protein shares homology with CD26/DPPIV (32%), the active site serine residue is replaced by glycine, resulting in loss of enzyme activity. In contrast to CD26/DPPIV, it is not widely expressed but is present chiefly in brain and pancreas (49, 50).

Based on experiments with CD26/DPPIV enzyme inhibitors, it has become clear that CD26/DPPIV-related family members are targets of drugs initially thought to be specific for CD26/DPPIV. For example, the dipeptidyl peptidase inhibitor, val-boro-pro triggered tumor regression and rejection in WEHI 164 fibrosarcoma and EL4 and A20/2J lymphoma models. Furthermore, treatment with this inhibitor induced upregulation of cytokine and chemokine expression in the tumor and draining lymph nodes. Antitumor activity and stimulation of cytokine and chemokine production was unchanged in $CD26^{-/-}$ mice (51). Therefore, it is currently thought that this DPPIV inhibitor targets FAP-alpha in the tumor stroma as well as cytoplasmic DPP8 and DPP9, inducing a cytokine-mediated immunological response.

5. CD26/DPPIV EXPRESSION IN HUMAN CANCERS

The exact role CD26/DPPIV plays in various cancers remains to be elucidated, partly due to its variable expression on these tumors. In general, it is strongly expressed on some cancers, while being absent or present at low levels in others. Furthermore, given the plethora of its biological functions, including its ability to associate with several key proteins and its cleavage of a number of soluble factors to regulate their function, it is likely that the CD26/DPPIV effect on tumor biology is at least partly mediated by the effect of these biological functions on specific tumor types.

5.1. Cancers associated with high CD26/DPPIV expression

5.1.1. Mesothelioma

CD26/DPPIV was shown to have the highest activity among cell surface aminopeptidases in human mesothelial cells (52). Since mesothelial cells are in constant contact with bodily fluids such as ascites, the influence of ascites on DPPIV activity was measured. Mesothelial cells cultured in the presence of malignant ascites from ovarian carcinoma patients exhibited an increase in DPPIV activity of up to 200% over the control, whereas no significant increase was observed for benign ascites. Fractionation of the ascites revealed that the activity was present in the fraction containing small peptides (<3 kD) and was responsible for elevating both CD26/DPPIV mRNA and protein expression on the cell surface (52). More recent studies confirm that CD26/DPPIV is expressed at a high level on the surface of malignant mesothelioma cells, but not on cells derived from a benign mesothelioma (53). Malignant mesothelioma is an aggressive cancer involving the mesothelioma cells lining the pleura and is resistant to conventional treatments. Depletion of CD26/DPPIV by siRNA resulted in loss of binding to ECM proteins, fibronectin and collagen. Similarly, our recent work showed that incubation with the murine monoclonal antibody 14D10 or a humanized antibody against CD26/DPPIV caused loss of binding to ECM proteins and upregulation of $p27^{kip1}$. Antibody treatment of mice inoculated with human malignant mesothelioma cells inhibited tumor growth and enhanced survival when either 14D10 or the humanized CD26 antibody was injected, whereas the isotype matched control had no effect. In addition, both CD26/DPPIV-specific antibodies reduced formation of metastases (53).

5.1.2. Renal

CD26 has also been shown to be expressed on renal carcinoma cells (54, 55), including the cell lines Caki-1, Caki-2, ACHN, and VMRC-RCW (56). We recently treated Caki-2 cells, which strongly express CD26/DPPIV, with the murine monoclonal anti-CD26 antibody 14D10. Following treatment, cells arrested in G1/S, accompanied by an induction of $p27^{kip1}$, down-regulation of cyclin-dependent kinase 2, and dephosphorylation of retinoblastoma protein. When mice inoculated with human renal carcinoma cells were injected with the CD26-specific antibody, tumor growth was inhibited and survival was significantly enhanced (56).

5.2 . Cancers associated with variable CD26/DPPIV expression

5.2.1. Colon cancer

CD26/DPPIV level was found to be correlated with a differentiated phenotype in both HT-29 and Caco-2 colon cancer cells (57). Neither protein stability nor glycosylation was affected by the state of differentiation. Instead, expression appeared to be controlled at the transcriptional level, since CD26/DPPIV mRNA level was low in undifferentiated cells, but increased as differentiation progressed. Although CD26/DPPIV is not expressed in the adult colon, it has been shown in some cases to be re-expressed in some colon cancers and cell lines.

CD26/DPPIV was down-regulated by adenosine in HT-29 colorectal carcinoma cells, a process mediated by an increase in tyrosine phosphatase activity leading to decreased tyrosine phosphorylation of MAP kinase ERK (1/2) (58). Another study showed that while CD26/DPPIV expression was lower in the colon carcinoma cell lines tested, FAP-alpha was expressed at higher levels in cancer cells and adjacent stromal cells compared to normal colorectal tissue. In addition, a correlation was found between FAP-alpha expression and lymph node metastasis, with high FAP-alpha expression in colorectal cancer tissue being associated with lymph node metastasis (59, 60).

Conflicting reports have been published regarding the presence of soluble CD26/DPPIV molecules in the serum of patients with colorectal carcinoma. One study indicated that serum levels in healthy donors were significantly higher than those in colorectal carcinoma cancer patients (61). However, in another report, a higher level of soluble CD26/DPPIV was detected in patients diagnosed with colorectal cancer, being highest in those with metastatic disease (62). While the reason for the observed difference is unclear, one possibility may be due to the detection methods used-an ELISA assay in the former, and an assay for enzyme activity in the latter.

Meanwhile, tetraspanins are integral membrane proteins that play a role in organizing multimolecular complexes in the plasma membrane. Several studies have demonstrated a link between the expression of these proteins and metastasis. In a recent study utilizing a colon cancer model consisting of cell lines derived from the primary tumor and two metastases, CD26/DPPIV and the tetraspanin Co-029 (identified by mass spectrometry) were present only on the metastatic cancer cells (63).

5.2.2. Glioma

In a recent study using glioma cell lines, no simple correlation could be demonstrated between CD26/DPPIV expression and the degree of malignancy. The lack of an unambiguous result was due to the contribution of DPPIV-like enzymatic activity contributed by dipeptidyl peptidase IV activity and/or its structural homologs (DASH) (64). In an earlier report by the same group of investigators, a positive correlation had been made between the degree of transformation and DPPIV activity, but at the time the contribution of other DPPIV-like proteins was not fully appreciated (65).

5.2.3. Hematological malignancies

Immunofluorescence analysis revealed expression of CD26/DPPIV on peripheral blood lymphocytes of patients with B chronic lymphocytic leukemia (B-CLL), but not on peripheral B cells from normal donors. CD26/CPPIV could also be induced in normal B cells following treatment with interleukin-4, with RT-PCR analysis indicating that expression was regulated at the level of transcription (66). In contrast, the expression of CD26/DPPIV was decreased in the PBMC of patients with adult T cell leukemia/lymphoma (ATLL) compared with cells from normal donors. Again, expression was regulated at the level of transcription (67). Down-regulation appears to result from methylation of CpG islands in the promoter region (68).

CD26/DPPIV has also been shown to be a marker for aggressive T-large granular lymphocyte lymphoproliferative disorder (T-LGL LPD). Our work indicated that patients with CD26-positive disease were more likely to require therapies for cytopenia and infections associated with the disease than those with CD26-negative T-LGL. Furthermore, CD26-related signaling may be aberrant in T-LGL as compared to T-lymphocytes from normal donors (69). Disease aggressiveness is also correlated with CD26/DPPIV expression in other subsets of T-cell malignancies including T-lymphoblastic lymphoma/T-acute lymphoblastic leukemia (LBL/ALL), as those with CD26-positive T-LBLALL had a worse clinical outcome compared to patients with CD26- negative tumors (70, 71).

Two CD26/DPPIV inhibitors were used to elucidate the function of CD26/DPPIV in clones of the human histiocytic lymphoma cell line U937 expressing different levels of CD26/DPPIV. The presence of these inhibitors suppressed DNA synthesis and cytokine production in high expressors while having no effect in low expressors, and these findings support the notion that CD26/DPPIV plays a role in the proliferation and cytokine production of transformed cells (72). Meanwhile, we showed that treatment with anti-CD26 monoclonal antibody inhibited adhesion of the human CD30+ anaplastic large cell T-cell lymphoma cell line Karpas 299 to fibronectin. Furthermore, depletion of CD26 in Karpas 299 cells by siRNA decreased tumorigenesis and increased survival of SCID mice inoculated with these cells (38). We also showed that treatment with anti-CD26 monoclonal antibody inhibited the growth of T -leukemia cell line Jurkat transfected with CD26 through G1/S cell cycle arrest, associated with concurrent activation of the ERK signaling pathway and increased p21 expression (73). Likewise, we demonstrated that anti-CD26 monoclonal antibody treatment of the CD26-positive T-lymphoma line Karpas 299 resulted in *in vitro* and *in vivo* antitumor activity, with associated enhanced survival of SCID mice inoculated with Karpas 299 cells (74).

On the other hand, CD26/DPPIV was not detectable on tumor samples from most patients with the T-cell lymphoma subtype mycosis fungoides/Sezary syndrome, with low expression in the remaining patients in one published study (75). These findings have been corroborated by other investigators (76, 77). Therefore, the absence of CD26/DPPIV expression can be used in the clinical setting as a marker for the diagnosis of mycosis fungoides/Sezary syndrome, as suggested by our work (75). Meanwhile, a recent study suggested that the skin-homing characteristic of Sezary syndrome tumor cells is mediated by the interaction between the chemokine receptor CXCR4 and its ligand SDF-1-alpha, and is influenced by the fact that Sezary syndrome cells do not express CD26/DPPIV. The presence of exogenously added soluble CD26/DPPIV led to cleavage of SDF-1-alpha, interference with SDF-1alpha-CXCR4 interaction, and decreased SDF-1-alpha-

mediated migration of Sezary syndrome cells. Conversely, inhibition of DPPIV enzyme activity induced SDF-1-alpha-mediated cell migration. Therefore, the CD26/DPPIV-regulated interaction of SDF-1-alpha-CXCR4 could play a key role in the skin-homing ability of Sezary syndrome cells and be responsible for the cutaneous manifestations typically associated with this disease in the clinical setting (78).

Our recent work with B and T-tumor cell lines also demonstrated an association between CD26/DPPIV and the key intracellular protein topoisomerase II alpha, which is a target for such widely used topoisomerase II inhibitors as doxorubicin and etoposide. Overexpression of CD26/DPPIV by the use of stable transfectants in the CD26-negative T-leukemia line Jurkat and B-lymphoma line Jiyoye resulted in increased p38 phosphorylation and enhanced level of topoisomerase II alpha, associated with increased sensitivity to the topoisomerase II inhibitors (79-82). Conversely, depletion of CD26/DPPIV expression through small interfering RNA transfection of the CD26-positive T-cell lymphoma line Karpas 299 resulted in dephosphorylation of p38 and decreased topoisomerase II alpha level, leading to decreased sensitivity to doxorubicin and etoposide (38). Since the topoisomerase II inhibitors doxorubicin and etoposide are chemotherapeutic agents that are widely used in hematologic malignancies, these findings have potentially important implications in the clinical setting. Also importantly, siRNA-mediated down-regulation of CD26 resulted in decreased tumorigenicity of Karpas 299 cells in a SCID mouse xenograft model, implying a direct role for CD26 in tumor growth and development (38). These findings further support a therapeutic approach involving targeted therapy against CD26 for selected human cancers.

Certain hematological malignancies respond well to ADA inhibitors, suggesting that the interaction between CD26/DPPIV and ADA might play a role in tumor progression. Since pentostatin (2'-deoxycoformycin) is a potent inhibitor of ADA and CD26/DPPIV expression is integral to ADA cell-surface expression and function, the effect of pentostatin was tested on human leukemia/lymphoma T cell lines expressing different levels of CD26/DPPIV (83). Since *in vitro* exposure to pentostatin alone was usually insufficient to slow growth and induce apoptosis, adenosine or deoxyadenosine was used in conjunction with pentostatin. These studies unexpectedly showed that the expression of CD26 was inversely correlated with the ability of pentostatin to inhibit tumor cell growth and induce apoptosis. Meanwhile, our work showed that treatment of T-lymphoma patients with pentostatin resulted in a specific reduction in the level of circulating CD26-positive T-lymphocytes, potentially associated with immunosuppression (84).

5.2.4. Hepatocellular carcinoma

Cytochemical analysis showed that all hepatocellular carcinomas displayed an altered distribution of CD26/DPPIV activity, with the appearance of three representative patterns: distorted canaliculi with abnormally high activity; loss of activity in canaliculi, with activity restricted to isolated spots; and pseudoacinar structures of hepatocytes with basolateral and apical activity. For most pathological non-neoplastic liver disease, distribution did not significantly differ from that found in normal liver, and was localized in the bile canalicular plasma membrane (85).

In rat liver tissue, CD26/DPPIV enzyme activity in the plasma membranes of two different hepatomas was only 3% of the activity measured in normal livers. In contrast, CD26/DPPIV activity in the serum of rats with hepatomas was on average 150% of the normal value. Immunofluorescence staining with a CD26/DPPIV antibody revealed loss of surface expression, suggesting that CD26/DPPIV was shed into the serum (86).

5.2.5. Lung cancer

CD26 expression in lung cancer appears to be dependent on the specific histologic subtype. When the expression of CD26/DPPIV was compared at the mRNA and protein levels in non-small-cell lung cancer cell lines and normal bronchial epithelial cells, CD26/DPPIV was detected in normal epithelial cells, but was reduced or not detectable in NSCLC cell lines. Downregulation occurred at the RNA level (13). Lung adenocarcinoma appears to be the exception, as CD26/DPPIV is expressed only in this subtype, suggesting that it might be useful as a marker to distinguish it from other types of lung cancers (87). Interestingly, multiple molecular forms of CD26/DPPIV are observed in normal and cancerous lung tissues. CD26/DPPIV from lung cancer tissue consists of more basic molecular forms than that from normal lung tissue, suggesting that the molecular properties of CD26 in the two types of lung tissues are different (88).

5.2.6. Prostate cancer

Studies evaluating the expression of CD26/DPPIV in prostate cancer tissue have yielded mixed results. In one study, CD26/DPPIV activity was found to be twice as high in prostate cancer tissue compared to benign prostatic hyperplasia tissue, as determined by biochemical and quantitative histochemical methods (89). Measurement of DPPIV activity in secretions and different tissue zones also revealed higher activities in patients with cancer (90). In contrast, a different group has shown that loss of CD26/DPPIV was correlated with an increase in basic fibroblast growth factor (bFGF) in metastatic prostate cancer cells. Re-expression of CD26/DPPIV reversed the expression of bFGF and downstream effectors of the bFGF pathway, MAP kinase (ERK1/2) and urinary plasminogen activator (u-PA) (91). The opposite trend was observed using a different cell type, 1-LN. Plasminogen 2 isoforms (Pg 2-gamma, Pg 2-delta, and Pg 2-epsilon) have been shown to bind to CD26/DPPIV via their sialic acid residues, leading to a $[Ca^{2+}]_i$ response (15). In 1-LN cells, although all three isoforms were capable of binding to CD26/DPPIV, only Pg 2-epsilon induced expression and secretion of MMP-9 which led to increased invasion (16). Incubation with monoclonal antibodies to CD26/DPPIV, MMP-9, or u-PA blocked Pg 2-mediated invasion.

5.2.7. Thyroid cancer

CD26/DPPIV was expressed in nearly all cases of thyroid follicular and papillary carcinoma, whereas a lower

percentage of follicular adenoma cases stained positive for CD26/DPPIV, suggesting its potential usefulness as a marker for distinguishing thyroid cancer from benign tumors (92, 93). In addition, one study suggested that the co-expression of CD26/DPPIV and galectin-3 proteins and mRNAs may help in the diagnosis of differentiated thyroid carcinoma as compared to normal thyroid tissues or benign thyroid lesions (94). In an attempt to detect proteins that might contribute to the aggressive behavior of anaplastic thyroid carcinoma, five cases were selected because both undifferentiated and differentiated areas were present. Decreased levels of CD26/DPPIV were detected in undifferentiated areas compared with the differentiated areas, suggesting that it may play a role in regulating tumor aggressiveness and serve as a marker of disease prognosis (95).

5.2.8. Ovarian cancer

Five ovarian cell lines, HRA, SKOV3, TAOV, NOS4, and NOS2 were compared with respect to their CD26/DPPIV expression and invasive potential. HRA and SKOV3 expressed a low level of CD26/DPPIV, but exhibited the highest invasive potential. In contrast, TAOV, NOS4, and NOS2 expressed a high level of CD26/DPPIV, but exhibited low invasive potential (96). Transfection of the SKOV3 cell line with CD26/DPPIV resulted in increased adhesion, however, migration and invasion were substantially reduced (97). In addition, nude mice inoculated with CD26/DPPIV-transfected SKOV3 cells lived approximately twice as long as those receiving the parental or vector-transfected cells. In a separate study, SKOV3 cells were transfected with CD26/DPPIV cDNA, and binding to various substrates was measured. The CD26/DPPIV transfectants bound both collagen and fibronectin-coated plates to a greater extent than the parental cell line or transfectants expressing vector only. Inhibition of DPPIV activity had no effect on adhesion. For CD26/DPPIV transfectants, adhesion rates to mesothelial cells were twice that of parental cells and cells transfected with empty vector (98). Further studies with CD26/DPPIV transfectants indicated that expression levels of MMP-2 and MT1-MMP were reduced, while tissue inhibitors of matrix metalloproteinases were enhanced (96).

5.3 Cancers associated with low CD26/CPPIV expression

5.3.1. Breast cancer

The role of CD26/DPPIV in breast cancer is an area for future research. CD26/DPPIV expressed on the surface of rat lung capillary endothelia was shown to be a receptor for rat breast cancer cells that display fibronectin on their surface. CD26/DPPIV enzyme activity was not involved in binding. Furthermore, the extent of binding to CD26/DPPIV-expressing lung cells was shown to be proportional to the amount of fibronectin on the breast cancer cells (36). In addition, peptides containing the fibronectin CD26-binding domain blocked the CD26/DPPIV-fibronectin interaction and decreased pulmonary metastasis of the breast cancer cells (37). These findings are consistent with an earlier study on the capacity of CD26/DPPIV to act as an adhesion molecule in which outside-out endothelial cell membrane vesicles were used as a model system. In this study, a monoclonal antibody was generated that prevented adhesion of lung-derived endothelial membrane vesicles to lung-metastatic breast and prostate carcinoma cells. This antibody was found to be specific for CD26/DPPIV (99). In studies with F344 rat substrains, cells expressing lower levels of CD26/DPPIV exhibited lower *in vivo* adhesion and fewer colonies in lung tumors, following i.v. inoculation of rat syngeneic mammary adenocarcinoma cells, MADB106. It is not clear whether CD26/DPPIV in MADB106 cells played a role in adhesion or metastasis since these cells did not express CD26/DPPIV *in vitro* but acquired expression following injection into rats (100).

5.3.2. Endometrial cancer

CD26/DPPIV was found to be expressed on normal endometrial glandular cells, but its expression on endometrial adenocarcinoma was down-regulated with increasing grade of neoplasm. Therefore, downregulation of CD26/DPPIV expression was correlated with neoplastic transformation and tumor progression (101).

5.3.3. Melanoma

While CD26/DPPIV is present at high levels on normal melanocytes, the process of malignant transformation results in the loss of expression, which occurs at the stage when melanocytes become independent of exogenous growth factors (12, 102). Interestingly, re-expression of CD26/DPPIV rescued expression of FAP-alpha (12). Since these two proteins form heterodimers, these findings suggest that expression of either one regulates the localization of the other. When matrigel invasion assays were used in a study involving two melanoma cell lines, LOX and C8161, parental cells and cells transfected with the empty vectors were found to be highly invasive. However, the invasiveness of cells transfected with CD26/DPPIV was reduced by more than 75%. Decreased metastatic potential did not appear to require either the 6 amino acid cytoplasmic tail or DPPIV activity (103). FAP-alpha expression has been shown to correlate with the invasive phenotype of human melanoma and carcinoma cells (104). LOX cells with higher levels of FAP-alpha exhibited a more invasive phenotype than those with lower levels (9).

5.3.4. Oral cancer

CD26/DPPIV activity in serum and expression on peripheral blood T lymphocytes are decreased in patients with oral cancers compared to normal controls (105, 106). To better understand the biochemical mechanism involved in CD26/DPPIV down-regulation, the effect of cytokines produced by the squamous cell carcinoma cell line KB was studied in peripheral blood T cells. The factor present in KB culture media which was responsible for CD26/DPPIV down-regulation in T cells, and decreased activity in serum, was identified as tumor growth factor beta 1 (TGF-beta 1) (107).

6. SUMMARY AND PERSPECTIVE

It is likely that the pleiotropic effects of CD26/DPPIV account for its varied roles in different

cancers. Among its key attributes are its ability to associate with other key molecules and its cleavage of biological factors to regulate their functions. For example, it can bind to plasminogen 2-epsilon, triggering an intracellular [Ca^{2+}] flux which leads to secretion/activation of MMP-9 in 1-LN cells (16). It can also form heterodimers with FAP-alpha, colocalizing at pseudopodia and causing secretion/activation of MMPs in migratory fibroblasts and endothelial cells (5, 11). Both of these activities suggest that CD26/DPPIV is involved in tumor invasiveness. On the other hand, its ability to bind fibronectin and collagen not only predicts a potential role in invasion, but also perhaps an inability to migrate due to tight cell-cell adhesions mediated by CD26/DPPIV, as in the case for melanoma cells (103). Cleavage of cytokines and chemokines by CD26/DPPIV also enables it to act as either a tumor suppressor or activator. For example, SDF-1-alpha is one of the best CD26/DPPIV substrates *in vitro*, but whether it contributes significantly to the metabolism of SDF-1-alpha *in vivo* needs to be further studied. While many bioactive peptides are qualified to be CD26/DPPIV substrates, substrate recognition and cleavage efficiency are probably regulated at least partly by the proteins associated with CD26/DPPIV and the tumor-specific microenvironment, which can modulate substrate accessibility to the enzyme active site. The local concentration of the putative substrate is also important for its interaction with CD26/DPPIV. Therefore, the specific biological functions of CD26/DPPIV are likely to vary depending on its location, tumor cell type, oligomeric state, and the concentration of ligands and cofactors. In addition, multiple isoforms exist for both soluble and membrane-associated CD26/DPPIV, factors which add another layer of complexity to the role of this multifaceted molecule in tumor biology. Meanwhile, its various functions in tumor development would indicate that CD26 may therefore be an appropriate novel target for cancer therapy. Indeed, our studies, as well as work done by others, suggest that targeting CD26/DPPIV with specific agents may be an effective therapeutic approach for selected cancers, which would be logical in view of the key role CD26/DPPIV plays in cancer biology.

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Abbreviations: DPPIV: dipeptidyl peptidase IV; FAP-alpha: fibroblast activating protein-alpha; ADA: adenosine deaminase; ECM: extracellular matrix; siRNA: small interfering RNA; Mannose-6-P/IGFIIR: mannose-6-phosphate/insulin-like growth factor II receptor; Pg 2: plasminogen 2; MMP-9: metalloproteinase 9; SDF-1-alpha: stromal cell-derived factor-1-alpha/CXCL12; HEK293: human embryonic kidney cells 293; NSCLC: non-small-cell lung carcinoma cells; DPP8: dipeptidyl peptidase 8; DPP9: dipeptidyl peptidase 9; DPP10: dipeptidyl peptidase 10; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; bFGF: basic fibroblast growth factor; u-PA: urinary plasminogen activator

Key Words: CD26, dipeptidyl peptidase IV, DPPIV, DPP4, FAP-alpha, cancer, Hematologic Malignancies, Targeted Therapy, Review

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