Differential Binding of Drugs Containing the NGR Motif to CD13 Isoforms in Tumor Vessels, Epithelia, and Myeloid Cells

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ABSTRACT

The NGR peptide motif is an aminopeptidase N (CD13) ligand that targets angiogenic blood vessels. NGR-containing peptides have proven useful for delivering cytotoxic drugs, proapoptotic peptides, and tumor necrosis factor-α (TNF) to tumor vasculature. Given that CD13 is not only expressed in the angiogenic endothelium but also in other cell types, the mechanism(s) for the tumor-homing properties of NGR-drug conjugates remains elusive. We have examined the expression of CD13 in normal and neoplastic human tissues and cells by using two anti-CD13 monoclonal antibodies. The immunoreactivity patterns obtained with cultured cells and tissue sections from kidney, breast, and prostate carcinomas suggest that different CD13 forms are expressed in myeloid cells, epithelia, and tumor-associated blood vessels. Both, direct binding assays with a CNRGC-G-TNF conjugate (NGR-TNF) and competitive inhibition experiments with anti-CD13 antibodies showed that a CD13 isoform expressed in tumor blood vessels could function as a vascular receptor for the NGR motif. In contrast, CD13 expressed in normal kidney and in myeloid cells failed to bind to NGR-TNF. Consistently with these results, neither murine 125I-NGR-TNF nor 125I-TNF accumulated in normal organs containing CD13-expressing cells after administration to mice. These findings may explain the selectivity and the tumor-homing properties of NGR-drug conjugates and may have important implications in the development of vascular-targeted therapies based on the NGR/CD13 system.

INTRODUCTION

In vivo screening of peptide-phage libraries has proven to be a powerful tool for the discovery of ligands that selectively home to tumor vessels (1–3). Among the targeting probes identified thus far, a peptide containing the NGR motif has been coupled to different anticancer compounds, such as doxorubicin, proapoptotic peptides, and TNF, and shown to enable targeted delivery of these drugs to tumor vessels (4–6), e.g., the antitumor activity of NGR-TNF in animal models is 10–30 times stronger than that of TNF, whereas its toxicity is similar (5). Evidence suggests that aminopeptidase N (CD13) is an important receptor targeted by NGR-containing conjugates (5, 7). However, in addition to endothelial cells of angiogenic vessels, most cells of myeloid origin, including monocytes, macrophages, granulocytes, and their hematopoietic precursors, express CD13 (8–10). This peptidase is also abundantly expressed in the brush border of epithelial cells from renal proximal tubules and small intestine, in prostatic epithelial cells, in bile duct canaliculi, in mast cells, and, in some cases, in fibroblasts and smooth muscle cells (9, 11–14). In most of these cells, CD13 immunoreactivity localizes to the cell membrane or in the apical part of epithelial cells (15). However, cytoplasmic staining (15–17) and detection of soluble CD13 in human plasma have also been reported (18, 19). In addition, stromal fibrillar components of certain connective tissues contain CD13 immunoreactivity (20). Given the widespread distribution of CD13 throughout the body, the mechanisms involved in the tumor-homing properties of NGR-drug conjugates still remain unclear.

To analyze this specificity, we have investigated the expression of CD13 in various human tissues and tumors using two mAbs and studied the CD13/NGR-TNF interaction in tissues expressing high levels of CD13. In addition, we have studied the interaction of human NGR-TNF with normal and neoplastic tissues in vitro, as well as the biodistribution of radio-labeled murine NGR-TNF and TNF in a mouse model. Our findings suggest that different CD13 isoforms are expressed within tumor vessels, normal epithelia, and myeloid cells. Moreover, we show that NGR-TNF can bind to vessels within or close to tumor nodules but not to vessels in normal tissues and to epithelial cells of CD13-rich organs. On the basis of these observations, we propose that the mechanism involved in the tumor-homing properties of NGR-drug conjugates relies on recognition of a CD13 isomorph selectively expressed within tumor associated vessels.

MATERIALS AND METHODS

Cell Lines and Reagents. Mouse RMA-T lymphoma cells of C57BL/6 origin (21) were prepared and cultured as described previously (22). Molt-4 cells (acute lymphoblastic leukemia) transfected with the human CD13 cDNA (CD13/Molt-4) were prepared as described (7, 23). THP-1 (acute monocytic leukemia) cells were obtained from Dr. F. Blasi (San Raffaele H Scientific Institute, Milan, Italy). Human macrophages were isolated from peripheral blood mononuclear cells by standard techniques. mAb 13C03 (antihuman CD13, IgG1) was from Neomarkers, LabVision Corp. (Fremont, CA); mAb WM15 (antihuman CD13, IgG1) was from Pharmingen (San Diego, CA); mAb JC/70A (antihuman CD31, IgG1) was from DAKO (Copenhagen, Denmark); mAb QBEND10 (antihuman CD34, IgG1) was from Serotec (Oxford, United Kingdom); mAb 78 (IgG1) was kindly supplied by Dr. E. Barbanti (Pharmacia-Upjohn, Milan, Italy). mAb 78 is an antihuman TNF antibody able to form stable complexes with soluble TNF (Kd: 3.2 × 10^10 M^-1) and neutralize its interaction with membrane receptors (24).

Human recombinant TNF and NGR-TNF (consisting of human NTF, fused with the COOH terminus of CNRGC-G) were prepared by recombinant DNA technology and purified from Escherichia coli cell extracts, as described (5). The protein concentration was measured with a commercial protein quantification assay kit (Pierce, Rockford, IL). The in vitro cytolytic activity of NGR-TNF, estimated from a standard cytolytic assay with L-M mouse fibroblasts (25), was (4.6 × 10^10) ± 0.4 units/mg, whereas that of purified human TNF was (5.45 × 10^10) ± 3.1 units/mg. The hydrodynamic volume of NGR-TNF was similar to that of TNF, a homotrimeric protein (26), in gel filtration chromatography on a Superdex 75 HR column (Pharmacia, Uppsala, Sweden). The molecular mass of NGR-TNF subunits was 17938.0 ± 1.9 kDa (expected for NGR-TNF, 17939.4 kDa) by electrospray mass spectrometry (27). Complexes of human NGR-TNF and anti-TNF mAb 78 (termed NGR-TNF/78) were prepared by incubating a mixture of 1 μg/ml NGR-TNF and 1 μg/ml mAb 78, both in PBS containing 2% BSA for 20 min (20°C). A mixture of TNF and mAb 78 (termed TNF/78) was prepared in the same way using human TNF instead of NGR-TNF.
Recombinant murine IFN-γ fused with CNGRCG (NGR-IFN-γ) was prepared by recombinant DNA technology, essentially as described for NGR-TNF, and purified by immunoaffinity chromatography using an antimouse IFN-γ. Reducing and nonreducing SDS-PAGE of the final product showed a single band of 16 kDa. The synthetic peptide CgA (60–68), corresponding to the chromogranin A fragment 60–68, was prepared as described previously (28).

**Immunohistochemical Studies.** Surgical specimens of human tissues were obtained from the Department of Histopathology, San Raffaele H Scientific Institute. Sections (5–6 μm thick) of Bouin-fixed (4–6 h), paraffin-embedded specimens were prepared and adsorbed on polylysine-coated slides. Antigens were detected using the avidin-biotin complex method as follows: tissue sections were rehydrated using xylenes and a graded alcohol series, according to standard procedures. Tissue sections were placed in a vessel containing 1 mM EDTA and boiled for 7 min using a microwave oven (1000 W). The vessel was then refilled with 1 mM EDTA and boiled again for 5 min. The tissue sections were left to cool and incubated in PBS containing 0.3% hydrogen peroxide for 15 min to quench endogenous peroxidase. The samples were then rinsed with PBS and incubated with 100–200 μl of PBS containing 2% BSA (PBS-BSA; 1 h at room temperature), followed by the primary antibody or NGR-TNF/78 complex in PBS-BSA (overnight at 4°C). The slides were then washed three times (3 min each) with PBS and incubated with PBS-BSA containing 2% normal horse serum (PBS-BSA-normal horse serum; Vector Laboratories, Burlingame, CA) for 5 min. The solution was then replaced with

Fig. 1. Expression of CD13-, CD31-, and NGR-TNF-binding sites in normal (left panels) and neoplastic (right panels) renal tissues. Representative photomicrograph of tissues immunostained with mAb 13C03 (anti-CD13, diluted 1:2; A and B), 0.5 μg/ml WM15 (anti-CD13; C and D), JC/70A (anti-CD31, diluted 1:50; E and F), 1 μg/ml NGR-TNF/78 (G and H), 1 μg/ml TNF/78, (I and L), and 1 μg/ml mAb 78 (anti-TNF; M and N). g, kidney glomeruli; t, tumor cells; arrowheads, vessels. A–N, ×400.
3 µg/ml biotinylated horse antimouse IgG (H+L; Vector Laboratories) in PBS-BSA-normal horse serum and further incubated for 1 h at room temperature. The slides were washed again and incubated for 30 min with Vectastain Elite Reagent (Vector Laboratories) diluted 1:100 in PBS. A tablet of 3,3′-diamino-benzidine-tetrahydrochloride (Merck, Darmstadt, Germany) was then dissolved in 10 ml of deionized water containing 0.03% hydrogen peroxide, filtered through a 0.2-µm membrane, and overlaid on tissue sections for 5–10 min. The slides were washed as above and counterstained with Harris’ hematoxylin.

**FACS Analysis.** Expression of CD13- and NGR-TNF-binding sites by cultured human macrophages (stimulated with 1 µg/ml lipopolysaccharide for 16 h), THP-1, MOLT-4, and CD13/MOLT-4 cells was measured by FACS. Before analysis, the cells were preincubated with Dulbecco’s PBS (BioWhitaker) containing 30% human serum and 2% fetal bovine serum for 5 min, followed by 1 µg/ml WM15, 1 µg/ml NGR-TNF/78, or mAb 13C03 (diluted 1:2) in the same buffer (14 min, 4°C). After washing, the cells were incubated with a goat antimouse-FITC secondary antibody (Sigma Chemical Co.) diluted in the same buffer (14 min, 4°C) and fixed with 2% formaldehyde in PBS.

**In Vivo Biodistribution Studies.** Murine 125I-NGR-TNF (1.41 µCi/µg) and 125I-TNF (1.8 µCi/µg) were prepared using Iodo-Gen precoated tubes (Pierce) according to the manufacturer’s instructions. 125I-NGR-TNF or 125I-TNF was injected i.p. into C57BL/6 mice weighing 19–20 grams (Charles River Laboratories, Calco, Italy; 1/2 g in 125 µl of 0.9% sodium chloride containing 0.1 mg/ml endotoxin-free human serum albumin). After 6 or 24 h, the animals were sacrificed and surgically dissected. The radioactivity in tissues was quantified by γ-scintillation counting of 125I.

### RESULTS

**Different CD13 Forms Are Expressed within Tumor Vessels and Epithelia.** The expression of CD13 in normal and neoplastic tissues was evaluated by indirect immunohistochemistry using two anti-CD13 mAbs (13C03 and WM15). The immunoreactivity of these mAbs with a renal cell carcinoma and non-neoplastic kidney was first investigated. The 13C03 epitope, but not the WM15 epitope, was expressed in the brush border of the renal proximal tubule epithelial cells (Fig. 1, A and C). The weak cytoplasmic staining of kidney tubules by WM15 is not likely to be specific, as it was observed also when the antibody was omitted (data not shown). Although none of these epitopes were expressed by neoplastic cells (Fig. 1, B and D), both epitopes were expressed by stromal cells within and around the tumor nodules; arrowheads, vessels. A, B, and D–H, ×400; C, ×630.

**Table 1 Binding of antibodies to renal cell carcinoma vessels (mAb WM15) and proximal tubules of normal kidney (mAb 13C03), by immunohistochemistry, in the presence of various competitors**

<table>
<thead>
<tr>
<th>Competitor</th>
<th>WM15</th>
<th>13C03</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NGR-TNF (25 µg/ml)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>NGR-IFNγ (50 µg/ml)</td>
<td>–</td>
<td>NDb</td>
</tr>
<tr>
<td>CNGRC (100 µg/ml)</td>
<td>–</td>
<td>NDb</td>
</tr>
<tr>
<td>TNF (25 µg/ml)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Human serum albumin (25 µg/ml)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Synthetic CgA (60–68; 100 µg/ml)</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
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a The competitor in PBS containing 2% BSA was added in the blocking step and mixed with the primary antibody.
b ND, not determined.
neoplastic lesion. The majority of stained cells in tumor stroma corresponds to endothelial cells, as suggested by similar staining patterns obtained with an anti-CD31 mAb, a well-known marker of endothelial cells (Fig. 1F). Of note, both epitopes were either not expressed or expressed very weakly in vessels of normal kidney, either within renal glomeruli or in connective tissue (Fig. 1, A and C). These results suggest that: (a) different antigenic forms of CD13 are expressed within normal epithelial cells and endothelial cells of tumor-associated vessels; (b) the 13C03 epitope is expressed on both isoforms; and (c) the WM15 epitope is differentially expressed on these isoforms.

A CD13 Isoform Associated with Tumor Vessels Is a Receptor for NGR-TNF in Renal Cell Carcinoma. Next, we compared the expression of CD13 with that of NGR-binding sites by immunohistochemistry. Sections of normal kidney and renal cell carcinoma were incubated with human NGR-TNF precomplexed with the antihuman TNF mAb 78 (NGR-TNF/78). Controls with TNF/78 complexes or mAb 78 alone were also included. These complexes offer the advantage that they can be used as a single reagent in parallel with other antibodies. The staining patterns obtained with NGR-TNF/78 were very similar to those of WM15 and distinct from those of 13C03. Like WM15, NGR-TNF/78 interacted with tumor-associated vessels but not with the brush border of renal proximal tubule epithelial cells (Fig. 1, G and H). No binding was observed with controls, such as TNF/78 or mAb 78 alone (Fig. 1, I–N). These results indicate that: (a) the binding of mAb 78 to endogenous TNF is negligible; (b) the binding of TNF/78 to TNF receptors is undetectable; and (c) the binding of NGR-TNF/78 depends on the interaction of the NGR domain with an NGR receptor. Accordingly, the binding of NGR-TNF/78 was totally competed by coincubation with an NGR-IFNγ conjugate (data not shown). These and the above results suggest that the NGR receptor and WM15 antigen colocalize in tumor-associated vessels.

To establish that the CD13 isoform recognized by the WM15 antibody is the one that is recognized by the NGR peptide, we performed competitive binding assays. Various reagents were used in these experiments. The binding of WM15 to tumor-associated vessels was inhibited by NGR-TNF, NGR-IFNγ, and CNGRC, but not by other control reagents lacking the NGR motif (Table 1). In contrast, the binding of 13C03 to the brush border of renal proximal tubule epithelial cells was not competed by NGR-TNF.

In summary, NGR-TNF interacts with a receptor expressed in renal cell carcinoma-associated vessels that corresponds to a CD13 isoform recognized by mAb WM15.

CD13 Expression in Breast and Prostate Cancer Metastasis and in Normal Tissues. To test whether other tumors express the CD13 isoform recognized by NGR-TNF and mAb WM15, we analyzed human breast cancer tissue sections. In this case, an anti-CD34 mAb was used to identify vessels around tumor nodules (Fig. 2A) and in normal adipose tissue in the same tissue section (Fig. 2B). NGR-TNF/78 recognized an antigen on the endothelial lining of vessels close to tumor nodules but did not stain normal vessels present in adipose tissue distant from the tumor (Fig. 2, C and D). Cytoplasmic staining of tumor cells with NGR-TNF/78 but not with TNF/78 or
mAb 78 alone was also observed (Fig. 2, E–G). Binding to vessels and tumor cells was competed with NGR-IFN/H-9253 (Fig. 2H), suggesting that in both cases, binding depended on the NGR domain.

To investigate whether NGR-TNF can also bind to vessels associated with metastatic tumors, we performed immunohistochemical analyses of bone metastasis of human breast and prostate cancer. Analysis of breast cancer bone metastasis showed staining of vessels in a manner reminiscent of that of anti-CD13 mAbs (Fig. 3). The staining was not limited to endothelial cells, as other cells around large vessels, presumably pericytes and smooth muscle cells, were positive. Of note, staining of fibrillar components of fibrotic tissues occurred with both 13C03 and WM15, possibly related to a shed form of the membrane antigen. Interestingly, NGR-TNF/78 bound very weakly or not at all to these forms.

Analysis of prostate cancer bone metastasis showed vessel staining after incubation with NGR-TNF/78 within and around the tumor nodules (Fig. 4). Additionally in this case, staining was not limited to the endothelial cells, as weak staining of neoplastic and stromal cells occurred with both NGR-TNF/78 and WM15.

Normal vessels in kidney, liver, small intestine, and placenta tissue sections were not stained by NGR-TNF/78 (data not shown).

In conclusion, the results suggest that human NGR-TNF can bind to a receptor expressed in vessels within or close to human primary and metastatic tumors but not to normal tissues.

**Characterization of CD13 in Myeloid and Lymphoid Cells.** To further characterize the CD13 isoform recognized by 13C03, WM15, and NGR-TNF/78, we investigated the binding of these reagents to myeloid and lymphoid cells. FACS analysis showed that WM15 recognizes THP-1 cells (acute monocytic leukemia) and lipopolysaccharide-stimulated human macrophages but not Molt-4 cells (acute lymphoblastic leukemia; Fig. 5). In contrast, neither 13C03 nor NGR-TNF/78 immunoreacted with these cell lines. Notably, these reagents failed to react with Molt-4 cells transfected with the human CD13 cDNA. In addition, NGR-TNF (50 μg/ml) did not inhibit the binding of WM15 to THP-1 cells (data not shown). The lack of detectable binding of NGR-TNF/78 to CD13/Molt-4 cells apparently contrasts with the results of previous studies showing that these cells bind phages expressing the CNGRC peptide on their surface (7). An explanation for this discrepancy is that these cells could express a very low fraction of CD13 molecules able to bind both NGR-phages and NGR-TNF/78. Whereas bound phage particles could be easily detected, even when present in low numbers, the binding of low amounts of NGR-TNF is undetectable by FACS. In addition, we have found that immunodetection of phage particles is at least two to three orders of magnitude less sensitive than colony counting in bacterial infection assay. Thus, although a number of phages can be detected as binders compared with controls, the amount of NGR-binding CD13 isofrom is quite low. In any case, the high level of CD13 expression and the lack of detectable binding of NGR-TNF strongly suggest that most CD13 molecules on these cells are unable to bind NGR-TNF.

Granulocytes, monocytes, and lymphocytes present in the vessel

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**Fig. 4.** Expression of CD34- and NGR-TNF-binding sites in bone metastasis of prostate cancer. Representative photomicrograph of tissues immunostained with 1 μg/ml mAb 78 (A), QBEND10 (diluted 1:100; B and C), 1 μg/ml WM15 (D), 1 μg/ml TNF/78 (E), and 1 μg/ml NGR-TNF/78 (F). t, tumor cells; arrowheads, vessels. A and B, ×200; C–F, ×400.

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R. Pasqualini and W. Arap, unpublished observations.

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lumen of kidney, breast, and prostate tissue sections, as well as tumor-infiltrating lymphocytes, were not stained by NGR-TNF/78 in immunohistochemistry assays. Of note, NGR-TNF/78 and WM15 but not TNF/78 immunoreacted with scattered granular cells, presumably mastocytes, within the neoplastic tissues (data not shown).

Biodistribution of 125I-NGR-TNF and 125I-TNF in Mice. To rule out the possibility that CD13 isoforms expressed by epithelial cells in kidney or other CD13-rich organs bind NGR-TNF in vivo, we examined the biodistribution of radiolabeled murine NGR-TNF and TNF in a mouse model. 125I-NGR-TNF and 125I-TNF (1 µg/g mouse) were administered to C57BL/6 mice. The kidney:blood ratios of 125I-NGR-TNF and 125I-TNF 6 or 24 h after administration were similar, suggesting that NGR-TNF does not accumulate in the kidney (Fig. 6). This finding agrees with the results of the above in vitro studies showing a lack of binding of NGR-TNF to CD13 isoforms expressed in normal epithelial cells, tumor-associated vessels, and myeloid cells. Moreover, the results of direct and competitive binding experiments with NGR-TNF and anti-CD13 mAbs suggest that a tumor vessel-related CD13 isoform but not those associated with epithelial and myeloid cells functions as an NGR receptor.

The binding of WM15 to tissues but not 13C03 was competed with NGR-TNF or CNGRC peptide. This suggests that the NGR binding site sterically overlaps, at least partially, with the WM15 epitope on human CD13. The steric overlapping and the similar staining pattern observed with WM15 and NGR-TNF in tissue sections do not necessarily indicate that the structural determinants of WM15 epitope and NGR binding site are identical or have the same accessibility in all organs.

DISCUSSION

NGR-containing peptides, identified by in vivo screening of phage libraries, have proven useful for preparing anticancer drugs that target tumor vessels. Previous studies showed that aminopeptidase N (CD13) is an important receptor for NGR-containing conjugates (7). This protein is an abundant myeloid differentiation antigen that is also expressed by many nonhematopoietic cell types. For example, in the intestinal brush border, aminopeptidase N constitutes 6–8% of the total proteins and is thought to play an important role in protein digestion (9). Other studies have shown a strong expression of CD13 in the brush border of renal proximal tubules (14). Accordingly, using mAb 13C03, we observed marked expression of CD13 antigen in the apical part of epithelial cells of proximal tubules in normal kidney. Nevertheless, the results of immunohistochemical and biodistribution studies indicate that NGR-TNF does not bind to these cells in vitro and does not accumulate in the kidneys or other CD13-rich organs in vivo. These data suggest that NGR-drug conjugates bind receptors with a more restricted expression pattern than CD13. Immunohistochemical analysis of normal and neoplastic tissues with anti-CD13 antibodies that recognize different epitopes (13C03 and WM15) showed that different CD13 isoforms are expressed in normal epithelial cells, tumor-associated vessels, and myeloid cells. Moreover, the results of direct and competitive binding experiments with NGR-TNF and anti-CD13 mAbs suggest that a tumor vessel-related CD13 isoform but not those associated with epithelial and myeloid cells functions as an NGR receptor.
tissues. Indeed, we found that NGR-TNF does not recognize WM15-positive myeloid cells, by FACS analysis, suggesting that the structures of NGR binding site and WM15 epitope are different or differentially accessible in these cells. Thus, the CD13 isoform that binds NGR-TNF is likely a subpopulation of WM15-antigenic forms.

The selectivity of NGR-TNF for tumor-associated endothelial cells was not absolute. Other cells present in the tumor stroma and perinodular connective tissue, likely fibroblasts and mastocytes, were also stained. In addition, we observed a weak reactivity of NGR-TNF with breast and prostate cancer cells. Because it is known that breast cancer cells usually express CD13 (14), it is possible that the staining of tumor cells was related to expression of CD13. We do not know whether these cells are accessible to systemically administered NGR-TNF. If they are, they may represent an additional homing site for targeted delivery of NGR-drug conjugates to tumors. In any case, expression of CD13 by tumor cells is not a prerequisite for tumor targeting, because we have shown previously that even the growth of CD13-negative tumor cells in vivo is efficiently inhibited by NGR-TNF when the tumors are well established and vascularized (5).

It is not known what the structural determinants responsible for differential binding of anti-CD13 mAbs and NGR-TNF to CD13 isoforms are. CD13 is synthesized as a 130-kDa intracellular precursor of 967 amino acids and post-translationally modified in the Golgi to produce a 150–240 kDa mature cell surface molecule (13, 29, 30). In the mature protein, 25–30% of the molecular weight is carbohydrate. Previous investigations have shown that differential or incomplete utilization of O-glycosylation sites results in at least five isoforms that are differentially recognized by antibodies, likely attributable to variable masking of protein epitopes (31). We have found that enzymatic deglycosylation of soluble CD13, isolated from human placenta, with O-glycanase and neuraminidase does not increase the binding of NGR-TNF in various ELISA and ligand blotting assays (data not shown). Moreover, treatment of normal kidney tissue sections with these enzymes did not increase to binding of NGR-TNF to the brush border of the epithelial cells (data not shown). These results argue against the hypothesis that carbohydrates mask the NGR binding site in nonfunctional CD13 isoforms. As alternative hypotheses, it is possible that the differential reactivity of NGR-TNF with different cells is related to different conformations of CD13, e.g., previous studies showed that the binding of CD13 to natural peptide substrates or antibodies induces conformational changes and exposure of cryptic epitopes (32). Alternatively, association with other proteins or elements present in the tumor microenvironment or in tissues can cause differential reactivity or availability to NGR-TNF. Additional work is necessary to clarify this point.

It has been reported that the biological function of CD13 varies depending on tissue microenvironment. CD13 exists as a membrane bound or a soluble form, both of which catalyze the removal of NH2-terminal residues, preferentially neutral, from small peptides (9). It has been suggested that CD13 plays a role in antigen processing (33), in neuropeptide and cytokine degradation (34, 35), in cell cycle control and differentiation (13, 36, 37), and in tumor invasion and extracellular matrix degradation (38, 39). Two recent reports showed that CD13 is also important in angiogenesis and is activated by angiogenic signals (7, 40). The presence of the NGR binding site in tumor vessels could be required for the selective interaction of this site with other compounds potentially mimicked by NGR during angiogenesis and tumor invasion. NGR-TNF could be a valuable tool for the identification of this CD13 isoform, as well as for the characterization of its biological function.

The observation that human NGR-TNF can bind vessels in human neoplastic tissue section could also have important clinical implications. Previously, we showed that systemically administered murine NGR-TNF is 10–30 times more efficient than murine TNF in decreasing the tumor burden of animals bearing well-established lymphomas, but both TNF and NGR-TNF were equally effective in animals with freshly implanted, and hence avascular, tumors (5). Co-administration of murine NGR-TNF with an antitumor CD13 antibody or a CNGRC peptide markedly decreased its antitumor effects, suggesting that the NGR domain of NGR-TNF can interact with murine CD13. The results of the present work suggest that NGR-TNF can also bind human CD13. Human NGR-TNF might have, therefore, more potent antitumor properties than human TNF in patients with vascularized tumors, as observed previously with murine TNF in mice. Indeed, several lines of evidence suggest that the antitumor activity of TNF largely depends on selective obstruction and damage to tumor-associated vessels (41–45). Because the clinical use of TNF as an anticancer drug is limited to locoregional treatments attributable to dose-limiting systemic toxicity, it is possible that targeted delivery of TNF to vessels via the NGR domain may allow systemic and/or locoregional treatment of patients with lower toxicity and stronger antitumor effects.

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REFERENCES

DIFFERENTIAL EXPRESSION OF CD13 ISOFORMS


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