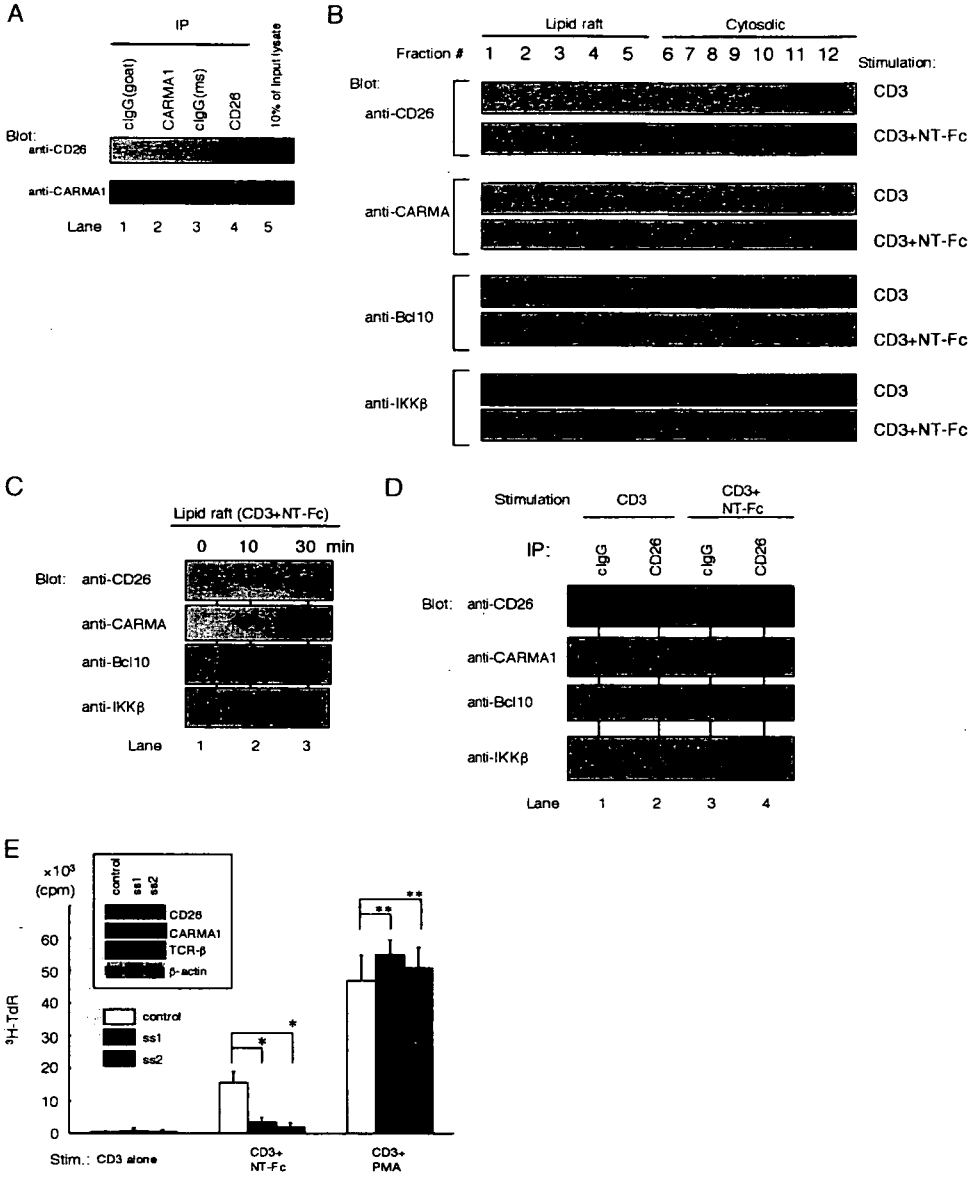

FIGURE 7. **CARMA1 plays an important role in CD26-mediated costimulation by caveolin-1 in human peripheral blood T-cells.** *A*, purified T-cells were lysed, and IP assays were conducted with anti-CARMA1 pAb (*goat*), anti-CD26 mAb (mouse (*ms*)), or control Ig (*cIgG*). IP complexes as well as 10% of input lysates were then separated using SDS-PAGE and immunoblotted with the indicated antibodies. Similar results were obtained in three independent experiments. *B*, purified T-cells were stimulated for 10 min with anti-CD3 alone (0.05 μg/ml) or with anti-CD3 plus NT-Fc (5.0 μg/ml), and lysates were prepared by sucrose gradient centrifugation as described under "Experimental Procedures." The distribution of CD26, CARMA1, Bcl10, and IKKβ was determined by immunoblotting with specific antibodies. Similar results were obtained in three independent experiments. *C*, purified T-cells were stimulated for 0, 10, and 30 min with anti-CD3 plus NT-Fc, and lysates were prepared by sucrose gradient centrifugation as described under "Experimental Procedures." The distribution of CD26, CARMA1, Bcl10, and IKKβ was determined by immunoblotting with specific antibodies. Similar results were obtained in three independent experiments. *D*, purified T-cells were stimulated with anti-CD3 alone (*lanes 1* and *2*) or with anti-CD3 plus NT-Fc (*lanes 3* and *4*), and lipid raft fractions were prepared as described in *A*, and immunoprecipitation of lipid rafts with control IgG (cIgG) (*lanes 1* and *3*) or anti-CD26 mAb (*lanes 2* and *4*) was performed as described under "Experimental Procedures." IPs were resolved in SDS-PAGE and immunoblotted with indicated antibodies. Similar results were obtained in three independent experiments. *E*, purified T-cells were transfected with sense-siRNA (ss1 and ss2) of CARMA1 gene or mismatched siRNA (*control*) using HVJ-E vector. Cell lysates were resolved by SDS-PAGE and immunoblotted with indicated antibodies, followed by stripping and reprobing with anti-β-actin antibody (*inside box*). Purified T-cells treated with siRNA were stimulated and subjected to T-cell proliferation assay as described in Fig. 3*A*. Values shown are means ± S.E. of determinations from triplicate cultures of five independent donors. * shows points of significant decrease ($p < 0.05$), and ** indicates points of no significant change compared with controls.

DISCUSSION

In this study, we showed that caveolin-1 is the costimulatory ligand for CD26, and that ligation of CD26 by caveolin-1 induces T-cell proliferation and NF-κB activation with costimulation of TCR/CD3. Moreover, we showed that the cytoplasmic tail of CD26 in T-cell interacts with CARMA1, resulting in signal transduction leading to NF-κB activation and that ligation of CD26 by caveolin-1 recruits a complex of CD26, CARMA1, Bcl10, and IKKβ to lipid rafts.

Enhancement of CD26 expression in autoimmune diseases may correlate with disease severity (40, 41), because patients with autoimmune diseases such as Grave's disease and rheumatoid arthritis have increased levels of CD26 + T-cells in their peripheral blood as well as inflamed tissues, including thyroid and synovial fluids and membranes (9, 42). These findings imply that CD26 + T-cells play a role in the inflammation process and subsequent tissue destruction. Originally characterized as a T-cell activation antigen, human CD26 is preferentially expressed on the CD4+ memory T-cell subset and is up-regulated after T-cell activation (2, 3, 10). Along with its enhanced expression on activated T-cells, various lines of evidence have converged to demonstrate that CD26 is functionally associated with T-cell signal transduction processes relating to T-cell activation (2, 10, 11, 43). However, the precise mechanism involved in T-cell activation via CD26 in response to memory antigen such as tetanus toxoid remains to be clearly characterized, including the identification of its costimulatory ligand and the associated proximal signaling molecules. Recently, we demonstrated that CD26 binds to caveolin-1 on APC and that residues 201–211 of CD26 along with the serine catalytic site at residue 630, which constitute a pocket structure of CD26/DPPIV, contribute to binding to the caveolin-1 scaffolding domain (14). This region in CD26 contains a caveolin-binding domain ($\Phi X\Phi XXXX\Phi XX\Phi$; Φ and X depict aromatic residue and any amino acid, respectively), specifically WVYEEEVFSAY in CD26 (2, 44). These

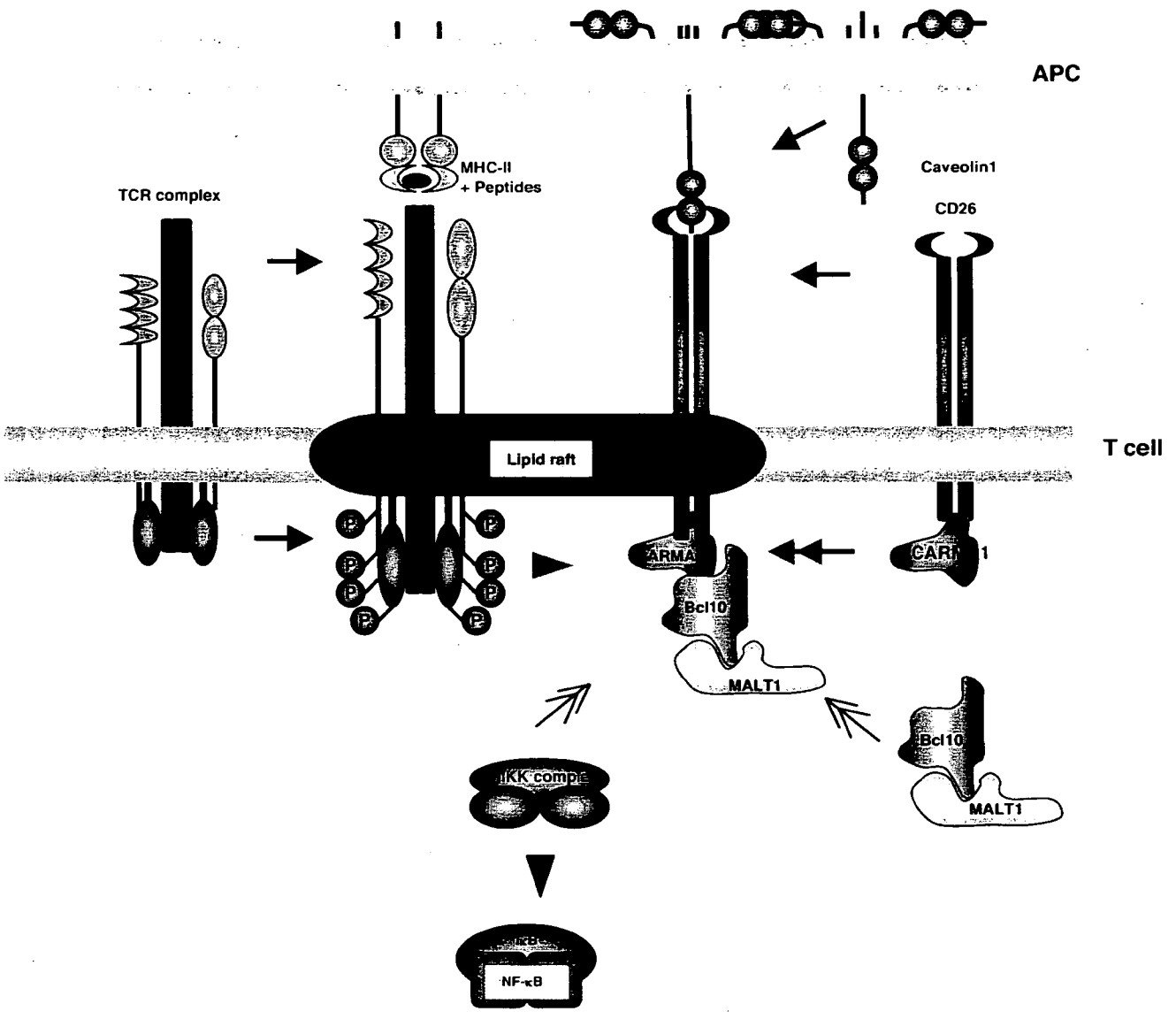

FIGURE 8. **A model for signaling by TCR and CD26 costimulation.** Stimulation of cells through TCR complexes leads to phosphorylation of cytoplasmic immunoreceptor tyrosine-based activation motifs by ligation of peptide-loaded major histocompatibility complex class II (*bold arrows*) and recruitment and activation of phosphatidylinositol 3-kinase and PKCθ (*gray arrowhead*). Meanwhile, caveolin-1, of which the N-terminal extracellular regions are presented on antigen-loaded APC, ligates CD26, which exists as dimers on the cell surface and recruits lipid rafts (*gray arrows*) while interacting with CARMA1 (*gray double-headed arrow*). The recruitment of CARMA1 along with CD26 to lipid rafts also recruits the CARMA1-Bcl10-IKKs complex (*black double-headed arrows*), leading to activation of the IKK complex (*black double-headed arrows*) and finally activation of NF-κB.

observations strongly support the notion that DPPIV enzyme activity is necessary to exert T-cell costimulatory activation via CD26 as demonstrated in our previous report using CD26 specific mAbs (13).

To examine the binding of caveolin-1 to CD26 in T-cells, we used soluble Fc fusion proteins containing the N-terminal domain of caveolin-1 (NT-Fc) (Fig. 1), and we found that NT-Fc binds specifically to CD26 to induce T-cell proliferation in the presence of TCR/CD3 costimulation (Figs. 2 and 3). Moreover, the binding affinity between caveolin-1 and CD26 ($K_d \sim 2 \times 10^{-5}$ M), as determined by the Biacore system (Fig. 2*C*), is comparable with that of other costimulatory molecules with important roles in immune responses and their associated ligands, such as CD2-CD5 ($K_d \sim 10^{-6}$ M), CD80-CD28 ($K_d \sim 10^{-7}$ M), and CD86-CD28 ($K_d \sim 10^{-6}$ M) (45–47). Until now, CD26-mediated T-cell costimulation was performed using anti-CD26 mAbs, resulting in various CD26 functions (4, 7, 48, 49). Assuming that the affinity between antigen and antibody is higher ($K_d \sim 10^{-9}$ M) than that of a ligand-receptor system, and that ligand-specific conformations are capable of differentially activating distinct signaling partners (50), ligand-dependent pathways may be predicted to have different signals associated with the antigen-antibody system and ligand-receptor system.

We have demonstrated previously that ligation of CD26 by the anti-CD26 mAb 1F7 induces T-cell costimulation and IL-2 production by CD26-transfected Jurkat T-cell lines, while increasing tyrosine phosphorylation of signaling molecules such as ZAP70, p56^{lck}, and CD3ζ was observed (2, 7, 12). In addition, we have shown that ligation of the CD26 molecules by the anti-CD26 mAb 1F7 increases the recruitment of CD26 molecules with CD45RO to lipid rafts, resulting in increased tyrosine phosphorylation of signaling molecules (33). However,

the precise proximal signaling pathway of CD26 has not yet been identified, particularly in view of the fact that the cytoplasmic tail of CD26 contains only 6 amino acid residues without a common signaling motif structure. Moreover, it has been unclear whether the short cytoplasmic tail is responsible for signal transduction associated with CD26-mediated costimulation. In this study, using recombinant CD26-CD10 chimeric receptor, we showed that the cytoplasmic tail of CD26 is indeed responsible for T-cell costimulation induced by anti-CD3 plus caveolin-1 (Fig. 4*D*). Furthermore, to explore the proximal signaling molecules interacting with the cytoplasmic tail of dimeric CD26, we used proteomic analyses with Fc fusion proteins containing the cytoplasmic amino acid residues of CD26 (Fig. 5, *A* and *B*) to identify that CARMA1 binds to the cytoplasmic tail of dimeric CD26 (Fig. 5*C*). Moreover, we demonstrated here that a PDZ domain in CARMA1 is necessary for binding to CD26 (Fig. 5*G*). The importance of CARMA1 in CD26-mediated costimulation is also shown by rescue experiments using the CARMA1-deficient Jurkat T-cell line JPM50.6 (Fig. 6*D*) and using siRNA against CARMA1 in APB-T-cells (Fig. 7*E*). CARMA1, containing caspase-recruitment domain and MAGUK domains, plays an essential role in the NF-κB activation and IL-2 expression induced by CD3-CD28 or CD28-PMA stimulation (18, 22). After being phosphorylated, CARMA1 functions as a signaling intermediate downstream of PKCθ and upstream of IKK in the TCR signaling transduction pathway leading to NF-κB activation (39, 51). Because MAGUK domain-containing proteins are generally involved in the organization of multiprotein complexes at the interface of the cytoplasmic membrane (52), it is possible that CARMA1 associates with as yet undefined membrane proteins in the immunological synapse of T-cells. In this regard, our present data suggest a novel mechanism for CAMRA1 function as it complexes with Bcl10 and IKK to transduce CD26-costimulatory signals. Moreover, as shown Fig. 5*C*, cytoskeletal proteins were also observed in the complex in the pulldown assays with CD26 aa1–10-Fc. Because MAGUK domain-containing proteins are generally involved in the organization of multiprotein complexes in the cytoskeleton (52), the downstream signaling of CD26 may also be associated with cytoskeletal assembly via CARMA1. The association of CD26, CARMA1, and the cytoskeleton will be elucidated in future studies.

CD26/DDPIV is reported to exist as homodimers, a structural organization that allows access of substrates to DPPIV catalytic activity (36, 37). Although DPPIV activity is crucial for CD26-mediated T-cell costimulation (13, 30), the exact role played by DPPIV in this process is unclear. Our previous study showed that the enzymatic pocket structure of the DPPIV catalytic site is necessary for binding of CD26 to caveolin-1, leading to the up-regulation of CD86 expression on APC (14, 15). In this study, we found that monomeric CD26 H750E, which has a 300-fold decrease in catalytic activity (36), does not bind to CARMA1 (Fig. 5*E*), resulting in loss of CD26-mediated T-cell costimulation by anti-CD3 plus caveolin-1 (Fig. 4*D*). Therefore, dimerization of CD26 is not only necessary for binding to caveolin-1 but also serves as a scaffolding structure for the cytoplasmic signaling molecule CARMA1. The precise binding position of CARMA1 in the cytoplasmic domain of CD26 remains to be elucidated in future work, because PDZ domains bind primarily to specific C-terminal motifs (X(S/T)X(V/L), where X depicts any amino acid) or internal target motifs as well as other PDZ domains (52).

Based upon this study, we propose the following model to explain the sequence of events leading from CD26-CD3 costimulation to NF-κB activation (Fig. 8). In CD3-CD26 costimulation, TCR engagement by peptide-loaded major histocompatibility complex class II presented on APC activates phosphatidylinositol 3-kinase via phosphorylation of immunoreceptor tyrosine-based activation motifs in TCR, leading the recruitment of PKCθ and IKK complex in lipid rafts (16, 18, 25, 38). Concomitantly, CD26 ligation by caveolin-1 on APC recruits CD26-interacting CARMA1 to lipid rafts, resulting in the formation of a CARMA1-Bcl10-MALT1-IKK complex, and this membrane-associated Bcl10 complex then activates IKK through ubiquitination of the NF-κB essential modulator. This study involving Jurkat T-cell lines and human peripheral T-cells represents a different cellular system than those with murine T-cells, where other investigators previously described a role for CD26 in thymic development of murine T-cells (53, 54). Our objective with this study was to define a costimulatory ligand for CD26 and proximal signaling molecule of CD26 in human T-cells, with a future aim of analyzing the *in vivo* role of CD26-mediated T-cell immunity.

In conclusion, we have now demonstrated that CD26 on the T-cell surface binds to caveolin-1, hence identifying the first endogenously expressed CD26 costimulatory ligand in the immune system. Moreover, the caveolin-1-CD26 interaction results in strong T-cell costimulation as a result of the recruitment of a molecular complex consisting of CARMA1-Bcl10-MALT1-IKK in lipid rafts. Our findings will therefore serve as a foundation for future insights into the regulation of T-cell costimulation via the CD26 molecule.

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CD26/Dipeptidyl Peptidase IV as a Novel Therapeutic Target for Cancer and Immune Disorders

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Abstract: CD26 is a 110 kDa surface glycoprotein with intrinsic dipeptidyl peptidase IV (DPPIV) activity that is expressed on numerous cell types and has a multitude of biological functions. An important aspect of CD26 biology is its peptidase activity and its functional and physical association with molecules with key roles in various cellular pathways and biological programs. CD26 role in immune regulation has been extensively characterized, with recent findings elucidating its linkage with signaling pathways and structures involved in T-lymphocyte activation as well as antigen presenting cell-T-cell interaction. Recent work also suggests that CD26 has a significant role in tumor biology, being both a marker of disease behavior clinically as well as playing an important role in tumor pathogenesis and development. In this paper, we will review emerging data that suggest CD26 may be an appropriate therapeutic target for the treatment of selected neoplasms and immune disorders. Through the use of various experimental approaches and agents to influence CD26/DPPIV expression and activity, such as anti-CD26 antibodies, CD26/DPPIV chemical inhibitors, siRNAs to inhibit CD26 expression, overexpressing CD26 transfectants and soluble CD26 molecules, our group has shown that CD26 interacts with structures with essential cellular functions. Its association with such key molecules as topoisomerase IIα, p38 MAPK, and integrin β1, has important clinical implications, including its potential ability to regulate tumor sensitivity to selected chemotherapies and to influence tumor migration/metastases and tumorigenesis. Importantly, our recent *in vitro* and *in vivo* data support the hypothesis that CD26 may indeed be an appropriate target for therapy for selected cancers and immune disorders.

Key Words: CD26, dipeptidyl peptidase IV (DPPIV, DPP4), adenosine deaminase (ADA), autoimmune, T-cell.

1. INTRODUCTION

CD26 was described in 1966 as an enzyme with intrinsic dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) activity [1]. CD26/DPPIV selectively removes the N-terminal dipeptide from peptides with Pro or Ala in the third amino acid position (NH_2-X-X-↓-Ala/Pro-X...), where X is any amino acid and ↓ indicates the splice site [2]. DPPIV was later found to be the same as CD26, which is a 110 kDa extracellular membrane-bound glycoprotein that is expressed on numerous cell types and has a multitude of biological functions. Morrison *et al.* showed that adenosine deaminase (ADA)-binding protein-2 is identical to CD26 [3]. CD26 is a multifunctional type II transmembrane Ser peptidase that has an extracellular domain with DPPIV enzymatic activity and a short cytoplasmic domain. It interacts with extracellular molecules and is also involved in intracellular signal transduction cascades.

CD26 is important in immunology, autoimmunity, HIV, diabetes, and cancer. Interacting directly with various other cell surface and intracellular molecules, CD26 can regulate receptor specificity *via* its DPPIV activity and the function of various chemokines and cytokines. CD26 is expressed at low density on resting T-cells, but is upregulated with T-cell activation. Therefore, CD26 may have an important functional role in T-cells and overall immune function. CD26 associates with other important immunologic cell surface receptors such as CD45 and ADA. The multifunctional activities of CD26 are dependent on cell type and intracellular or extracellular conditions that influence its role as a proteolytic enzyme, cell surface receptor, co-stimulatory interacting protein and signal transduction mediator; as well as its role in adhesion and apoptosis. CD26/DPPIV has been the subject of recent reviews by Fleischer, 1995 [4], De Meester *et al.*, 1999 [5], Kahne *et al.*, 1999 [6], Hildebrandt *et al.*, 2000 [7], Gorrell *et al.*, 2001 [8], Langner and Ansorge, 2000 [9] and 2002 [10], Boonacker and Van Noorden, 2003 [11], as well as by our group [12-16]. In this review we stress the importance of CD26 in clinical conditions including various cancers and recent laboratory developments that elucidate its potential role as a novel therapeutic target.

2. STRUCTURE

CD26 is in the prolyl oligopeptidase/S9 enzyme family, which is characterized by a Ser-Asp-His catalytic triad in the C-terminal region. In humans and most mammals, the prolyl oligopeptidase gene family includes: prolyl endopeptidase (PEP), acylaminoacyl peptidase (ACPH), CD26/DPPIV, and three proteins highly related to CD26 -- fibroblast activation protein (FAP), DPPX, and DPP10 – as well as proteins with lower homology to CD26 -- DPP8 and DPP9. These proteins have moderate amino acid homology, but highly conserved structural features [17]. This includes higher conservation in

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the C-terminal (an α/β-hydrolase domain) than in the N-terminus (a β-propeller region) [18]. The N-terminus also contains the consensus sequence (DW(V/L)YEEE), and the first two Glu (E) amino acids in this sequence are necessary for enzyme activity [19].

According to the HUGO Gene Nomenclature Committee (www.gene.ucl.ak./nomenclature/), the official gene symbol for CD26 is DPP4 and the official name is dipeptidyl-peptidase 4. The CD26 gene is located on chromosome 2q24.3 [20]. The gene is 70 kb and contains 26 exons, ranging from 45 to 1.4 kb in length [20]. The 5'-flanking region does not contain a TATA box or CAAT box, commonly found in housekeeping genes. CD26 does contain a 300 bp G-C rich region with potential binding sites for NFκB, AP2, or Sp1 [21]. CD26 expression is activated by interferons and retinoic acid in chronic lymphocytic leukemia (CLL) *via* Stat1α and the GAS response element (TTCnnnGAA located at bp -35 to -27) in the CD26 promoter [22]. A hepatocyte nuclear factor 1 binding site at position -150 to -131 of the CD26 gene regulates CD26 expression in human intestinal (Caco-2) and hepatic epithelial (HepG2) cell lines. Mutation of the CD26 promoter by the site directed mutagenesis approach resulted in transcription levels of 5-10% of the activity of the non-mutated controls [23]. The Ser recognition site (G-W-S-Y-G) is split between exons 21 and 22 (Fig. **1**). This differentiates CD26 from DPP8 and DPP9 – the putative ancestral genes for CD26/DPPIV/DPP4 in humans – which contain the Ser recognition site in one exon [18]. The CD26 cDNA contains a 3465 bp open reading frame that encodes a 766 amino acid protein [24]. The CD26 amino acid sequence has 85% amino acid identity with the mouse and rat CD26 genes and 37% amino acid identity with *D. melanogaster* (See Table **1** for other species). Chihara *et al.* recently characterized the *D. melanogaster* gene *omega*, one of five putative DPPIV genes, and described the evolutionary relationships of human DPPIV with other members of the prolyl oligopeptidase/S9 enzyme family [25]. Abbott *et al.* found two CD26 transcripts (4.2 and 2.8 kb), both of which were expressed at high levels in the placenta and kidney and at moderate levels in the lung and liver [20]. However, only the 4.2 kb mRNA was expressed at low levels in skeletal muscle, heart, brain, and pancreas.

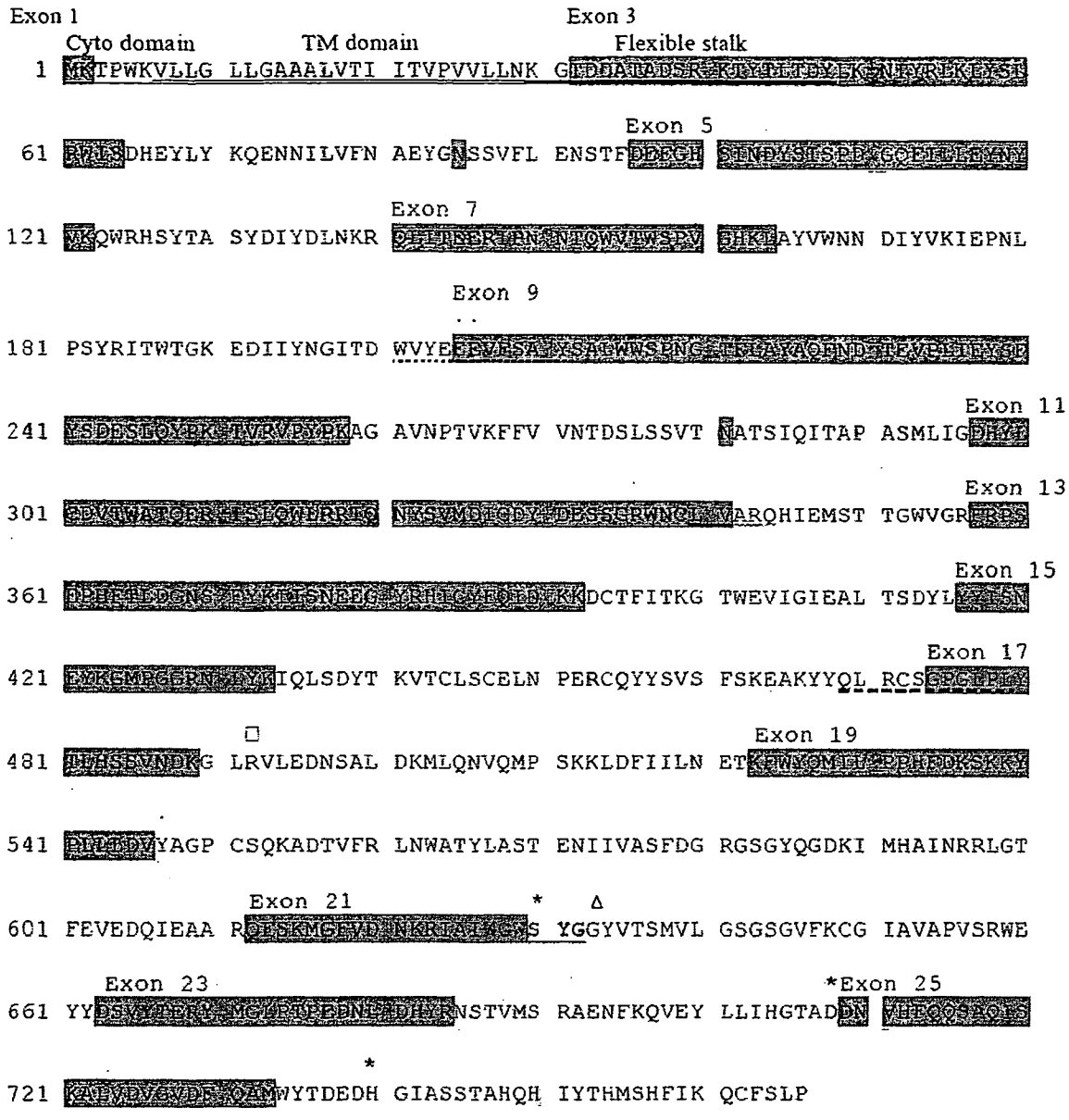
CD26 has a short (6 amino acid) cytoplasmic domain, a transmembrane region, and an extracellular domain with DPPIV activity (Fig. **1** and Table **2**). The three-dimensional crystal structure of CD26 was determined from porcine kidney at a resolution of 1.8 Å [26]. The crystal structure of recombinant human CD26 was determined in the dimeric state at 2.5 Å resolution with the inhibitor Val-pyrrolidide (Fig. **2**) [27]. The last C-terminal 200 amino acids are similar to those of structural homologs prolyl oligopeptidase and proline imidopeptidase, which contain an α/β-hydrolase fold. It is interesting to note that the Pro residues are the only cyclic amino acids and may effect the susceptibility of proximal peptide bonds to cleavage [28, 29]. The catalytic site (Ser630-Asp708-His740) is located in a large cavity (also called a central tunnel), formed between the α/β-hydrolase domain and eight-bladed β-propeller domain, which contains the consensus sequence (DW(V/L)YEEE), that is common to S9b proteases [19, 27]. Single amino acid point mutations in the β-propeller motif identified Glu205 and Glu206 as essential for DPPIV enzyme activity [19]. The central tunnel and α/β-hydrolase domains both participate in inhibitor binding. This is not seen in classic Ser proteases in the chymotrypsin and subtilisin families [30, 31]. The propeller domain excludes large peptides occupying the catalytic pocket by steric hindrance, so there is only oligopeptidase (not polypeptidase) activity. The catalytic site is in the center of the lower face of the β propeller. The amino acids lining the opening to the catalytic site pocket control substrate specificity [32]. Boonacker and Van Noorden extensively review CD26 structure or activity homologs [11]. Substrate-assisted catalysis is one proposed mechanism for CD26/DPPIV substrate specificity [33].

3. EXPRESSION

CD26 is expressed in many tissues. Abbott *et al.* found two CD26 transcripts (4.2 and 2.8 kb), both of which were expressed at high levels in the placenta and kidney and at moderate levels in the lung and liver [20]. The 4.2 kb transcript was expressed at low levels in skeletal muscle, heart, brain, and pancreas [20]. Other organs expressing CD26 include: brain, endothelium [34], heart [20], intestine (colon adenocarcinoma, fetal colon expression disappears at birth) [3], kidney [3, 35], liver [3], lung [20], skeletal muscle [20], pancreas [20], and placenta [20]. In the hematopoietic system CD26 is found on CD4+ T memory cells, T-cells in lymphoblastic lymphoma (LBL), acute lymphoblastic leukemia (ALL), CD30+ anaplastic large cell lymphoma (ALCL), and T-large granular lymphocyte leukemia (T-LGLL). Soluble CD26 is found in serum. Expression of CD26 and CD40 ligand in the hematologic malignancies appears to be mutually exclusive [36, 37].

3.1. CD26 Isoforms

Kahne *et al.* have described various CD26 isoforms, some of which are associated with cell activation [38]. They found at least 5 enzymatically active DPPIV isoforms in activated lymphocytes and 11 immunoreactive DPPIV isoforms with isoelectric points between pH 3.5 and 5.9. Other isoelectric focusing studies have also demonstrated isoforms of CD26 [38-41]. Schmauser *et al.* showed with a series of experiments that purified CD26 consisted of several proteins with isoelectric points from 5.5 to 7.0 [41]. *In vitro* desialylation of CD26 followed by both isoelectric focusing and SDS-PAGE showed that isoelectric points were due to differences in the degree of sialylation [41]. Lectin affinity blotting with Sambucus nigra agglutinin and Maackia amurensis agglutinin revealed that CD26 was predominantly sialylated *via* an alpha 2,6-linkage. DPPIV activity is found only in basic isoforms not in acidic isoforms. A switch to acidic CD26 isoforms has been observed with HIV infection [40]. Neuraminidase treatment of HIV+ subject T-cells cleaves sialic acid residues and reduces the amount of acidic isoforms of CD26/DPPIV [40]. Mavropoulos *et al.* found a change in CD26 isoforms from acidic to a more neutral isoelectric point after anti-tumor necrosis factor-alpha therapy for rheumatoid arthritis [42]. Following cell mitogenic stimulation, one isoform may translocate from the cytoplasm to the cell surface membrane, which suggests isoform specific functions associated with cellular activation [38].



= odd numbered exons
N = glycosylation sites
GWSYG = serine recognition site
* = Ser630, Asp708, His740 active site triad
MKTPWK = amino acids 1-6 - cytoplasmic domain
VLLGLLGAAALVTLITVPVVLLN = amino acids 7-29 - transmembrane domain
KGTDDATADSRKTYTLTDY = amino acids 30-48 – flexible stalk
WVYEEEVFSAY = amino acids 201-211, Ser603 - caveolin-1 binding site
LVAR = amino acids 340-343 - ADA binding site
QLRCSGPGLPL = amino acids 469-479 - fibronectin binding site
· = Glu205,Glu206, Tyr547 – essential for DPPIV activity
□ = non-synonymous cSNP Arg492Lys
H = His750 – required for CD26 dimer formation
Δ = Gly633Arg mutation results in retention and degradation of mutant protein in the endoplasmic reticulum

Fig. (1). CD26/DPPIV amino acid sequence, gene structure, and selected sites. CD26 has a short (6 amino acid) cytoplasmic domain, a transmembrane region, and an extracellular domain with DPPIV activity (see also Table 2).

Table 1. CD26 Gene and Protein Identities Between Human and Other Species

Species	Gene		
H.sapiens	**DPP4**	**aa%ID**	**nt%ID**
vs. P. troglodytes	DPP4	99.6	99.6
vs. C. familiaris	LOC478767	88.7	90.2
vs. M. musculus	Dpp4	85.2	86.6
vs. R. norvegicus	Dpp4	85.2	85.7
vs. G. gallus	RCJMB04_29g21	69.5	71.0
vs. D. melanogaster	CG11034	37.0	46.0
vs. A. gambiae	ENSANGG00000007979	39.8	46.0
vs. S. pombe	SPACUNK4.08	33.0	46.1
vs. S. cerevisiae	DAP2	34.9	45.6
vs. K. lactis	KLLA0E11462g	32.8	44.6
vs. E. gossypii	ACR073C	35.6	44.7
vs. M. grisea	MG07745.4	33.0	43.0
vs. N. crassa	NCU02515.1	33.8	45.9

From HomologGene

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=homologene&dopt=AlignmentScores&list_uids=3279. Accessed 4/10/2006

Slimane *et al.* found that the glycosylation step of sialylation regulates apical targeting and secretion of DPPIV in kidney (MDCK) and intestinal (Caco-2) cell lines [43]. Treatment with a glycosylation inhibitor (GalNac-alpha-O-benzyl) resulted in a lower molecular weight protein with decreased apical secretion and increased basolateral secretion. This suggests a role for glycosylation/sialylation in regulation of DPPIV directional secretion. Various monoclonal CD26 antibodies can at least partially differentiate CD26/DPPIV isoforms. CD26 isoforms also have different patterns of immunostaining and enzymatic staining (Gly-Pro-beta-methoxy-naphthylamide). CD26/DPPIV putative isoforms may also include redundant enzymes that could perform substrate conversion such as attractin, DPPII, DPPIV-β, or DPPVIII [11].

3.2. CD26/DPPIV Activity

Boonacker and Noorden describe at least 5 functions for CD26/DPPIV which include: 1) Ser protease, 2) receptor, 3) co-stimulatory protein, 4) adhesion molecule, and 5) mediator of apoptosis [11]. Knock out mice that do not express CD26/DPPIV are viable, have only minor physiological defects related to glucose tolerance, and do not have impaired growth [44]. Therefore, at least some functions of CD26/DPPIV are redundant and able to be performed by other proteins.

DPPIV is a Ser protease that cleaves dipeptides from the N terminus of peptides (NH_2-X-X-↓-Ala/Pro-X...), where X is any amino acid and ↓ indicates the cleavage site [2]. In the catalytic triad, Ser630-Asp708-His740, the His residue acts as a proton acceptor for the acyl-enzyme intermediate and acts as a proton donor for the subsequent deacylation step [45]. CD26/DPPIV substrates with Pro in the N-terminal position (P_1) usually have large hydrophobic/aromatic side chains, while substrates with Ala at P_1 have Asp, Glu, Gln at the amino acid just C-terminal to the Ala/Pro cleavage site (P_1') using the nomenclature of Schecter and Berger [46]. The next amino acid (P2') position is often a small polar amino acid (Gly, Ser, or Ala) and a variety of amino acids may occupy the P3' position.

CD26 substrates include growth factors, chemokines, neuropeptides and vasoactive peptides. Boonacker and Van Noorden reviewed an extensive list of possible CD26/DPPIV substrates in physiologic processes, inflammation, and neuropsychiatry [11]. Selected CD26 substrates and functions after DPPIV cleavage are summarized in Table 3. Lambeir *et al.* ranked chemokines by k_{cat}/K_m values, with these values giving a measure of the substrate specificity [47]. The ratio k_{cat}/K_m has a unit of a second order rate constant and is expressed as 1/(concentration * time). The higher the k_{cat}/K_m values, the higher the substrate specificity for CD26/DPPIV. Substrates with a k_{cat}/K_m ratio in the 10^5-10^6 $M^{-1} s^{-1}$ range are very good substrates, i.e high affinity and rapid turn-over. The rate of CD26/DPPIV catalysis is inversely related with the amino acid length of substrate [47, 48]. The importance of CD26/DPPIV activity is expanded in the sections below.

Table 2. CD26 Amino Acid Domains Associated With Various CD26 Structures and Functions

Amino Acid	Structure or Function
1-6	Cytoplasmic domain
7-29	Transmembrane domain
30-48	Flexible stalk
29-766	Extracellular domain
39-51, 506-766, 630, 708, 740	α/β-hydrolase domain[27]
55-497	β-propeller domain[27]
201-211	Binds caveolin-1 along with Ser 630[85]
Glu205, Glu206	Essential for DPPIV enzyme activity[19]
Leu340, Val341, Ala342, Arg343	ADA binding site. CD26 deletion, human-rat swap, and point mutation experiments showed that the CD26 amino acids L340, V341, A342, and R343 are essential for ADA binding[50]
469-479	Fibronectin binding site[108]
Tyr547	Required for catalytic activity[216]
Ser630	Catalytic site[31] Binds caveolin-1 with amino acids 201-211[85]
Ser630, Asp708, His740	Catalytic triad[31]
His750	Required for CD26 dimer formation[217]

3.3. CD26 Polymorphisms and Mutations

Several polymorphisms in the DPPIV/DPP4 gene coding region have been deposited in the National Center for Biotechnology Information single nucleotide polymorphism (SNP) database (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=1803). The functional significance of coding region SNPs (cSNPs), non-cSNPs, or other polymorphisms has not, to our knowledge, been determined. Synonymous (non-amino acid changing) polymorphisms include the following (N.D. indicates not determined): Leu8 (T->C, exon 2, frequency: N.D.), Ile405 (C->T, exon 14, frequency: heterozygosity 0.056), Gly645 (C->T, exon 22, frequency: N.D.), Tyr661 (C->T, exon 22, frequency: N.D.). The non-synonymous cSNP Arg492Lys (G->A, exon 18, frequency: N.D.) functional significance has not yet been determined. CD26 (-/-) knockout mice are viable and have improved glucose tolerance, showing increased insulin secretion after a glucose bolus [49]. CD26 experimental mutations include:

- Glu205 and Glu206 mutants (DPPIV negative activity [19])
- Leu294, Leu340, Val341, Ala342, Arg343, Thr440, and Lys441 mutants (ADA binding [50, 51])
- Ser630Ala (DPPIV negative activity)
- Gly633Arg (retention and degradation of mutant protein in the endoplasmic reticulum [52])
- *D. melanogaster* DPPIV (-/-) mutant (*ome*^{1}) (changes in fly size, development time, fertility of males [25])

4. IMMUNE REGULATION

4.1. CD26 and T-cell Function/Regulation

The exact function of CD26/DPPIV activity in T-cell biology is not completely understood. CD26 is preferentially expressed on the CD4+ T memory cell subset of T-cells (CD45RO+/CD29-) and is upregulated after T-cell activation [53]. CD26 is a co-stimulatory molecule for T-cell signal transduction [39, 54-56]. However, DPPIV activity is not necessary for CD26-mediatied T-cell stimulation in certain experimental systems [57, 58]. CD26/DPPIV processing of chemokines may be an important immune regulator *via* reducing attraction of leukocytes in the T helper (Th)-1 response. Th cells stimulate antibody synthesis in B-cells and activate MHC-restricted cytotoxic T-cells [53, 54]. The T-cell activated increase in CD26 surface expression is three to six-fold higher in Th1 cells than Th2 cells; however, Th1 and Th2 show similar DPPIV activity due to posttranslational alterations in CD26 that effect the K_m and V_{max} values for DPPIV [59]. The signal transduction activity of CD26/DPPIV is directly proportional to cell surface CD26 expression level. CD26 has variations in expression that may be important in regulating T-cell development and the immune response more generally. The co-stimulatory function of CD26 remains incompletely understood, because DPPIV activity seems to play an important but not essential role in the co-stimulatory function of CD26/DPPIV in immune regulation [60]. In particular, DPPIV substrate cleaving action has a central set of effects, which may be related to CD26 signaling or independent of signaling. For instance,

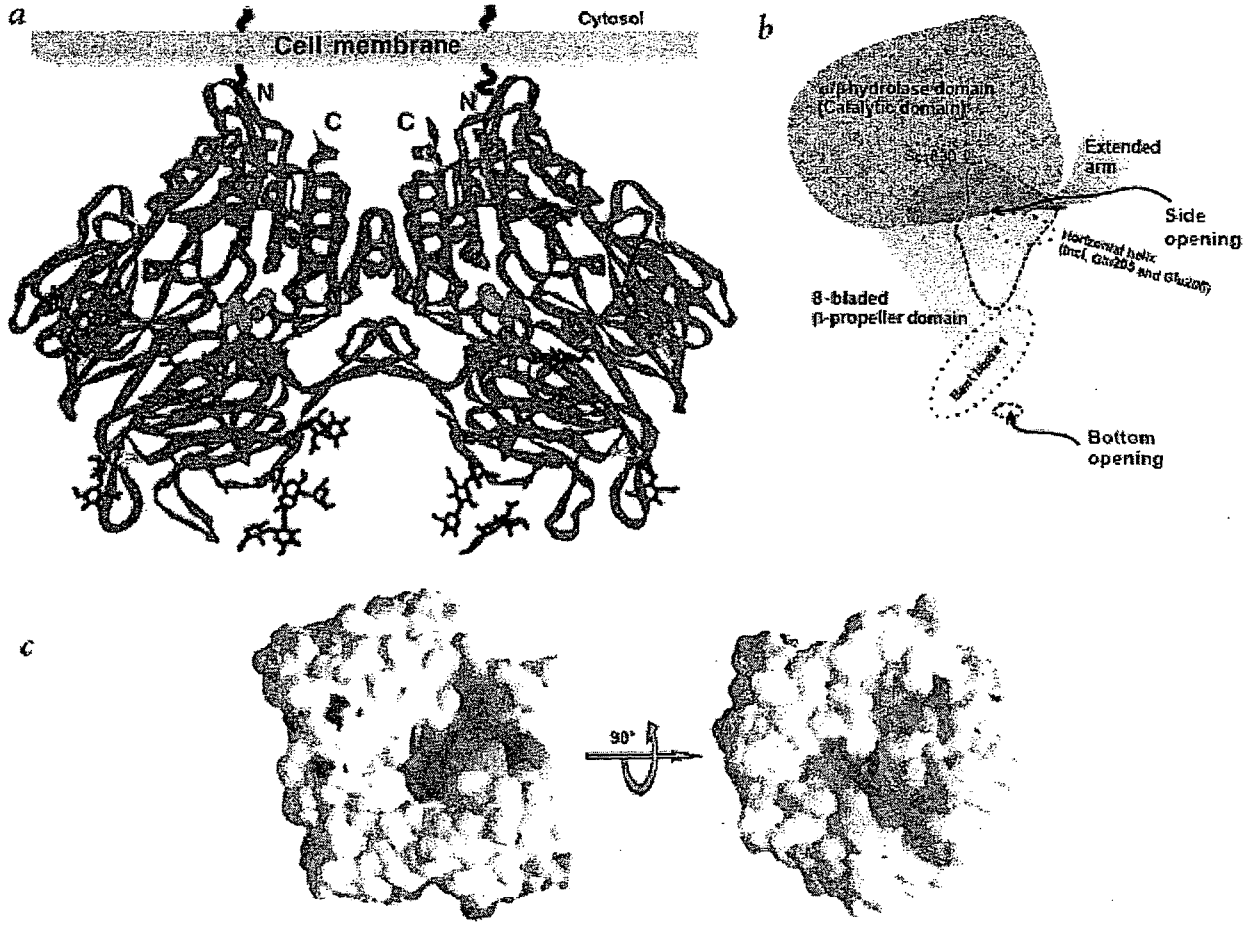


Fig. (2). Three-dimensional crystal structure of CD26. The crystal structure of recombinant human CD26 was determined in the dimeric state at 2.5-Å resolution with the inhibitor Val-pyrrolidide [27]. Figure and figure legend are reprinted by permission from Macmillan Publishers Ltd: **2003**, *Nat. Struct. Mol. Biol.*, *10*(1), 19-25(Fig. **1**), copyright.

a. DPPIV forms a homodimer (subunit A shown in green and subunit B in magenta). Each subunit consists of two domains: an α/β-hydrolase domain and a β-propeller domain. The full-length DPPIV is a type II transmembrane protein in which amino acids 7-28 constitute the membrane spanning region. The α/β-hydrolase, located closest to the membrane, consists of amino acids 39-51 and 506-766, and contains the active triad Ser630, Asp708 and His740. The eight bladed β-propeller is formed by residues 55-497. The inhibitor Val-pyrrolidide is shown in CPK and colored by element: carbon (gray), nitrogen (blue) and oxygen (red). Five S-S bridges (yellow) have been identified in each molecule: Cys328-Cys339 (blade 5), Cys385-Cys394 (blade 6), Cys444-Cys447 (blade 7), Cys454-472 (blade 8) and Cys649-Cys762 (hydrolase). Carbohydrates (blue) have been located in the electron density map at seven of nine possible N-glycosylation positions in both subunits, holding up to three branched proximal glycoside units per position. The glycosylation sites are all situated in the β-propeller domain, except one, and clustered mainly to subdomain two (blade 2-5) of the β-propeller.

b. Schematic illustration of DPPIV showing the individual domains and important structural elements.

c. The surface of molecule A of DPPIV colored by electrostatic potential. The negatively charged surface is red and positively charged surface is blue, viewed from the side and the bottom of the propeller. The inhibitor, Val-pyrrolidide is shown in CPK (yellow). The figure was generated by GRASP [215].

Jurkat cells (derived from a human T-cell leukemia) transfected with CD26 show greater activation than CD26- Jurkat cells or cells transfected with CD26 mutated at the DPPIV enzymatic site [60]. A human Jurkat T-cell line was made deficient of DPPIV activity by a Ser 603 to Ala mutation (Ser630Ala) [60]. When stimulated by anti-CD3 and anti-CD26 antibodies or the combination of anti-CD3 and phorbol esters, the wild type CD26(DPPIV+) transfected Jurkat cells showed more IL-2 production than the mutant CD26 (DPPIV-) or vector-only control transfected cells. In addition, DPPIV activity can change T-cell response to a variety of external stimuli through CD26 and/or the CD3/T-cell receptor (TCR) complex to regulate IL-2 production. The DPPIV activity of CD26 is therefore important in enhancing cell activation in response to external stimuli [14]. However, in a murine T-cell transfection system, transfectants with mutant (no enzyme activity) CD26(DPPIV-) had completely normal signaling compared to wild type CD26 [61]. Also, a C-terminal truncated CD26 lacking the Cys-rich and catalytic domains was sufficient to transmit a co-stimulatory signal in T-cells [62].

Besides its function in T-cell activation, CD26 is a collagen receptor and has a potential role in thymic ontogency

Table 3. Selected CD26 Substrates and Functions After DPPIV Cleavage

Substrate	Function after cleavage
Collagen	Collagen degradation by gelatinase activity allows cells movement through the extracellular matrix may allow lymphocyte migration in thymus from cortical region to medulla during maturation [66]. CD26 has also been reported to have a weak endopeptidase activity [65].
Eotaxin	Eotaxin is an important mediator of allergic reactions. Truncated eotaxin has reduced chemotactic activity for eosinophils and impaired binding and signaling properties through the CC chemokine receptor 3. This physiological processing may be an important down-regulatory mechanism, limiting eotaxin-mediated inflammatory responses [27].
GHRF	GHRF is a substrate for DPPIV; therefore, inhibition of CD26/DPPIV may be a target for children with growth hormone deficiency [218].
GIP	Become non-insulinotropic[132-134]
GLP-1	Become non-insulinotropic[132-134]
Glucagon1-29	glucagon inactivation[219]
IP-10	Reduced lymphocyte chemotaxis[77]
I-TAC	Reduced lymphocyte chemotaxis[77]
LD78β isoform of MIP-1α	Improved monocyte chemotactic activity [125]. LD78β(3-70) is one of the most potent CCR5 binding chemokines and inhibitors of macrophage tropic HIV-1 infection [126]. CD26 does not cleave MIP-1α or the related chemokine LD78α.
MDC	CD26 cleaves both Gly1-Pro2 and Tyr3-Gly4 instead of just 2 amino acids. Decreased chemotactic activity of lymphocytes and dendritic cells, but not monocytes due to reduced signaling through CCR4 [220].
RANTES	- Altered receptor specificity and reduced activity by elimination of binding through the CCR1 chemokine receptor [110]. - abolished CCR3 binding by truncated RANTES, but no change in signaling through CCR5. - Cleavage affects monocyte chemotaxis and HIV-1 infection [214].
SDF-1	Decreased SDF-1 mediated anti-HIV and chemotactic activity [111, 112]. Truncated SDF-1 lacks the ability to induce migration of hematopoietic cells isolated from mouse bone marrow. Mice treated with CD26 inhibitors during G-CSF mobilization had a significant reduction in hematopoietic progenitor cells mobilized [200, 201].

CCR – CC chemokine receptor

GHRF – growth hormone releasing factor

GIP – gastric inhibitory polypeptide or glucose-dependent insulinotropic polypeptide (an incretin)

GLP-1 – glucagons-like peptide-1 (an incretin)

IP-10 – Interferon (IFN) gamma-inducible protein-10

I-TAC – IFN-inducible T-cell α chemoattractant

LD78β isoform of macrophage inflammatory protein-1α

MDC – macrophage-derived chemokine, also known as stimulated T-cell chemotactic protein-1 (STCP-1)

MIP1α – macrophage inflammatory protein 1α

RANTES – regulated on activation, normal T-cell expressed and secreted

SDF-1α – stromal-derived factor 1α (also called CXCL12, chemokine (CXC motif) ligand 12)

[63, 64]. CD26 has also been reported to have weak collagen endopeptidase activity [65]. Gelatinase activity, resulting in collagen degradation, allows cell movement through the extracellular matrix which may facilitate lymphocyte migration in the thymus from the cortical region to the medulla during maturation [66]. DPPIV activity is higher on neonatal T-cells compared to adult T-cells. Development of the neonatal T-cell repertoire is a more dynamic and faster process than in adults and was associated with levels of CD26/DPPIV [67]. CD26 crosslinking results in the Tyr phosphorylation of several signaling proteins (similar to those after TCR/CD3 stimulation). Engagement of the CD26-CD3 complex causes T-cell activation and enhanced IL-2 production. Since CD26 has a short conserved cytoplasmic domain of only 6 amino acids, T-cell signal transduction may be mediated by physical interactions of other molecules with the cytoplasmic domain. Soluble recombinant CD26 with DPPIV activity enhances proliferation of peripheral blood lymphocytes induced by the recall antigen tetanus toxoid [68, 69]. However, soluble CD26 without DPPIV activity does not enhance T-cell

proliferation. Soluble CD26 alone does not trigger mitosis and does not enhance T-cell proliferation to mitogens such as phytohemagglutinin and anti-CD3. These findings suggest that DPPIV activity is necessary for T-cell proliferation. Antibody-induced activation of CD26 leads to CD26 recruitment into lipid rafts, followed by CD26-CD45 association, and increased phosphorylation of T-cell signal transduction/activation proteins [70, 71]. T-cell activation results in protein redistribution in lipid rafts followed by TCR/CD3 association with signal-transduction molecules [72-75]. Lipid raft co-stimulatory molecules include CD2, CD5, CD9, and CD44 [76]. CD26 truncated IP-10 and I-TAC show reduced lymphocyte chemotaxis as a result of impaired CXCR3 mediated calcium signaling [77].

Inflamed tissues and peripheral blood from patients with autoimmune diseases such as multiple sclerosis, Grave's disease, and rheumatoid arthritis have increased CD26 levels, which correlate with disease severity [78-82]. In addition, T-cell that migrate through endothelial cell monolayers *in vitro* express high levels of CD26 [83]. These studies suggest that CD26+ T-cells mediate the inflammatory process and subsequent tissue damage in autoimmune diseases. As discussed in the "CD26 Isoforms" section, CD26 sialylation is associated with different CD26 isoforms and may play a role in the regulation and localization of DPPIV activity. The role of CD26 in human autoimmune disorders and other diseases is described in the "Clinical" section.

4.2. Natural Killer (NK) Cells

CD26 is expressed on NK cells. Shingu *et al.* found that NK cell cytotoxicity against breast adenocarcinoma (MADB106) cells was decreased in CD26 mutant rats (DPPIV- and reduced CD26 surface expression), suggesting that DPPIV activity was associated with NK cytotoxicity [84]. In that study, tumor cells were CD26- by immunoreactivity *in vitro*; however, after incubation with rat serum or after *in vivo* inoculation the tumor cells became CD26+. These results suggest serum interaction with tumor cells induces cell surface expression of CD26, which may play a role in tumor adhesion and metastasis.

4.3. Monocytes/Antigen Presenting Cells

Exogenous soluble CD26 *via* its DPPIV activity upregulates the expression of the co-stimulatory molecule CD86 on monocytes at the mRNA and protein levels [68]. Mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR) mediated internalization of soluble CD26 into monocytes, enhancing tetanus toxoid-induced T-cell proliferation. CD26 upregulates expression of CD86 on antigen presenting cells (APCs), leading to increased APC to T-cell interaction and enhanced T-cell proliferation [68]. CD26 binds to caveolin-1 on APCs *via* amino acids 201 to 226 and the catalytic site Ser630 [85]. On monocytes expressing tetanus toxoid antigens, the CD26-caveolin-1 interaction resulted in caveolin-1 phosphorylation, NFκB activation, and upregulation of CD86. On the other hand, decreasing caveolin-1 resulted in inhibition of CD26-mediated upregulation of CD86 and tetanus toxoid induced T-cell proliferation. Ohnuma *et al.* concluded that the CD26-caveolin-1 interaction on APCs upregulates CD86 and CD86-CD26 interaction on T-cells, leading to antigen-specific T-cell activation [85].

4.4. Adenosine Deaminase (ADA)

CD26 binds to adenosine deaminase (ADA, also referred to as adenosine aminohydrolase, EC 3.5.4.4), an enzyme that plays a key role in purine metabolism and in the development and function of lymphoid tissues [3, 50, 86, 87]. ADA is a ubiquitously expressed, 41 kDa enzyme, that is present in the cytosol and in a lesser amount on T- and B-cell surfaces. ADA cell surface expression is increased when the cell is activated. ADA has much higher activity in lymphocyte development, perhaps due to its association with CD26 [88]. ADA binding to CD26 on the surface of T lymphocytes increases proliferation [89]. Exogenous ADA binding to CD26 causes a co-stimulatory response in T-cell activation events independent of enzymatic activity [89, 90]. ADA catalyzes the irreversible deamination and detoxification of adenosine to inosine and 2' deoxyadenosine to 2' deoxyinosine through hydrolysis of the purine group [3, 86, 91]. There are two isoforms of ADA activity, ADA1 and ADA2. ADA1 is a complex of a 40 kDa monomeric protein with a 200 kDa, noncatalytic combining protein. ADA1 associates with CD26 on the cell surface and accounts for 90% of adenosine deamination. ADA2 is a 110 kDa protein found in macrophages along with ADA1. ADA2 is the predominant ADA isoform present in human serum [88]. ADA2 is increased in many autoimmune diseases and in many cancers.

The HIV gp120 protein inhibits binding between ADA and CD26, which inhibits adenosine breakdown and may be involved in the pathogenesis of HIV-related diseases [92]. The gp120-CD26 interaction occurs at a site different than the ADA binding site [90]. Adults with defective ADA-CD26 interactions can be healthy, suggesting redundancy [93]. Jurkat cells with CD26 and ADA on the cell surface abrogate the usual inhibitory effect of adenosine on T-cell proliferation and IL-2 production. In Jurkat cell lines lacking either ADA or DPPIV activity the following were observed: 1) co-immunoprecipitation of both CD26 and ADA1 showed that DPPIV enzyme activity is not required for the physical interaction of CD26 and ADA and 2) CD26 and ADA are only associated on the cell surface, not in the cytoplasm [87]. Additional experiments have suggested that ADA on the cell surface is from the cytoplasm of other cells.

Congenital ADA1 deficiency is a cause for severe combined immunodeficiency (SCID) [94]. Morrison *et al.* showed that ADA-binding protein-2 is identical to CD26 [3]. CD26 may be involved in regulating the extracellular concentration of ADA, and mutations in ADA may cause SCID [86]. 2'-deoxycoformycin (dCF, pentostatin), an ADA inhibitor, has been used in phase II clinical trials in T-cell hematologic malignancies. When ADA is inhibited by pentostatin in recurrent T-cell lymphoma, circulating CD26+ T-cells were decreased in treated patients [95]. This is consistent with a CD26/ADA physical interaction and may have immunologic implications for treated patients. Clustered charged amino acids of ADA comprise a binding site for CD26 [96]. Dong *et al.* used a variety of CD26 deletion, human-rat swap, and point mutation experiments to show that the CD26 amino acids Leu340, Val341, Ala342, and Arg343 are essential for ADA binding [50]. Huhn and colleagues found that the ADA binding site is in a cysteine-rich region of the CD26 extracellular domain [97]. The binding

sites for ADA were determined by studies utilizing several monoclonal antibodies, point mutations, and deletion constructs, with critical amino acids being those at Leu294, Val341, Leu340 to Arg343, and Thr440/Lys441 but not the 214 residues C-terminal to Ser552 [51]. The authors concluded that the 47 and 97 amino acid spacing was consistent with a β-propeller fold consisting of repeated β sheets of about 50 amino acids [51]. The Arg142Gln mutation impairs binding to CD26 but does not cause immune deficiency [93].

The CD26-ADA interaction highlights the importance of CD26 in critical biologic functions and interactions with other cell structures.

5. INTERACTION WITH PROTEINS AND OTHER CELL STRUCTURES

5.1. Integrin β1

To elucidate the role of CD26 in tumor behavior, we examined the effect of CD26 depletion by small interfering RNA transfection of the T-anaplastic large cell lymphoma cell line Karpas 299. We showed that the resultant CD26-depleted clones lost the ability to adhere to fibronectin and collagen I. Because anti–integrin $ß_1$ blocking antibodies also prevent binding of Karpas 299 to fibronectin and collagen I, we then evaluated the CD26-integrin $ß_1$ association. CD26 depletion does not decrease integrin $ß_1$ expression but leads to dephosphorylation of both integrin $ß_1$ and p38 mitogen-activated protein kinase (MAPK) [98]. Moreover, the p38 MAPK inhibitor SB203580 dephosphorylates integrin $ß_1$, while binding of the anti-CD26 antibody 202.36 dephosphorylates both p38 MAPK and integrin $ß_1$ on Karpas 299, leading to loss of cell adhesion to the extracellular matrix. These results indicate that CD26 mediates cell adhesion through p38 MAPK-dependent phosphorylation of integrin $ß_1$. Finally, *in vivo* experiments showed that depletion of CD26 is associated with loss of tumorigenicity and greater survival.

5.2. CD45/Lipid Rafts

A lipid raft is a cholesterol-rich microdomain in cell membranes. CD26 interacts with lipid rafts in peripheral blood T-cells, leading to modification of cellular signaling events [70, 71]. Non-activated peripheral blood T-cells treated with the anti-CD26 antibody 1F7 increased CD26 recruitment to lipid rafts, which increased Tyr phosphorylation of c-Cbl, Zap70, Erk1/2, $p56^{Lck}$, and TCR-zeta. Lipid raft disturbing agents such as cytochalasin D and nystatin can disrupt Tyr phosphorylation from CD26 crosslinking. CD45 is a T-cell membrane-linked Tyr phosphatase which interacts with CD26 at cytoplasmic domain 2 of CD45 [71]. CD45 activity can be changed by interaction with CD26. The CD26-CD45 interaction occurs outside lipid rafts. This non-raft association of CD26-CD45 is associated with decreased CD26 mediated T-cell activation.

5.3. M6P/IGFIIR

Mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR) binds CD26 *via* M6P residues in the carbohydrate moiety of CD26 [99]. This interaction is important for CD26-mediated T-cell activation and T-cell migration [99, 100]. CD26 internalization can be mediated by M6P/IGFIIR on T-cells [99]. After T-cell activation, CD26 mannose 6 phosphorylation increases, which leads to CD26 internalization. Experimental studies show that, CD26 crosslinking induces co-localization with M6P/IGFIIR. Competition with exogenous M6P inhibits the internalization of CD26 by crosslinking and inhibits CD26-mediated T-cell activation. CD26 treatment with either a glycosidase or phosphatase completely abrogates CD26-M6P/IGFIIR binding, showing that both glycosylation and phosphorylation of CD26 are required for M6P/IGFIIR binding [99]. The controls, glucose-6-phosphate and mannose 1-phosphate had no effect, demonstrating the specificity of the CD26-M6P/IGFIIR interaction and role in T-cell activation [99].

5.4. CD95 and Apoptosis

CD26(DPPIV+) cells were less apoptotic than CD26 (DPPIV-) transfected Jurkat T-cells. The CD26(DPPIV -) cells had increased CD95 (Fas/Apo-1) [101]. CD95 is a member of the nerve growth factor/tumor necrosis factor receptor family that mediates apoptosis. CD95 is not upregulated in hepatoma cells – a difference from Jurkat T-cells [101]. After stimulation with an immobilized anti-CD26 mAb, a human hepatoma cell line underwent apoptosis. Apoptosis could be increased by a phosphatase inhibitor, suggesting mediation through a Tyr kinase [102].

5.5. Collagen/Fibronectin

CD26 binds to collagen and fibronectin in a variety of experimental conditions [63, 103-105]. The CD26 binding site for collagen is in a Cys rich region [104]. Endothelial cell adhesion molecules are partly responsible for the distinct organ distribution of cancer metastases. In a rat model, CD26 on lung capillary endothelium was shown to specifically bind to fibronectin on the breast cancer cell surface [106]. CD26 can induce cell activation *via* binding to collagen receptors on mouse fibroblasts and on human T cells [63, 103]. CD26 has also been reported to have a weak collagen endopeptidase activity [65]. Gelatinase activity, resulting in collagen degradation, allows cell movement through the extracellular matrix, which may allow lymphocyte migration in thymus from the cortical region to the medulla during maturation [66]. In an experimental system, T-cells were able to migrate through an endothelial monolayer on a collagen gel when CD26/DPPIV was highly expressed [83]. Antibodies to CD26 can decrease fibronectin-associated hepatocyte adhesion to collagen [107]. The fibronectin binding site has been identified by nitrocellulose binding assays using ^{125}I-labelled CD26. The fibronectin binding site is different than the site of DPPIV activity and is between amino acids 469-479 (see Table **2**). Competitive peptide inhibitors to CD26 and phenylmethylsulfonyl fluoride enhanced fibronectin-CD26 binding, potentially *via* altering CD26 binding sites [105].

CD26 on lung endothelium acts as a "vascular address" for cancer cells with surface polymeric fibronectin. Cheng *et al.* found CD26 binding sites in type III repeats 13, 14, and 15 using fibronectin protein fragments [108]. The consensus motif T(I/L)TGLX(P/R)G(T/V)X mediates CD26 binding and was confirmed by protein motif swapping experiments. Also, peptides containing the CD26-binding domain of fi-

Table 4. CD26/DPPIV Inhibitors

Drug	Disease	Phase/Reference
Small Molecules		
biarylaminoacyl-(*S*)-2-cyano-pyrrolidines and biarylaminoacylthiazolidines	N/A	Pre-clinical [221]
dCF (2'-deoxycoformycin, Pentostatin)	T-cell hematologic malignancies	Clinical [193]
Ile-thiazolidide (sitagliptin, Januvia)	DM	Phase II
LAF237 (vildagliptin, Galvus)	DM	Phase III
Lys[Z(NO2)]-piperidide	N/A	Pre-clinical [206]
Lys[Z(NO_2)]-pyrrolidide	N/A	Pre-clinical [27]
Lys[Z(NO2)]-thiazolidide	N/A	Pre-clinical [206]
NVP-DPP728	DM, glucose intolerance	Mice [144], Phase I [143], Phase II[222]
Prodipine	Cardiac transplant	Rat [198]
PSN9301	DM	Phase II
PT-100 (Val-boro-Pro)		Pre-clinical [154]
Val-pyrrolidide	N/A	Pre-clinical [27, 207]
Zn^{+2}	N/A	Pre-clinical [208]
Chemotherapy		
Doxorubicin	various	Clinical
Etoposide	various	Clinical
Antibodies		
1F7	N/A	Pre-clinical [39, 53, 210]
5F8	N/A. Less potent than 1F7	Pre-clinical
6A3	N/A	Pre-clinical [106]
14D10	Renal cell cancer	Pre-clinical [178]
202.36	N/A	Pre-clinical [223]
YSCMA	Cancers and autoimmune disorders	Pre-clinical

N/A – not applicable

bronectin type III repeat 14 blocked CD26-poly-fibronectin adhesion and impeded pulmonary metastasis [108].

5.6. CXCR4

The chemokine (CXC motif) receptor 4 (CXCR4, also called fusin) is a G-protein-linked chemokine receptor that acts as a co-receptor with CD4 for HIV infection. CXCR4 physically associates with CD26 in human lymphocytes as well as T- and B-cell lines [109]. The endogenous CXCR4 ligand stromal-derived factor 1α (SDF-1α) promotes the co-internalization of CXCR4 and CD26. CD26 cleaves SDF-1α; therefore, the CD26-CXCR4 complex may modulate SDF-1α downstream activity in the immune system. [110-113]. The SDF-1α-CXCR4 interaction results in increased chemotaxis and antiviral activity of Th2 cells, but not Th1 cells. Sezary syndrome (SS) is a rare form of cutaneous T-cell lymphoma characterized by metastases to the skin and blood involvement. Narducci *et al.* recently described Sezary CD26- cells lacking SDF-1 proteolysis, which may be a mechanism for skin homing to tumor cells [114].

6. CLINICAL

6.1. Autoimmune Diseases

Studies from our laboratories and other have shown that CD26 expression is tightly regulated on human T-cells and that it has a key role in T-cell function [53, 55, 115]. CD26 expression on T-cell surface is higher in multiple sclerosis and Graves disease [78, 80]. CD26/DPPIV appears to play a critical role in the effector functions of CD4- T-cells in experimental autoimmune encephalomyelitis systems [116].

CD26+ T-cells are more sensitive to TCR-mediated activation [117], and CD26 is expressed preferentially on the subset of CD4+ helper cells in response to recall antigens [53]. These results strongly suggest that CD26 plays an important role in the pathophysiology of autoimmune diseases, as further described in the sections below.

6.2. HIV/AIDS

CD4 is necessary for binding human immunodeficiency virus (HIV), but is not sufficient for efficient viral entry and subsequent infection [118]. Co-expression of human CD4 and CD26 in mouse NIH 3T3 cells allowed them to be infected by HIV. Therefore, CD26 inhibitors might be effective therapeutic agents against HIV. HIV patients have a reduced immune response to antigen even before the CD4 count decreases [119-121]. This CD4+ cell memory function occurs on cells that co-express CD26/DPPIV. So, CD26+/CD4+ cells decrease prior to a general decline in CD4 counts [122]. DPPIV levels are decreased in HIV-1 infection and inversely correlated with HIV-1 RNA levels [111]. Furthermore, the immunologic antigen response can be restored by adding soluble CD26/DPPIV *in vitro* [123]. RANTES(1-68) when cleaved by CD26/DPPIV results in 5 fold higher infection rates of mononuclear cells by M-tropic HIV strains. Therefore, CD26 may act as an endogenous chemotaxis inhibitor.

CD26 cleaves SDF-1, reducing SDF-1 anti-HIV activity and potentially facilitating entry of HIV into cells [112, 124]. SDF-1 secreted from CD26+ cells loses both SDF-1 mediated anti-HIV and chemotactic activities [111, 112]. CD26 activation to LD78β(3-70) results in improved monocyte chemotactic activity [125]. LD78β(3-70) is one of the

Table 5. CD26/DPPIV Cancer Summary

Cancer	Expression	Increased CD26	Ref.
ATLL	-/decreased	Less aggressive —	[187]
Breast	ND	Associated with lung metastases and increased topoisomerase IIα	[106, 108]
CLL	+	ND	[185]
Colon	+	Serum levels before surgery associated with a worse DFS	[153]
CTCL	Loss of CD26 suggested as a diagnostic marker for CTCL	ND	[189]
Endometrial	+	No correlation with stage. Increased CD26 associated with lower grade cancer.	[146]
Glioma	+	Increased in poorly differentiated glioma	[156]
HCC	+/- altered distribution	ND	[158]
Melanoma	-	decr cell invasion	[161, 162, 164]
Lung	+ (AdCA) - (other subtypes)	Cell cycle arrest? Reduced anchorage independent growth.	[159, 160]
Oral	-	ND	[167, 168]
Ovarian	+/-	decr intraperitoneal dissemination and prolonged survival	[171, 172]
Prostate	+	Equivocal effect on growth	[173-175]
Renal	+	anti-CD26 monoclonal antibodies inhibited a renal clear cell carcinoma cell line and human renal cell carcinoma in a mouse xenograft model	[178]
SS/MF	+/-	ND	[189, 195]
Thyroid	+	Increased in well differentiated thyroid cancer vs. other subtypes	[179-183]
T-ALCL/ALL/LBL	+	Increased CD26 associated with worse survival	[36, 37]
T-LGLL	+	Decreased CD26 - more neutropenia Increased CD26 – more aggressive	[188-190]

ND – not determined

most potent chemokine receptor 5 (CCR5) binding chemokines and inhibitors of macrophage tropic HIV-1 infection [126]. However, CD26 does not cleave MIP-1α or the related chemokine LD78α. DPPIV activity is inhibited by the HIV protein Tat and the 9 N-terminal amino acids of Tat (MDPVDPNIE) by binding at the CD26/DPPIV active site [127]. Isoelectric focusing studies have demonstrated isoforms of CD26 [38-40]. DPPIV activity is found only in the basic isoforms, but not in the acidic isoforms. A switch to acidic CD26 isoforms has been observed with HIV infection [40]. Neuraminidase treatment of HIV+ subject T-cells cleaves sialic acid residues and reduces the amount of acidic isoforms of CD26/DPPIV [40].

6.3. Rheumatoid Arthritis

Rheumatoid arthritis is a chronic, inflammatory autoimmune disease that primarily affects the joints, but also has systemic effects. CD26/DPPIV is overexpressed on the peripheral blood T-cells of subjects with rheumatoid arthritis. CD26/DPPIV+ T-cells induce the inflammation and tissue destruction characteristic of rheumatoid arthritis by migration to and activity in the rheumatoid synovium [81]. DPPIV can alter the chemokine milieu toward pro-activation and attraction of Th1 cells *via* cleavage of RANTES, eotaxin, MDC, SDF-1α and SDF1-β. IL-12 and IL-15 upregulate CD26 *in vitro*. Cordera *et al.* studied IL-12, IL-15, soluble CD26, and ADA serum levels from 35 patients with active and inactive rheumatoid arthritis as well as that of controls [128]. Patients' serum had higher IL-12 and IL-15 levels. This was independent of disease activity and included patients receiving steroid treatment. Soluble CD26 was inversely correlated with the number of swollen joints. This suggests that interleukins and CD26 are associated with the inflammation and immune activity in rheumatoid arthritis. Ohnuma *et al.* found CD26+ T-cells infiltrating the rheumatoid synovium using immunohistochemical studies [129]. They also noted high expression of caveolin-1 in the rheumatoid synovium vasculature and synovocytes. These data suggest that the CD26-caveolin-1 upregulation of CD86 on activated monocytes leads to antigen-specific T-cell activation in rheumatoid arthritis. DPPIV inhibitors may be useful for suppressing the immune system in rheumatoid arthritis and other autoimmune diseases. DPPIV inhibitors inhibit a rat model of rheumatoid arthritis in a dose-dependent manner [130]. Mavropoulos *et al.* found that anti-tumor necrosis factor-alpha therapy increases DPPIV activity and decreases autoantibodies to the chaperone protein Bip (GRP78) and phosphoglucose isomerase (PGI) in patients (n=15) with rheumatoid arthritis [42]. They also noted a change in CD26 isoforms from acidic to a more neutral isoelectric point after therapy [42].

6.4. Chronic Fatigue Syndrome

Maher *et al.* recently found that compared to controls, chronic fatigue syndrome patients had significantly higher CD26+ lymphocytes [131].

6.5. Diabetes

CD26 plays an important role in glucose homeostasis, and is a potential target for therapy in type 2 diabetes [49]. The insulin-releasing hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are CD26/DPPIV substrates and become non-insulinotropic after truncation by CD26 [132-135]. The active form of GLP-1, GLP-1_{7-36}, is metabolized to GLP-1_{9-36} and acts as an antagonist at the GLP-1 receptor [136, 137]. GLP-1 is released following meals and plays an important role in the regulation of postprandial glucose levels by increasing the amount of insulin secreted in response to the meals. GIP is truncated by 2 amino acids after exogenous administration in patients [138]. Inhibition of CD26 may improve the efficacy of GIP and GLP-1 in diabetes by increasing incretin levels [139]. GLP-1 and DPPIV based drugs for diabetes were recently reviewed by Salehi and D'Alessio [140].

DPPIV deficient mice have improved glucose tolerance and show increased insulin secretion after a glucose bolus [49]. Additionally, glucose tolerance is improved in a rat model treated with Ile-thiazolidide (sitagliptin; MK-0431; Januvia; Merck) and NVP-DPP728 (Novartis AG) [141, 142]. With the Ile thiazolidide, obese rats had more improvement than lean rats [141]. NVP-DPP728 is a specific and selective inhibitor of CD26 and improved glucose control over 4 weeks in human type 2 diabetics [143]. Reimer *et al.* found DPPIV inhibition over a longer duration (8 weeks) in mice fed oral NVP-DPP728 [144]. This was associated with increased GLP-1, increased insulin levels, and increased glucose transporter isoform-2 (GLUT-2) expression in both normal and glucose-intolerant mice. Val-pyrrolidide improved glucose tolerance in mice fed high-fat diet and in Fischer rats [145].

GLP-1 mimetics such as exendin-4 are naturally resistant to DPPIV activity. Exenatide (Byetta, Amylin Pharmaceuticals) is a synthetic form of exendin, which received FDA approval in April 2005. Both NVP-DPP728 (Novartis) and LAF237 (vildagliptin; Galvus; Novartis) improved glucose control when given for 4 weeks to subjects with type 2 diabetes. Both could be administered by mouth and had minimal side effects. The oral, small molecule CD26/DPPIV inhibitor, PSN9301 (OSI Pharmaceuticals), showed positive results in humans in a phase II clinical trial. That study showed that following 14 days of therapy, PSN9301 substantially reduced glucose levels after an oral glucose tolerance test and was generally well tolerated with no episodes of hypoglycemia. These findings together support the potential usefulness of DPPIV/CD26 as a targetable molecule for diabetes mellitus type 2.

6.6. Obstetrics and Gynecology

CD26 is expressed in both the proliferative and to a lesser degree the secretory phase of normal uterine endometrium [146]. Placental CD26/DPPIV modulates fetal metabolism by cleavage of fetoplacental circulating bioactive peptides, including incretins, which regulate fetal growth [147]. In a stress induced abortion murine model, the CD26/DPPIV inhibitor, Ile-thiazolidide, inhibited an increased abortion rate in stressed mice versus control mice (37.2% versus 13.6%, $P < 0.01$) [148]. The role of CD26 in gynecologic neoplasms is discussed in the Cancer section below.

7. CANCER

CD26 status may be altered in cancer and effect the growth and metastatic potential of various cancers. CD26

absence is associated with cancer development for some cancers, while CD26 presence is associated with a more aggressive phenotype in other cancers. The role of CD26 in cancer has been reviewed [14]. Besides its expression on the tumor cell surface, CD26 can also be found in the serum, and its levels may correlate with cancer status and tumor biology for certain cancers.

Endothelial cell adhesion molecules are partly responsible for the distinct organ distribution of cancer metastases. In a rat model, CD26 on lung capillary endothelium was shown to specifically bind to fibronectin on the breast cancer cell surface [106]. CD26 binds to collagen and fibronectin in a variety of experimental conditions and CD26 may have a role in cancer migration and metastasis by association with extracellular matrix proteins [63, 103-105]. Also, CD26 may interact with extracellular matrix protein to recruit activated lymphocytes to sites of inflammation or cancer [83]. CD26 can induce matrix metalloproteinase-9 (MMP-9) expression in cancer cells, which may facilitate metastasis [149]. A truncated CD26 containing amino acids 31-766, in which the fibronectin-binding site was preserved could mask breast cancer cell surface-associated fibronectin complexes, causing a dose-dependent inhibition of adhesion to endothelial CD26 and impeding lung colony formation. Since cancer cells accumulate fibronectin on their surface mainly during hematogenous spread, such anti-metastatic treatment may have important clinical implications [150]. CD26/DPPIV association with plasminogen may lead to signal transduction that regulates expression of MMP-9 in prostate cancer cells [149]. CD26/DPPIV expression in cancer cells may downregulate cancer progression by promoting differentiation, but CD26/DPPIV also regulates properties of local invasion and metastasis. Therefore, the role of CD26 needs to be evaluated individually for each tumor type.

7.1. Solid Tumors

7.1.1. Breast Cancer

Cheng *et al.* found that CD26 expressed on rat lung capillary endothelium mediated lung metastases of breast cancer cells by association with fibronectin [106]. They studied the Fischer 344/CRJ rats, which have a CD26 Gly633Arg substitution, that leads to retention and degradation of the mutant protein in the endoplasmic reticulum, as a "protein knockout" model to characterize the previously established role of CD26 in metastasis [52]. They found that lung metastases from the highly metastatic MTF7 rat breast cancer cell line were reduced by only 33% relative to normal Fischer 344 rats. Detailed immunohistochemical experiments revealed low levels of mutant enzymatically inactive CD26 on lung endothelial cells. When the mutant CD26 was purified it had identical adhesion qualities for breast cancer cells as wild type DPPIV. The CD26/fibronection-mediated adhesion and metastasis are effectively competed by soluble CD26, anti-CD26 mAb 6A3, and anti-fibronectin antiserum [106]. Furthermore, peptides containing the fibronectin CD26-binding domain blocked the CD26-fibronectin interaction and significantly decreased pulmonary metastasis of breast cancer and melanoma cell lines [108]. The utilization of fibronectin by cancer cells and fibronectin self-association in the blood suggests that CD26/fibronectin binding may be a mechanism for lung metastasis [106].

CD26 is associated with increased topoisomerase IIα levels and tumor sensitivity to the topoisomerase II inhibitors, such as doxorubicin and etoposide. Recent studies suggest that topoisomerase IIα level is a prognostic factor in breast cancer that is independent of stage, Her-2/neu status, and histological grading [151]. Furthermore, anthracycline treatment did not reverse the negative prognostic effect of topoisomerase IIα expression. Others have found, in retrospective studies that topoisomerase IIα overexpression confers a higher probability of response to doxorubicin [152]. Topoisomerase IIα is currently being evaluated prospectively as a breast cancer predictive marker. The role of CD26 in breast cancer and the interaction of CD26 with topoisomerase IIα is an area for future research.

7.1.2. Colon Cancer

CD26 is found on the cell surface and its level correlates with disease status and tumor biology for certain cancers. In colorectal cancer, serum CD26 was not related to colon cancer grade, stage, or location [153]. The DPPIV inhibitor PT-100 (Val-boro-Pro; Point Therapeutics) improved the activity of trastuzumab in human Her2/neu+ colon cancer in xenograft models [154]. However, the anti-cancer activity of PT-100 was not changed in CD26(-/-) mice, suggesting non-CD26 mediated activity [154].

7.1.3. Endometrial Cancer

CD26 is expressed in both the proliferative and to a lesser degree the secretory phase of normal uterine endometrium [146]. In one study, CD26 may be involved in the transformation and progression of endometrial adenocarcinoma. CD26 expression was strong or moderate in grade 1 endometrial adenocarcinoma, weak or negative in grade 2 and 3. Thus, suggesting a loss of CD26 with higher grade cancer; however, there was no correlation between DPPIV expression and clinical stage [146]. Mizokami and colleagues demonstrated that SDF-1α and CXCR4 were expressed in human endometrial cancer with protein levels similar to that of CD26 [155]. These proteins were inversely correlated with the tumor grade. Also, exogenous SDF-1α significantly stimulated cell proliferation in vector-transfected cells but not in CD26-transfected cells [155]. Suggesting CD26 cleavage and abrogation of SDF-1α mediated cell proliferation.

7.1.4. Glioma

Low CD26/DPPIV activity is found in poorly differentiated gliomas and high activity is found in differentiated gliomas. Differentiated gliomas have higher levels of membrane-associated isoforms of CD26/DPPIV [156]. Sedo and colleagues studied "DPPIV activity and or structure homologs" (DASH) in 5 glioma cell lines of varying grade [157]. They concluded that there was "no simple correlation with the degree of malignancy of the original donor tumor or morphologic phenotype..." [157] They did note that changes in DASH composition as detected by non-denaturing polyacrylamide gel electrophoresis may reflect changes in substrate specificity and regulate changes in glioma activity.

7.1.5. Hepatocellular Carcinoma

In normal liver as well as cirrhotic and steatotic liver, CD26 is expressed in the bile canalicular domains of hepatocellular membranes. In hepatocellular carcinoma (HCC), CD26/DPPIV activity was lost (2/25 cases) or had altered distribution (23/25 cases) [158]. Gaetaniello and colleagues found Tyr phosphorylation of several proteins after antibody binding to CD26 in HCC cell lines. This effect was inhibited by CD45 Tyr phosphatase [102]. They found that a protein or proteins with kinase activity were associated with CD26 [102]. CD26 protects against apoptosis in Jurkat T-cells, while in the human hepatoma PLC/PRF/5 cells CD26 antibody binding is pro-apoptotic. CD26 expression is also pro-apoptotic in HepG2 cells.

7.1.6. Lung Cancer

CD26 is expressed in lung adenocarcinoma but not other subtypes of lung cancer [159]. CD26 expression and DPPIV activity are present in normal bronchial and alveolar epithelium, but non-adenocarcinoma lung cancers lose CD26 expression. CD26 downregulation may contribute to the loss of growth control in NSCLC cells [160]. NSCLC cells transfected with CD26 develop morphologic changes, altered contact inhibition, and reduced ability for anchorage-independent growth. An increased percentage of cells in G0-G1 was noted in CD26 expressing cells, indicating CD26 may promote cell cycle arrest [160].

7.1.7. Melanoma

CD26 is highly expressed in normal melanocytes, but not in melanoma cells, suggesting CD26 expression is lost in malignant transformation [161, 162]. Loss of CD26 was also associated with development of specific chromosome abnormalities [3]. When CD26/DPPIV is expressed in melanoma cells using a tetracycline-inducible expression system, melanoma growth was suppressed in cell line clones expressing higher levels of CD26. CD26 expression also decreased the melanoma growth in soft agar – indicating a CD26 association with anchorage-independent growth [162]. The highly sialylated plasminogens 2γ, 2δ, 2ε may bind to CD26 and mediate signaling by increasing intracellular calcium. [149] The plasminogen 2ε binding to CD26 stimulates MMP-9 expression in cancer cells, which may facilitate metastasis [149]. In a subsequent paper, that group showed that angiostatin 2ε can bind CD26 and abrogate calcium signaling and block invasion in a prostate cancer *in vitro* model. [163] Pethiyagoda *et al.* found that CD26/DPPIV inhibits invasion of malignant melanoma cell lines by transfecting CD26 into melanoma cell lines [164]. Invasion in Matrigel was decreased by 75%. This was also true for CD26 mutant transfectants that either lacked the extracellular protease activity (Ser630Ala) or the six amino acid cytoplasmic domain – demonstrating that neither DPPIV activity or the six amino acid C-terminal domain were necessary to inhibit invasiveness [162]. Expression of either wild-type CD26 (DPPIV+) or mutant CD26(DPPIV-) rescued expression of the cell surface Ser protease FAP-α. [162]. FAP-α can form a heterodimer with CD26 and may play a role in regulating melanocyte/melanoma growth [162]. CD26 can inactivate circulating growth hormone-releasing factor (GHRF), so releasing factor (GHRF), so decreased CD26 may result in cancer growth by increased GHRF [165]. A recent study found no effect of interferon-alpha (IFNα) on DPPIV activity in 18 patients with high-risk melanoma [166].

7.1.8. Oral Cancer

CD26 serum activity and expression on peripheral blood T lymphocytes is decreased in oral cancers compared to normal controls [167, 168]. Peripheral blood T lymphocytes exposed to phytohemagglutinin, concanavalin A and/or interleukin-2, had a decreased proliferative response and expression of CD26 in oral cancer patients compared to controls [168]. The mechanism for this downregulation appeared to be through squamous cell carcinoma derived TGFβ1 [169].

7.1.9. Ovarian Cancer

Expression of CD26 varies in ovarian cancer cell lines [170]. CD26 is negatively correlated with ovarian cancer invasive potential. Specifically, overexpression of CD26 in ovarian cancer leads to increased E-cadherin and tissue inhibitors of MMPs, resulting in decreased invasive potential [171, 172]. CD26 transfection in ovarian cancer cell lines decreased intraperitoneal dissemination and prolonged survival *in vivo* in mice [171].

7.1.10. Prostate Cancer

CD26/DPPIV biochemical activity was twice as high in prostate cancer compared to benign prostate hyperplasia (BPH) tissues. CD26/DPPIV activity was also increased in BPH adjacent to cancerous prostate tissue [173]. DPPIV activity was present in epithelial but not stromal BPH and cancer tissues. This suggests that paracrine factors produced by prostate cancer cells may modulate the local microenvironment to increase cancer growth. Johnson *et al.* found that lung endothelial cells with metastatic prostate cancer cells were enriched for CD26 compared to non-metastatic prostate cancer cells [174]. However, Wesley *et al.* suggested that CD26 inhibits the "malignant phenotype" of prostate cancer cells by inhibiting the bFGF signaling pathway [175]. Wilson *et al.* examined DPPIV activities in different prostatic tissue zones and in prostatic expressed secretions in relation to the presence of cancer [176]. They found that expressed prostate secretions in patients with cancer were higher than in men without cancer. DPPIV activities in the transitional and especially the peripheral zone biopsies were higher in cancer patients. They concluded that secreted DPPIV originates in the transitional and peripheral zones. Measuring DPPIV levels in prostate secretion or in post-digital rectal prostate examination urine may be a useful for tumor marker for prostate cancer [176]. Gonzalez-Gronow *et al.* found that in addition to its ability to inhibit tumor vascularization, angiostatin 2ε may also directly block prostate cancer metastasis by binding to CD26 on the surface of the 1-LN prostate cancer cell line [163]. CD26/DPPIV association with plasminogen may lead to signal transduction that regulates expression of MMP-9 in prostate cancer cells [149]. CD26 inhibitors may inhibit prostate cancer by blocking a CD26/DPPIV mediated signal transduction pathway that regulates MMP-9 expression by prostate cancer cells [149].

7.1.11. Renal Cancer

CD26 expression has previously been noted in renal cell carcinoma, with unclear significance [177]. CD26 has a high level of surface expression on the renal cell carcinoma cell lines Caki-1, Caki-2, VRMRC-RCW, and ACHN [178]. Inamato and colleagues recently showed that anti-CD26 monoclonal antibody inhibition of the Caki-2 cell line was associated with G1/S cell cycle arrest, enhanced $p27^{kip1}$ expression, down regulation of cyclin-dependent kinase 2 (CDK2) and dephosphorylation of retinoblastoma substrate (Rb) [178]. They also found that anti-CD26 monoclonal antibody therapy attenuated Akt activity and internalized cell surface CD26 leading to decreased CD26 binding to collagen and fibronectin. They also showed that anti-CD26 monoclonal antibodies inhibited human renal cell carcinoma in a mouse xenograft model [178].

7.1.12. Thyroid Cancer

Benign thyroid tissue is usually negative for CD26 expression, but CD26 expression has been observed in thyroid cancer. This difference has been exploited as a diagnostic marker for the differential diagnosis of benign thyroid disease versus thyroid cancer [179-183]. Kholova *et al.* suggest that CD26 positivity is limited to well-differentiated thyroid carcinomas including papillary carcinoma and is of limited value for diagnosing follicular and oncocytic thyroid malignancies [184].

7.2. Hematologic Malignancies

While the role of CD26 in the regulation of T-cell function and cleavage of substrates has been extensively studied, its involvement in cancer biology has only recently been examined. CD26 may contribute to the biology of hematologic cancers by expression on subsets of T-cells and influencing tumor growth by its DPPIV catalysis of substrates. CD26 may be a targetable molecule to exploit for selected malignancies.

7.2.1. Lymphoma and Leukemia

CD26 is overexpressed in B-cell CLL compared to normal resting B-cells. Treatment of B-CLL cells with interferons and retinoic acid increased CD26 expression by a STAT1α mediated process [185].

A study of the human T-lymphotropic virus 1 (HTLV-1) related cancer adult T-cell leukemia/lymphoma (ATLL) showed a reduction of surface CD26 expression in the peripheral blood mononuclear cells in all 20 patients studied [186]. Additionally, CD26 mRNA was undetectable in 7 of 8 ATLL patients. Quantification of HTLV-1 viral DNA by PCR in cells from subjects with CD26-, CD26+, and 17 HTLV-1 carriers showed that the CD26- cells had a higher HTLV-1 copy number than CD26+ cells. This suggests that HTLV-1 has tropism for CD26- cells *in vivo*. In a study of 49 patients with ATLL, 10 subjects that were carriers of HTLV-1, and 4 HTLV-1 infected cell lines, ATLL HTLV-I-infected cell had reduced or absent the CD26/DPPIV expression. CD26 expression decreased with the advancement of ATLL stage [187]. This appeared to be due to progressive aberrant methylation of CpG islands in the CD26 promoter proportional to increasing ATLL stage. This was confirmed with rescue experiments with the demethylating agent, 5-azacytidine.

CD26 expression is associated with a more aggressive clinical course in T-cell large granular lymphocyte leukemia (T-LGLL). T-LGLL patients with low expression of CD26 on T-LGLL cells had a more indolent course, while patients with high expression developed recurrent infections due to neutropenia. LGLL patients often have autoimmune diseases [188]. CD26 expression on T-LGLL is associated with inhibition of myeloid progenitors, possibly explaining the neutropenia seen in these patients with higher levels of CD26 expression [188, 189]. CD26 on T-LGLL cells is unable to transmit antibody-mediated activation signals, unlike CD26 on normal T-cells, so CD26-related signaling may be aberrant in T-LGLL [188]. In a recent report of a single institution long-term follow-up of 21 T-LGLL patients, 0 of 21 had CD26 expression [190].

In NHL, CD26 expression is found mainly in aggressive subtypes, such as T-lymphoblastic lymphoma (LBL)/T-acute lymphoblastic leukemia (ALL) and T-cell CD30+ anaplastic large cell lymphoma (ALCL) [36, 37]. CD26 and CD40L expression was mutually exclusive, with CD40L expressed on cells from more indolent diseases. CD26 expression in T-cell LBL/ALL was associated with a worse survival [36, 37]. The majority of patients with T-ALL express CD26 on the leukemic cell surface [191]. There appears to be high CD26/DPPIV expression on T-lymphoblasts but only moderate DPPIV activity [191, 192]. Aldinucci *et al.* showed that CD26 is a marker of poor prognosis in T-cell cancer and is a predictive marker of poor response to dCF (2'-deoxycoformycin, pentostatin) [193]. This effect was seen *in vitro* in CD26/ADA positive leukemia/lymphoma T-cell lines, primary CD26+ T-cell cancers, and normal T-cells (CD26+) [193]. Loss of CD26 appears to be characteristic of cutaneous T-cell lymphoma (CTCL) and has been suggested as a useful diagnostic marker [189]. Adams and colleagues administered oral PT-100 (Val-boro-Pro), a DPPIV inhibitor to mice with syngeneic tumors from lymphoma cell lines [154]. The PT-100 resulted in slowed growth, regression, and rejection of tumors. PT-100 also increased the activity of rituximab in human CD20+ B-cell lymphoma xenograft models. The putative mechanism was antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by innate effector cells that were responsive to the cytokines and chemokines up-regulated by PT-100 [154]. However, the PT-100 anti-cancer and cytokine stimulatory activity was abrogated in CD26(-/-) mice, suggesting that CD26/DPPIV is not a critical target for PT-100 in this model [154].

Ruiz *et al.* described CD26 expression detected on the surface of tumor cells from a patient with an aggressive hepatosplenic gamma-delta T-cell lymphoma [194].

7.2.2. Sezary Syndrome/Mycosis Fungoides (SS/MF)

CD26 expression is absent or weak in other T-cell lymphomas such as mycosis fungoides (MF) and Sezary syndrome (SS) [189, 195]. SS is a form of CTCL involving the blood and skin [114]. Loss of CD26 appears to be characteristic of CTCL and has been suggested as a useful diagnostic marker [189]. Chemokines and their receptors are involved

in recruitment and homing of cancer cells to tissues of several tumors including non-Hodgkin's T-cell lymphomas. SS cells express CXCR4 and the skin generates its ligand, SDF-1, which may represent a target for the main destination of SS cells metastasizing to the skin. SDF-1 is normally cleaved and inactivated by DPPIV mediated activity. An abnormal CD26-negative/dim T-cell population was found in a study of 66 of 69 samples from 28 SS/MF patients. These CD26 negative/dim T-cells were CD26- in 23 patients and weakly CD26+ in 5 patients [189]. Sokolowska-Wojdylo and colleagues found that absence of CD26 on CLA+ CD4+ T-cells was 100% sensitive for SS in 7 patients [196]. Also, the number of CD26-negative T-cells correlated with treatments in 2 patients for over 1 year in a longitudinal study. SS patients have decreased plasma DPPIV activity. Soluble CD26 reduces the SDF-1 mediated SS cell migratory response. Inhibition of DPPIV activity in the CD26+ CTCL cell line Hut78 increases SDF-1-induced migration of SS cells. The SDF-1-CXCR4 interactions may mediate SS cell affinity for skin as a metastatic site *via* the regulatory activity of CD26.

7.2.4. Hematopoietic and Organ Transplantation

Inhibition of CD26 is a potential mechanism to increase engraftment and donor specific tolerance in hematopoietic and organ transplantation [197]. Prodipine is a low-molecular-weight inhibitor of the diphenyl-phosphonate group of CD26, which prevented an increase in serum CD26/DPPIV commonly seen after cardiac allotransplantation associated with a delay in acute rejection in rat recipients [198]. Prodipine also abrogated acute rejection and prolonged cardiac allograft survival. CD26/DPPIV plays a role in alloantigen-mediated immune regulation and allograft rejection *in vivo*. In stem cell transplants, donor cell CD26 expression inhibits homing and engraftment [199]. Truncated SDF-1α lacks the ability to induce migration of hematopoietic cells isolated from mouse bone marrow. Mice treated with CD26 inhibitors during G-CSF mobilization had a significant reduction in hematopoietic progenitor cells mobilized [200, 201]. In a sublethally irradiated NOD/SCID mouse model, inhibiting CD26 on immature subsets of CD34+ cells enhanced engraftment [202]. This also increased the overall survival in the mouse recipient [203]. Christopherson and colleagues recently reported the effects of several cytokines on surface CD26 levels on CD34+ CD38- hematopoietic progenitor cells [203]. The percentage of CD26+ cells was increased by treatment with stem cell factor (SCF/steel factor/kit ligand), granulocyte-colony stimulating factor (G-CSF), and granulocyte macrophage-colony stimulating factor (GM-CSF). More modest surface CD26 increases were seen with treatment with erythropoietin (EPO) or trombopoietin (TPO) [203].

The engraftment kinetics for hematopoietic progenitor cells correlate with the efficiency of bone marrow homing [204]. CD26 down-regulation on mobilized hematopoietic progenitor cells has been proposed as a mechanism to improve migration and homing. However, Boenig and colleagues recently showed that CD26 inhibitors are ineffective for this purpose due to the lack of CD26 expression on mobilized hematopoietic progenitor cells [204]. Kobayashi *et al.* suggested that the CD26-CD45RA interaction outside of cell surface "lipid raft" microdomains could result in decreased T-cell activation signaling through CD26, resulting in an immature immune response and the relatively low incidence of severe graft-versus-host disease in cord blood transplantation [205]. Further studies of CD26/DPPIV may allow for improvements in transplant recipient immunosuppression.

8. CD26/DPPIV INHIBITORS

8.1. Small Molecule Inhibitors

The DPPIV-specific inhibitors Lys [Z(NO2)]-thiazolidide and Lys [Z(NO2)]-piperidide inhibit T-cell signal transduction events such as Tyr phosphorylation and Ca^{+2} flux [206]. CD26 inhibition decreased phorbol 12-myristate 13-acetate (PMA)-induced hyperphosphorylation of $p56^{lck}$ in a dose-dependent manner, without changing CD26 activity, protein kinase C, or intracellular cAMP [206]. CD26/DPPIV inhibitors suppress anti-CD3-induced Ca^{+2} flux, suggesting direct involvement in T-cell activation mediated by Tyr kinases, affecting both anti-CD3-induced Ca^{+2} mobilization and PMA-induced $p56^{lck}$ hyperphosphorylation [14]. Steinbrecher *et al.* showed that the DPPIV inhibitor Lys [$Z(NO_2)$]-pyrrolidide inhibited an experimental model of autoimmune encephalomyelitis, which is associated with elevated TGF-β1 levels and inhibition of CD4+ T-cell effector functions *in vivo* [207]. Both NVP-DPP728 and LAF237 improved glucose control when given for 4 weeks to subjects with type 2 diabetes. Both could be administered by mouth and had minimal side effects. The oral, small molecule CD26/DPPIV inhibitor PSN9301 (OSI Pharmaceuticals), showed positive results in humans in a phase II clinical trial. That study showed that following 14 days of therapy, PSN9301 substantially reduced glucose levels after an oral glucose tolerance test and was generally well tolerated with no episodes of hypoglycemia. These findings together support the potential usefulness of DPPIV/CD26 as a targetable molecule for diabetes mellitus type 2 treatment. The amino boronic dipeptide, PT-100 (Val-boro-Pro) is a DPPIV inhibitor, which can increase cytokine expression *via* FAP. FAP is expressed in the stroma of normal and malignant lymphatic tissue. PT-100 had *in vitro* tumor cytotoxicicity [154]. Also, oral administration of PT-100 in a syngeneic mouse tumor model inhibited the growth of fibrosarcoma, lymphoma, melanoma, and mastocytoma [154]. The mechanism of action involves tumor-specific cytotoxic T-lymphocytes and protective immunological memory. PT-100 treatment resulted in increased mRNA levels of cytokines associated with T-cell priming and chemoattraction of T-cells and innate effector cells. Immunodeficient mice had reduced inhibition of tumors. In xenograft models, PT-100 increased the activity of rituximab in human CD20+ B-cell lymphoma and increased the activity of trastuzumab in HER-2+ colon cancer, presumably through ADCC mechanism. However, the PT-100 anti-cancer and cytokine stimulatory activity was abrogated in CD26(-/-) mice, suggesting that CD26/DPPIV is not a critical target for PT-100 in this model [154]. Also, DPPIV enzyme activity was completely inhibited by 1 mM Zn^{+2} [208].

8.2 CD26 Antibodies

Anti-CD26 monoclonal antibodies exhibit antitumor activity *in vitro* in the T-cell CD30+ anaplastic large cell lymphoma cell line Karpas 299 [209]. The 1F7 monoclonal an-