**Summary.** In this review, we highlight major aspects of the biology of CD26, a dipeptidyl peptidase IV (DPPIV)-containing surface glycoprotein with multiple functions. In particular, we discuss findings demonstrating that CD26/DPPIV has an essential role in immune regulation as a T cell activation molecule and a regulator of chemokine function. We also review recent studies that identify key cellular molecules that physically associate with CD26 and the potential consequences of their interaction, including those with clinically-related implications. Furthermore, we present work suggesting a role for CD26 in the pathogenesis and behavior of selected human cancers, both solid tumors and hematological malignancies. We present recent studies that investigate the potential role of CD26 as a molecular target for novel treatment modalities for T cell lymphoid malignancies, with work involving the use of anti-CD26 monoclonal antibody, CD26-transfected cells as well as soluble CD26 molecules.

**Key words:** CD26/DPPIV, T cell activation, Chemokine, Hematological malignancies, Cell cycle

**Introduction**

CD26 is a 110 kD cell surface glycoprotein with diverse functional properties, and is expressed on a variety of tissues, including epithelial cells and selected leukocyte subsets (Morimoto and Schlossman, 1998). The isolated CD26 cDNA predicts a type II transmembrane protein comprising of 766 amino acids and belonging to the serine protease family. As members of this family share the consensus sequence Gly-Xaa-Ser-Xaa-Gly at the catalytic site (Brenner, 1988), the predicted CD26 amino acid sequence contains a putative catalytic site, Gly-Trp-Ser-Tyr-Gly at positions 628-632, which fits the consensus sequence, with Ser-630 being assumed to be the catalytic residue. Therefore, CD26 is a membrane-associated ectopeptidase with a short cytoplasmic domain comprising of 6 amino acids, a transmembrane region and an extracellular domain with dipeptidyl peptidase IV (DPPIV) activity, capable of cleaving NH2-terminal dipeptides from polypeptides with either L-proline or L-alanine at the penultimate position (Tanaka et al., 1992). The predicted amino acid sequence of human CD26 shows approximately 85% homology with the rat DPPIV enzyme (Hong and Doyle, 1987, 1988) and mouse thymocyte activating molecule (THAM) (Marguet et al., 1992). Work over the past decade has demonstrated that CD26 has a multitude of physiological roles, ranging from its role in immune regulation as a structure capable of transmitting T cell activation signals to its role as a regulator of biological processes through its cleavage of biological factors. Furthermore, recent work has also suggested that CD26 may be involved in the development of certain human cancers. In this review, we will examine data focusing on CD26 role in the regulation of various aspects of human immune system as well as its emerging role in cancer development, including its potential suitability as a novel target for therapy.

**CD26 and human immune regulation**

Regarding the role of CD26/DPPIV in immune regulation, work done by our group and others have suggested that CD26 has a key role in T cell function. Although it is constitutively expressed in the liver, intestine and kidney, CD26 expression level is tightly regulated on T cells, with its density being markedly elevated following T cell activation (Fox et al., 1984; Morimoto et al., 1989; Dang et al., 1990a). In clinical settings involving activated immune responses, such as in autoimmune diseases, CD26 levels of circulating T lymphocytes are often elevated (Hafler et al., 1985; Eguchi et al., 1989). In the peripheral blood of normal donors, CD26 is expressed on a subset of CD4+ memory...
T cells, and this CD4+CD26+ T cell population responds maximally to recall antigens (Morimoto et al., 1989). In addition, CD26+ peripheral blood T cells exhibit greater proliferation when stimulated with mitogenic signals than CD26- T cells, again consistent with the notion that CD26 is a marker of activated cells (Hafler et al., 1989). Besides 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, capable of transmitting signals relating to T cell activation. We have shown that CD26 is able to conduct co-mitogenic signals in conjunction with activation through the CD3/T cell receptor complex (TCR) or the CD2 pathway of mature human T lymphocytes. Binding of solid-phase immobilized anti-CD26 monoclonal antibody has a co-mitogenic effect in conjunction with submitogenic doses of anti-CD3 or anti-CD2 antibodies, associated with the IL-2 autocrine pathway. Likewise, crosslinking of CD26 enhances T cell activation induced by phorbol esters (Dang et al., 1990a). Similarly, CD26 conveys a co-mitogenic signal in CD3/TCR-mediated activation of human thymocytes. Interestingly, unlike the case with mature peripheral blood T cells, the ability of CD26 to mediate co-mitogenic signal in thymocytes is restricted to the CD3 pathway only and does not involve CD2-induced activation. Meanwhile, CD26 is mostly found on the surface of medullary thymocytes, the population of relatively mature thymocytes expressing high level of CD3. These findings hence suggested that CD26 may play a role in thymic differentiation and maturation via the differential engagement of the CD3 pathway (Dang et al., 1991). Furthermore, binding of anti-CD26 Ab leads to T cell activation in the presence of FcR+ antigen accessory cells, likely due to crosslinking of the antigen (Fleischer, 1987; Dang et al., 1990b). Meanwhile, antibody binding results in a decrease in CD26 surface expression due to its internalization. Importantly, this down-modulation of CD26 is associated with enhanced tyrosine phosphorylation of such key signaling molecules as CD3-zeta and p56<sup>ck</sup> (Hegen et al., 1997) as well as increased proliferative response to anti-CD3 and anti-CD2 stimulation (Dang et al., 1990c), additional evidence of CD26 ability to convey signals related to T cell activation.

**Effect of DPPIV enzyme activity on CD26-mediated T cell function**

Using the CD26 negative human T cell leukemia line Jurkat, we established stable CD26 transfectants to further investigate the role of CD26 in T cell activation. Consistent with findings relating to peripheral blood T lymphocytes, CD26-expressing Jurkat transfectants display enhanced intracellular calcium mobilization and IL-2 production when triggered with solid-phase immobilized anti-CD26 and anti-CD3 antibodies (Tanaka et al., 1993). By mutating human CD26 cDNA through oligonucleotide directed mutagenesis to encode a protein in which the putative catalytic serine residue at position 630 is replaced by alanine, we created Jurkat transfectants expressing a mutant CD26 antigen lacking DPPIV enzyme activity. The concurrent use of the wild type CD26+DPPIV+ and the corresponding mutant CD26+DPPIV- Jurkat transfectants allows for the characterization of the role of DPPIV in T cell activation mediated through CD26. Wild type CD26+DPPIV+ Jurkat transfectants consistently produce substantially higher level of IL-2 than the mutant CD26+DPPIV- or vector-only transfectants following stimulation with the combination of anti-CD3 and anti-CD26 antibodies or with stimuli not involving CD26, such as anti-CD3 and phorbol esters. These results hence suggested that the DPPIV enzymatic activity of CD26 serves to enhance cellular responses of CD26 Jurkat transfectants to external stimuli through CD26 and/or the CD3/TCR complex, resulting in augmented IL-2 production and T cell activation.

Additional evidence of an essential role for the DPPIV enzymatic activity of CD26 in T cell proliferation is seen from studies involving soluble CD26 molecules. Experiments using either soluble recombinant CD26 that contains DPPIV (sCD26+DPPIV+) or soluble CD26 mutated at the DPPIV site (sCD26+DPPIV-) have demonstrated that the presence of DPPIV enzymatic activity enhances peripheral blood T cell proliferation induced by the recall antigen tetanus toxoid. However, sCD26+DPPIV+ itself does not provide a mitogenic signal and does not augment T cell responses to such mitogenic stimuli as phytohemagglutinin and anti-CD3, suggesting that sCD26 may exert its enhancing effect on T cell response to recall antigens via its effect on antigen-presenting cells (Tanaka et al., 1994). Our recent work supports this conclusion by demonstrating that the exogenously added sCD26 directly affects CD14+ peripheral blood monocytes in the early stages of immune response to the recall antigen tetanus toxoid. Furthermore, the M6P/IGFIIIR appears to play a role in the transportation of sCD26 into monocytes, since M6P interferes with its uptake. In addition, the enhancing effect of sCD26 on tetanus toxoid-induced T cell proliferation does not result from trimming of the MHC-bound peptide on the monocyte surface, unlike the case described for another ectopeptidase, CD13 aminopeptidase N (Larsen et al., 1996). Rather, exogenously added sCD26 upregulates the expression of the costimulatory molecule CD86 on monocytes through its DPPIV activity, both at the protein and mRNA levels. Therefore, these recent data suggested that sCD26, particularly its DPPIV enzymatic activity, enhances T cell immune response to recall antigens through its direct effect on antigen-presenting cells (Ohnuma et al., 2001).

Multiple studies using chemical inhibitors of DPPIV activity of CD26 have also demonstrated that DPPIV plays a key role in T cell activation. For example, the chemical inhibitor Pro-boroPro inhibits antigen-specific T cell proliferation and IL-2 production in vitro (Flentke...
et al., 1991). In addition, the synthetic competitive DPPIV inhibitors Lys[Z(NO₂)]-thiazolidide, Lys[Z(NO₂)]-piperidide, and Lys[Z(NO₂)]-pyrrolidide significantly inhibit DNA synthesis and the production of IL-2, IL-10, IL-12 and IFN-gamma in pokeweed mitogen (PWM)-stimulated purified T lymphocytes. On the other hand, these inhibitors enhance secretion of the immunosuppressive cytokine TGF-beta1 by the PWM-stimulated T cells, hence suggesting that TGF-beta1 helps regulate CD26/DPPIV effect on T cell function (Reinhold et al., 1997). Furthermore, these DPPIV inhibitors directly affect such early signaling events in T cells as tyrosine phosphorylation and Ca²⁺ flux, further support for the notion that CD26 affects T cell function (Kahne et al., 1998). DPPIV inhibitors also inhibit lymphocyte proliferation induced by mitogenic lectins and immunoglobulin production by pokeweed mitogen-stimulated lymphocytes in vitro (Schon et al., 1987). DPPIV inhibitors have also been used in vivo studies to evaluate their potential effectiveness in treating clinical conditions involving immune activation. Lys[Z(NO₂)]-pyrrolidide treatment blocks DPPIV activity in vivo and results in the clinical improvement of experimental autoimmune encephalomyelitis, while increasing the secretion of the immunosuppressive cytokine TGF-beta1 (Steinbrecher et al., 2001). Meanwhile, treatment with the low-molecular-weight inhibitor prodipine suppresses systemic DPPIV activity, impairs host cytotoxic T lymphocyte responses and abrogates acute rejection of cardiac allografts in rat recipients, leading to prolonged allograft survival (Korom et al., 1997). Besides providing additional evidence of a key role for CD26/DPPIV in immune activation in vivo, these findings demonstrate that specific targeting of CD26/DPPIV to induce immunosuppression may have potential effectiveness in selected clinical conditions involving active immune system.

However, DPPIV enzyme activity does not appear to be necessary for CD26-mediated T cell function in certain experimental conditions. At short incubation time, inhibitors blocking up to 95% of DPPIV enzyme activity do not inhibit CD26-mediated cytotoxicity of T cell clones against Fc-receptor bearing target cells (Hegen et al., 1993; von Bonin et al., 1998). Meanwhile, studies using T cell receptor positive-mouse T cell hybridoma transfected with human CD26 showed that cells expressing mutant CD26 lacking DPPIV enzyme activity are able to be stimulated with anti-CD26 mAbs, as are cells expressing wild type CD26 (Steeg et al., 1995). Also, work using murine hybridomas transfected with human CD26 molecules demonstrated CD26 co-stimulatory effect is still present in mutants lacking most of the extracellular domain of the molecule, with the membrane proximal glycosylation rich domain of CD26 being sufficient for its co-stimulatory activity (Huhn et al., 2000). The reasons for the differing results regarding the role of DPPIV enzyme activity in CD26-mediated T cell function are not yet clearly elucidated. Relative specificity and non-specific toxicity of the chemical inhibitors of DPPIV used in various experiments are potential confounding factors. In addition, the biological effect of DPPIV activity may depend on the particular experimental model and to particular growth conditions, due to the potential association of the enzyme activity to differentially manifested cellular processes.

CD26-associated molecules

**CD26-CD45**

The fact that its cytoplasmic domain contains only 6 amino acids strongly suggests that CD26 is able to convey intracellular signals through its physical and functional association with structures involved in the signal transduction cascades. Recent work has indeed demonstrated that CD26 interacts with several molecules with key roles in T cell function. We showed that CD26 is co-modulated on the T cell surface with CD45RO, a membrane-linked tyrosine phosphatase critical to T cell signal transduction, while anti-CD26 antibodies are capable of precipitating CD45 from T cell lysates (Torimoto et al., 1991). Moreover, antibody-induced modulation of CD26 results in augmented phosphorylation of CD3-zeta tyrosine residues and increased p56lck tyrosine kinase activity. Since CD45 regulates the phosphorylation status of these structures, these findings suggest that one possible mechanism for CD26 ability to convey activation signals involves its association with the membrane-linked protein-tyrosine-phosphatase CD45, perhaps by modulating CD45PTPase enzymatic activity or by affecting the accessibility of critical substrates. Recently, we demonstrated that CD26 localizes into lipid rafts, and targeting of CD26 to rafts is necessary for CD26-mediated signaling. We found that the presence of CD26 in rafts is necessary for the tyrosine phosphorylation of several cellular proteins in T cells, including Erk 1/2, TCR-zeta, and ZAP70, following anti-CD26 binding. Importantly, disruption of the structure of membrane rafts with such agents as cytochalasin D and nystatin markedly prevents the tyrosine phosphorylation of these molecules. Moreover, anti-CD26-induced aggregation of CD26 causes co-localization of CD45 molecules into lipid rafts, a process that involves CD26 binding to the cytoplasmic domain of CD45. Therefore, CD26-CD45 interaction may account for changes in the status of key signaling proteins, such as increased p56lck activity, enhanced CD3-zeta chain phosphorylation and intracellular calcium mobilization, leading to the...
amplification of in vivo immune response (Ishii et al., 2001).

**CD26-ADA**

CD26 also physically associates with adenosine deaminase (ADA) as the ADA-binding protein (Kameoka et al., 1993; Morrison et al. 1993; De Meester et al., 1994). A key role for ADA itself in lymphocyte physiology is indicated by the fact that ADA deficiency is a cause of human severe combined immunodeficiency disease (SCID) (Morimoto and Schlossman, 1998). Anti-CD26 antibody co-immunoprecipitates a 43 kD band, which is identified as ADA by partial sequencing. ADA is associated with CD26 on T cell lines lacking either ADA or DPPIV enzyme activity, indicating that the physical association between these two molecules does not require their respective enzymatic activity. In addition, CD26 and ADA appear to co-localize on the cell surface but not inside the cells in in vitro studies, thereby suggesting that CD26 does not function to transport ADA to the cell surface, but rather binds to ADA present in the culture medium. Consistent with this notion are the findings that CD26 can associate with ADA secreted from other cells to form a CD26/ADA complex on the cell surface. In the absence of cell surface ADA, adenosine inhibits T cell proliferation and IL-2 production induced by various stimuli. However, cells expressing ADA and CD26 on the surface display greater resistance to adenosine-mediated inhibition. These data hence suggested an important role for the cell surface complex of ADA/CD26 in immunoregulation, potentially negating the suppressive effect of endogenous adenosine on T cell function (De Meester et al., 1994; Dong et al., 1996). Additionally, CD26-associated ADA has been suggested to have co-stimulatory function independent of its catalytic activity (Martin, 1995; Franco et al., 1998). Whereas many of the available anti-CD26 monoclonal antibodies recognize epitopes in the glycosylation-rich region of the antigen, the ADA binding site appears to be located in the cysteine-rich region of the extracellular domain of CD26 (Huhn et al., 1999). Meanwhile, studies using CD26 deletion, human-rat CD26 swap mutants and point mutations showed that residues L340, V341, A342, and R343 on the CD26 molecule are essential amino acids for ADA binding (Dong et al., 1997). Another study also reported that binding to CD26 by ADA and anti-CD26 antibodies that inhibit ADA binding involves discontinuous and overlapping sites on a predicted beta-propeller domain. Specifically, residues Leu294 and Val341 of CD26 appear to be essential for ADA binding, while the epitope of anti-CD26 antibodies that inhibit ADA binding contains amino acid region Leu340 to Arg343 as well as amino acids Thr440/Lys441 (Abbott et al., 1999).

A potential consequence of the interaction between CD26 and ADA in the clinical setting may be reflected in situations involving the use of ADA inhibitors to treat selected human diseases. For example, at MD Anderson Cancer Center, we conducted a phase II study to examine the effect of the ADA inhibitor pentostatin on relapsed T cell lymphomas (Dang et al., 2001). The objectives of the study are to determine the effectiveness of pentostatin in these diseases and to evaluate the lymphopenic effect of the drug on CD26+ T lymphocytes. Interim analysis of 11 evaluable cases showed an overall response rate of 45%. Intriguingly, pentostatin preferentially reduces the level of CD26+ T lymphocytes in the patients’ peripheral blood and bone marrow in both responders and non-responders. While the mechanism behind the preferential reduction of CD26+ T lymphocytes induced by treatment with the ADA inhibitor pentostatin is unclear at the present time, a potential explanation may be due to the physical association between CD26 and ADA on the T lymphocyte surface. Since the CD26+ T cell population represents activated T lymphocytes, with the CD4+CD26+ subset belonging to the helper/memory population, a decrease in the level of CD26+ T lymphocytes induced by pentostatin has potentially significant implications for immune system function in treated patients.

**CD26-M6P/IGFIIR**

Another molecule that binds to cell surface CD26 is recently identified to be the mannose-6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR), with the M6P residues in the CD26 carbohydrate moiety being critical for this interaction (Ikushima et al., 2000). Activation of peripheral blood T lymphocytes results in the mannose-6-phosphorylation of CD26. In addition, antibody-mediated crosslinking of CD26 induces co-localization of CD26 with M6P/IGFIIR, along with capping and internalization of CD26. Importantly, both the process of antibody-mediated internalization of CD26 and the T cell proliferation induced by CD26-mediated costimulation are inhibited by the addition of M6P, but not by glucose-6-phosphate or mannose-1-phosphate. These data therefore indicated that the binding of CD26 with M6P/IGFIIR is important for antibody-mediated CD26 internalization and that the interaction between mannose-6-phosphorylated CD26 and M6P/IGFIIR has an essential role in CD26-mediated T cell activation.

**CD26-CXCR4**

Recent work has also shown that CD26 physically and functionally associate with the chemokine receptor CXCR4 on the surface of human peripheral blood lymphocytes (Herrera et al., 2001). CD26 colocalizes with CXCR4 on T and B cell lines, while the two molecules co-immunoprecipitate from cellular membranes. Down-regulation of CXCR4 induced by its physiological ligand stromal cell-derived factor 1 alpha (SDF-1 alpha) also results in co-internalization of CD26.
on lymphocytes. Furthermore, treatment with SDF-1 alpha induces the formation of pseudopodia in which both CD26 and CXCR4 co-localize and in which ADA is absent. Since CD26 is capable of cleaving SDF-1 alpha and thereby affecting its biological function (Ohtsuki et al., 1998; Proost et al., 1998a; Shioda et al., 1998), the CD26/CXCR4 complex may be a functional unit allowing CD26 to directly modulate SDF-1 alpha activity.

**CD26 and cleavage of cytokines**

Recent studies have demonstrated that CD26/DPPIV cleaves a number of cytokines to alter their biological functions, by altering their chemotactic potency, impairing their signaling effects and modifying their receptor specificity. Initial studies by Oravecz et al. (Oravecz et al., 1997) demonstrated that the receptor specificity of CCL5 (regulated on activation, normal T cell expressed and secreted, RANTES) is altered by DPPIV-mediated cleavage, as signaling through CCR1 and CCR3 by RANTES is abolished by the 2 amino acid truncation mediated by CD26/DPPIV while signaling through CCR5 is not affected. Further studies showed that CD26/DPPIV-mediated cleavage of RANTES influences its activity in monocyte chemotaxis and HIV-1 infection (Proost et al., 1998b). Work done subsequently by various investigators further showed that an emerging aspect of CD26 biology is the ability of its intrinsic DPPIV enzyme activity to cleave selected chemokines to alter their biological effects. For example, the chemotactic and anti-HIV activities of mature SDF 1-alpha are both abolished by cleavage of membrane-bound as well as soluble CD26/DPPIV (Ohtsuki et al., 1998; Proost et al., 1998a; Shioda et al., 1998). In addition, eotaxin, an important mediator of allergic reactions due to its ability to attract eosinophils, TH2 lymphocytes and basophils, is a substrate for CD26/DPPIV. DPPIV-cleaved eotaxin exhibits reduced chemotactic activity for eosinophils and impaired binding and signaling via its receptor CCR3, while differentially regulating the chemotactic and antiviral properties of intact eotaxin (Struyf et al., 1999). Other chemokines demonstrated to exhibit altered receptor specificity and biological activity following CD26/DPPIV-mediated cleavage include macrophage-derived chemokine (MDC) (Lambeir et al., 2001; Proost et al., 1999), LD78 beta isoform of macrophage inflammatory protein-1 alpha (Proost et al., 2000; Struyf et al., 2001), and the interferon-inducible chemokines (Proost et al., 2001).

**CD26 and cancer**

CD26 binding to the extracellular matrix is an aspect of its biology with potential implications in neoplastic diseases. CD26 binds to collagen and fibronectin in a variety of experimental conditions (Bauvois, 1988; Piazza et al., 1989; Dang et al., 1990d; Loster et al., 1995). Through its interaction with the extracellular matrix proteins, CD26 may further affect immune regulation by recruiting activated lymphoid effectors to

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**Fig. 1.** Enhanced p21 expression following anti-CD26 treatment. T-cell CD30- anaplastic large cell lymphoma Karpas 299 cells were incubated overnight at 37 °C with medium alone, medium containing isotype control mAb or the anti-CD26 monoclonal antibody 1F7 (2 µg/ml). Cells were then harvested, and SDS-PAGE and immunoblotting studies were performed. Adapted from Ho et al., 2001.
inflammatory sites (Masuyama et al., 1992). In addition, this interaction may play a role in the clinical behavior and progression of selected cancers. For example, CD26 molecules found on lung endothelial cells specifically interact with fibronectin assembled on breast cancer cell surface, potentially promoting tumor cell adhesion and metastasis (Cheng et al., 1998). Intriguingly, interference with this interaction inhibits tumor metastasis in animal models (Abdel-Ghany et al., 1998).

Besides influencing tumor cell progression through its interaction with the extracellular matrix, CD26 may have a role in the development of certain cancers, as suggested by several studies examining its expression in selected tumors. Most lung adenocarcinomas are DPPIV+, while other histological types of lung carcinomas are DPPIV- (Asada et al., 1993). In addition, CD26 is highly expressed on differentiated thyroid carcinomas but is absent in benign thyroid diseases (Tanaka et al., 1995). Aberrant CD26 expression is also seen in hepatocellular carcinomas, with its expression in most cases examined deviating distinctly from its expression in normal hepatocytes (Stecca et al., 1997). In other instances, CD26 expression is down-regulated during neoplastic transformation and tumor progression, and up-regulated during cellular differentiation. DPPIV expression is significantly decreased in metastatic prostate cancer specimens as compared to primary tumors (Bogenrieder et al., 1997), while colon cancer cell lines undergoing enterocytic differentiation show enhanced DPPIV expression (Yoshioka et al., 1991; Darmoul et al., 1992). Similarly, CD26 level is negatively correlated with melanoma growth phase, being expressed in cases involving the in situ or invasive radial growth phase to being progressively lost in late tumor progression stages, i.e. the vertical growth phase and metastatic phase (Van Den Oord, 1998). Meanwhile, in a series of elegant experiments, Houghton and colleagues demonstrated a potentially essential role for CD26 in melanoma development (Houghton et al., 1988; Morrison et al., 1993; Wesley et al., 1999). In vitro malignant transformation of melanocytes results in loss of expression, while CD26 reexpression in transfected melanoma cells leads to phenotypic changes characteristic of normal melanocytes, further supporting the view that regulation of CD26 expression is an important event in tumor pathogenesis. Meanwhile, serum levels of CD26/DPPIV are altered in cancer patients compared with normal controls (Uematsu et al., 1996; Cordero et al., 2000) and in certain instances,
serum CD26 levels have been reported to correlate with tumor clinical behavior (Cordero et al., 2000). For example, in patients with colorectal cancer, pre-operative serum CD26 levels appear to have potential diagnostic and prognostic value (Cordero et al., 2000). Compared with healthy donors, patients with colorectal carcinoma have significantly lower levels of serum CD26. In addition, serum CD26 level is a distinct variable that has greater diagnostic efficiency than that demonstrated by other markers, especially in patients with early stage disease.

Besides its role in the pathophysiology of certain solid tumors, CD26 may also have a role in the development of selected hematological malignancies. B-chronic lymphocytic leukemia (B-CLL) cells express higher levels of CD26 protein and mRNA transcripts as compared to normal resting B cells (Bauvois et al., 1999). Moreover, treatment with interferons and retinoic acid enhances CD26 expression in B-CLL cells by engaging Stat1 alpha-mediated signaling (Bauvois et al., 2000). In addition, CD26 expression on T cell malignancies appears restricted to aggressive pathologic entities such as T cell lymphoblastic lymphomas/acute lymphoblastic leukemias (LBL/ALL) and T cells CD30+ anaplastic large cell lymphomas. Significantly, within the T cell LBL/ALL subset, CD26 expression is an independent marker of poor prognosis; although these findings were based on relatively small sample sizes and limited clinical data (Carbone et al., 1994, 1995). Recent work has also confirmed that the majority of patients with T cell ALL express CD26 on the tumor cell surface (Klobusicka and Babusikova, 1999). A strong correlation between CD26 antigen expression and DPPIV positivity in the majority of T-lymphoblasts is

**Fig. 4.** Effect of CD26/DPPIV expression on doxorubicin-mediated cell cycle arrest at G2-M. In A, CD26 Jurkat transfectants were cultured at 37 °C in doxorubicin-containing media for 24 h. Cells were then harvested, and cell cycle analyses were done with propidium iodide staining. Data are representative of three separate experiments. Adapted from Aytac et al., 2001.
also noted, while CD26 expression is not detected on DPPIV-negative T lymphoblasts. CD26 and its associated DPPIV enzyme activity are also found on the surface of an aggressive hepatosplenic gamma-delta T-cell lymphoma (Ruiz et al., 1998). Conversely, in the case of the relatively indolent disease mycosis fungoides (MF), the neoplastic cells, which are typically CD4+, most often do not express surface CD26 (Bernengo et al., 2001; Jones et al., 2001). At MD Anderson Cancer Center, we performed flow cytometric characterization of CD26 surface expression in peripheral blood samples of patients with MF and its related disorder Sezary syndrome (SS). In 66 of 69 (96%) samples from 28 patients, we identified the tumor cell population in the peripheral blood as being CD26 negative/dim, distinct from the normal peripheral blood T lymphocytes. In contrast, expression of CD7, a commonly used marker to identify circulating MF/SS cells, is much more variable, allowing recognition of a distinct abnormal T cell population in only 34 of 69 (49%) of involved MF/SS samples (Jones et al., 2001). These findings suggested that CD26 can be a novel marker capable of being used in the clinical setting to identify MF/SS patients with circulating tumor cells. In fact, based on results mentioned above, CD26 is now included as part of the panel of flow cytometric markers used at MD Anderson Cancer Center to routinely screen clinical samples from patients with a confirmed or suspected diagnosis of T cell malignancy.

**CD26 as a potential treatment target in hematological malignancies**

T cell hematological malignancies represent a group of heterogeneous diseases that are generally noted for their aggressive clinical behavior and their relative refractoriness to current treatment modalities (Melnyk et al., 1997; Gisselbrecht et al., 1998). We recently conducted studies investigating the potential role of CD26 as a novel treatment target for selected T cell

![Fig. 5](image-url)
malignancies, using as models human T cell lines. We demonstrated that binding of the anti-CD26 monoclonal antibody 1F7 inhibits the growth of the human CD30+ anaplastic large cell T-cell lymphoma cell line Karpas 299 in both in vitro and in vivo experiments (Ho et al., 2001). In vitro studies showed that binding of 1F7 induces cell cycle arrest at the G1-S checkpoint, associated with enhanced expression of the key cyclin-dependent kinase inhibitor p21 that is dependent on de novo protein synthesis (Fig. 1). Our work also suggested that the ability of anti-CD26 antibody to inhibit cell growth is epitope-dependent, since the anti-CD26 monoclonal antibody 5F8, recognizing a distinct epitope than that recognized by 1F7, is much less potent in inducing growth arrest. This differential effect is concordant with previous work examining the role of various epitopes of CD26 in human T cell function, with the 1F7 containing the CD26 domain being consistently demonstrated to be capable of transmitting intracellular signals to modify cellular activity (Dang et al., 1990a; Dong et al., 1998; Torimoto et al., 1992). Expanding on the in vitro results, we subsequently conducted experiments with a severe combined immunodeficient mouse tumor model in which SCID mice were treated with 1F7 following injection with Karpas 299 cells. Our findings demonstrated that 1F7 treatment significantly enhances survival of tumor-bearing mice by inhibiting tumor formation, suggesting therefore that anti-CD26 antibody treatment may have potential clinical use for CD26+ T cell hematological malignancies (Figs. 2, 3).

As previously reported (Morrison et al., 1993; Carbone et al., 1995), CD26 expression is associated with changes in tumor cell line behavior in vitro, and the clinical behavior of certain T-cell tumors appears to correlate with differences in CD26 expression level. Furthermore, the DPPIV enzymatic activity of CD26 has

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an essential role in the biology of selected lymphoid cell lines (Reinhold et al., 1994). These observations led to our hypothesis that CD26 expression or DPPIV activity affects tumor cell biology and potentially cellular sensitivity to antineoplastic agents. Doxorubicin is a widely used anthracycline antibiotic in cancer therapy and is a main component of chemotherapeutic regimens for the treatment of hematological malignancies and selected solid tumors. Using CD26-negative parental Jurkat and Jurkat cells stably transfected with wild type CD26 antigen (wtCD26); mutant CD26 molecule containing an alanine at the putative catalytic serine residue at position 630, resulting in a mutant CD26-positive/DPPIV-negative Jurkat transfectant (S630A); or mutant CD26 molecule containing point mutations at the ADA-binding residues 340-343, with amino acids L340, V341, A342 and R343 being replaced by amino acids P340, S341, E342, and Q343, resulting in a mutant CD26-positive/DPPIV-positive Jurkat transfectant incapable of binding ADA (340-4), we investigated the effect of CD26/DPPIV on cellular sensitivity to doxorubicin (Aytac et al., 2001). Evaluating doxorubicin-induced cell cycle arrest at the G2-M checkpoint, we found that wtCD26 cells exhibit greater drug sensitivity than parental cells; moreover, S630A transfectants are less sensitive to doxorubicin, like parental cells. Conversely, 340-4 transfectants display greater sensitivity to doxorubicin, similar to the wtCD26 transfectants (Fig. 4). We also demonstrated that doxorubicin treatment has greater effect on the key regulators of G2-M in wtCD26 cells than in parental or S630A cells. Specifically, in wtCD26 as compared to parental and S630A cells, we observed greater phosphorylation of the inhibitory residues of Thr14Tyr15 of p34cdc2, associated with inhibition of p34cdc2 kinase activity, enhanced serine-216 phosphorylation of cdc25C, and elevated level of cyclin B1 (Fig. 5). Since our data indicated that the presence of DPPIV enzymatic activity is essential to enhanced doxorubicin sensitivity, we investigated the effect of exogenous soluble DPPIV (sDPPIV) on Jurkat cells. We found that the addition of exogenous sDPPIV also enhances Jurkat sensitivity to doxorubicin (Fig. 6). Importantly, we demonstrated that B cell lymphoid cell lines Jiyoye and Namalwa also exhibit CD26-associated enhancement in drug sensitivity, with potential implications for CD26 role in the clinical setting (Fig. 7). Taken together, our recent observations strongly suggest that CD26 is an appropriate molecular target for novel treatment approaches for T cell lymphoid malignancies as well as selected other neoplasms.

In this review, we focus on key aspects of CD26 function, presenting data that expand on its well-established role in immune regulation as well as findings that indicate an increasingly significant involvement in cancer biology. Meanwhile, our recent studies suggest that CD26 may be an appropriate target for novel treatment modalities for selected hematological malignancies. In view of its multifaceted roles in immune function and cancer, future therapeutic strategies targeting CD26 and its intrinsic DPPIV enzyme activity may be of potential value in the clinical setting.

Acknowledgements. NH Dang is supported by a grant from the V Foundation and the MD Anderson Physician-Scientist Program. CMorimoto is supported by a grant-in-aid from the Ministry of Education, Science, Sports and Culture and from the Ministry of Health, Labor and Welfare, as well as by National Institute of Health Grant AR33713. We also would like to thank Drs. Ugur Aytac, Linus Ho, Kei Ohnuma and Kazuya Sato for their contribution and helpful discussion.
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CD26 in immune function and cancer


CD26 in immune function and cancer


Accepted May 31, 2002