[Frontiers in Bioscience 13, 5571-5579, May 1, 2008]

Host CD147 blockade by small interfering RNAs suppresses growth of human colon cancer xenografts

Dietmar Abraham[1], Karin Zins[1], Mouldy Sioud[2], Trevor Lucas[1], Seyedhossein Aharinejad[1]

[1]Laboratory for Cardiovascular Research, Center of Anatomy and Cell Biology, Medical University of Vienna, Waehringerstrasse 13, A-1090 Vienna, Austria, [2]Department of Immunology, Molecular Medicine Group, The Norwegian Radium Hospital, Montebello, Oslo 0301, Norway

TABLE OF CONTENTS

1. ABSTRACT

Tumor cells can stimulate matrix metalloproteinase (MMP) production by stromal cells through cell–cell interactions mediated by cell adhesion molecules such as extracellular matrix metalloproteinase inducer (human CD147/EMMPRIN, mouse CD147/Basigin). This study sought to characterize whether specific tumor-stromal cell interactions mediated by CD147 promote colon cancer growth by utilizing small interfering (si)RNAs directed against human CD147/EMMPRIN or mouse CD147/Basigin in co-cultures of cancer cells with macrophages and fibroblasts and established human SW620 colon cancer xenograft models in immune deficient mice. We show that blockade of host (mouse) CD147/Basigin expression, but not cancer cell-derived CD147/EMMPRIN, suppresses tumor growth in human colon cancer xenografts. Experiments *in vitro* indicated that colon cancer cell-stromal cell interactions mediated by CD147 lead to increased MMP-2 expression in fibroblasts but not macrophages. Furthermore, expression of host VEGF-A in both fibroblasts and macrophages is independent of CD147 *in vitro* and *in vivo*. Interestingly, inhibition of cancer cell-derived EMMPRIN leads to increased MMP-9 levels *in vivo*. Our findings provide new insights into CD147-mediated tumor-host interactions mediating colon cancer growth.

2. INTRODUCTION

The stromal microenvironment in which tumor cells develop profoundly influences many stages of tumor progression (1). At some point during tumor development, cancer cells begin to modulate resident tissue cells and induce a cytokine response to recruit stromal cells, which in turn stimulates tumorigenesis by releasing factors that act on the tumor cells (1-3). Unrestrained proliferation and loss of cellular control mechanisms characteristic of cancer are processes that are intimately linked to and controlled by reciprocal signaling between genetically altered tumor cells and the stroma. The tumor stroma is a mixture of those cell types necessary to build and sustain a tissue and consists primarily of fibroblasts, macrophages and endothelial cells. The stroma provides growth factors, a blood supply, an extracellular matrix and removes cellular waste products and dead cells. The stroma can keep premalignant cells in check and a phenotypically abnormal stroma can support tumor development. Thus, activation of the host stromal microenvironment is a critical step in tumor growth and progression (2), but the molecular basis for such interactions is complex (4, 5).

Tumor cells can stimulate matrix metalloproteinase (MMP) production by stromal cells via cytokines (6-8) or through cell–cell interactions mediated

Table 1. Primer sequences

Human	**sense/antisense**
β-2 microglobulin:	5′-GATGAGTATGCCTGCCGTGTG-3′/5′-CAATCCAAATGCGGCATCT-3′;
EMMPRIN/CD147	5′-TGGTACAAGATCACTGACTC-3′/5′-TTCAGGTTCTCAATGTGTAG-3′.
Mouse	
β-2 microglobulin	5′-CCTCACATTGAAATCCAAATGC-3′/5′-CGGCCATACTGTCATGCTTAAC-3′
Basigin/CD147	5′-GAGGCAATCACCAATAGC-3′/5′-TTCCGCCTCTTCTCATAG-3′
MMP-2	5′-CCGATTATCCCATGATGAC-3′/5′-ATTCCCTGCGAAGAACAC-3′
VEGF-A	5′-AGTACATCTTCAAGCCGTC-3′/5′-GCAGGAACATTTACACGTC-3′

by cell adhesion molecules such as the extracellular matrix metalloproteinase inducer (EMMPRIN) (9). EMMPRIN (also known as CD147, M6 leukocyte activation antigen and Basigin in the mouse) is a member of the immunoglobulin superfamily (10). The differentially glycosylated CD147 protein (32-60 kDa) contains two extracellular immunoglobulin domains, a transmembrane domain and a 39-amino acid cytoplasmic domain (9). Similar to other members of the Ig superfamily, CD147 forms homo-oligomers in the plasma membrane (11). Accumulating evidence suggests a prominent role for CD147 in mediating interactions both between tumor cells and between tumor cells and host stromal cells to promote a number of events during cancer progression including MMP production (9).

CD147 is highly expressed on the plasma membrane of cancer cells such as lung (12), breast (12) and ovary (13) and induces the production of MMPs from neighboring stromal cells (14, 15). Mouse CD147 (Basigin) was also demonstrated to have the same MMP stimulatory effect as human CD147/EMMPRIN (15). Although CD147 is primarily expressed by tumor cells, CD147 expression has been detected in stromal fibroblasts and endothelial cells within some tumors (13, 16). CD147 consistently induces the production of secreted MMPs by fibroblasts and also by cancer cells, most likely via homophilic interactions between CD147 molecules on adjacent cells (17). Human breast cancer cells overexpressing CD147 exhibit both accelerated growth and increased invasiveness associated with increased expression of MMP-2 and MMP-9 (18). CD147 can also induce breast cancer vascular endothelial growth factor (VEGF) secretion in both tumor and stromal compartments to promote angiogenesis and tumor progression. When fibroblasts are exposed to a CD147 stimulus, CD147 expression is upregulated in these cells. Newly synthesized CD147 is then presented on the cell surface and serves as a counter-receptor for CD147-dependent signaling between tumor cells and fibroblasts (19). In addition, the generation of soluble CD147 by proteolytic cleavage of membrane-associated CD147 (19) and microvesicular release (20) has been reported.

Importantly, increased expression of CD147 was recently identified in colorectal biopsies of colon cancer patients (21). However, although increasing evidence suggests that CD147 is an important mediator of malignant properties facilitating tumor growth, the function of this molecule in colon cancer is not clear.

3. MATERIALS AND METHODS

3.1. cDNA arrays

Total RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized with 2 μg total RNA and an avian myeloblastosis virus-reverse transcriptase (Promega, Madison, WI). Total RNA was used to generate [α-^{32}P]dCTP-labeled cDNA probes for MMP species-specific array analysis (GEArray Q series, SuperArray, Frederick, USA). Hybridizations were done according to the manufacturer's protocol with labeled cDNA probes from SW620 human colon cancer xenografts grown in nude mice (day 22) and SW620 colon cancer cells (22). The mRNA signals were detected and recorded in a Phosphorimager (Typhoon, Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) and quantified with ImageQuant software (Molecular Dynamics, Gladbeck, Germany).

3.2. Quantitative real-time RT-PCR

Samples were snap frozen in liquid nitrogen, homogenized and processed for PCR as described previously (23). For sequences of primers (VBC Genomics, Vienna, Austria) used, see Table 1. PCR data were analyzed with LCDA Version 3.1.102 (Roche, Mannheim, Germany). Relative quantification of the signals was performed by normalizing the signals of the different genes to β2-microglobulin as described previously (22, 23). Measurements were performed in duplicate.

3.3. Cell culture and analysis of small interfering RNA (siRNA) effects *in vitro*

Human SW620 colon cancer cells and mouse S3T3 fibroblasts (ATCC, Manassas, VA) were cultured in DMEM containing 10% FCS (22). Mouse macrophages (CRL-2470; ATCC) were cultured in L-929 fibroblast medium (24) that contains mouse colony-stimulating factor-1 (CSF-1). Three siRNA sequences (per molecule) directed against human EMMPRIN/CD147 and mouse Basigin/CD147 and two scrambled siRNAs were designed synthesized and tested for efficacy and optimal concentration *in vitro* (23). Cells were cultured until they reached 60% confluence, rinsed with PBS, refed with serum-free DMEM, and then transfected with 50 or 100 nM siRNA using Lipofectamine and Plus Reagent (Invitrogen, Carlsbad, CA) for 48h, according to the manufacturer's protocol. Then, mRNA was isolated and real time RT-PCR performed as described above. For sequences of the identified active human and mouse CD147 siRNAs used, see Table 2.

Table 2. siRNA sequences

Target	sense/antisense
human EMMPRIN/CD147	5´-GACCUUGGCUCCAAGAUACTT-3´/5´-GUAUCUUGGAGCCAAGGUCTT-3´
mouse Basigin/CD147	5´-GAGGCAAUCACCAAUAGCATT-3´/5´-UGCUAUUGGUGAUUGCCUCTT-3´
scrambled siRNA (control)	5'-GAAGCAGCACGACUUCUUCTT-3'/5´-GAAGAAGUCGUGCUGCUUCTT-3´

3.4. Co-culture experiments

Human SW620 colon cancer cells were treated with siRNA directed against EMMPRIN/CD147 and Basigin/CD147 (as a control for siRNA species specificity). Mouse fibroblasts were treated with siRNA directed against Basigin/CD147 and EMMPRIN/CD147 (as a control for siRNA species specificity) and mouse macrophages with siRNA directed against Basigin/CD147. Prior to siRNA treatment (100 nM), cells were allowed to adhere for 24 h. Three hours after transfection, SW620 cells (10^5 cells/6-well) were co-cultured with mouse fibroblasts or macrophages at a ratio of 1:1 for 48 h. Following treatment, RNA was isolated for real-time RT-PCR. Experiments were performed in triplicate.

3.5. Tumor models and siRNA treatment

The experiments performed in this study were approved by the Institutional Animal Care and Use Committee at the Medical University of Vienna. Pathogen-free male BALB/*c-nu/nu* (nude) mice (Harlan-Winkelmann, Borchen, Germany), 5 weeks of age, were weighed, coded and randomly assigned to 4 experimental groups of n=8. Mice were anaesthetized (ketamine hydrochloride/xylazine at 55/7.5 mg/kg, s.c.) and $8x10^6$ SW620 cells/100 µl culture medium were injected subcutaneously into the left flank as described previously (23). The control group received scrambled siRNA. Animals received human CD147/EMMPRIN, mouse CD147/Basigin or a combination of human and mouse CD147 siRNA treatment that was initiated on day 8. Mice were anesthetized and 10 µg siRNA were injected intratumorally with Lipofectamine® (Invitrogen) in a volume of 20µl. The treatment was cycled on days 11, 14, 17 and 20. On day 22, animals were sacrificed and the tumors were isolated, weighed, and prepared for molecular analyses as described above (22, 23).

3.6. Western blotting

Tissue samples were lyzed in solubilisation buffer supplemented with a complete-EDTA-free protease inhibitor cocktail and 50 µg/lane total cell lysates (TCL) were separated by SDS-PAGE and electrophoretically transferred onto Hybond C membranes (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) as described previously (22). Blots were probed with rabbit polyclonal antibodies against MMP-2, MMP-9, MT1-MMP (Chemicon, Hampshire, United Kingdom) and MMP-12 (Santa Cruz Biotechnology, CA) and goat polyclonal antibodies against human CD147/EMMPRIN (AF972; R&D Systems, Minneapolis, MN USA) and mouse CD147/Basigin (AF772; R&D Systems, Minneapolis, MN) and incubated with horse radish peroxidase conjugated secondary antibodies (Amersham). Proteins were immunodetected on the membrane using enhanced chemiluminescence (Supersignal-West-Femto, Pierce, Rockford, IL), and specific protein bands were quantified using Easy plus Win 32 software (Herolab, Wiesloch, Germany) as described previously (22, 23, 25).

3.7. Statistical Analysis

We used the Wilcoxon rank test to compare the data between the groups. All statistical tests were two-sided. Statistical tests were done with the use of SPSS software (version 10.0.7, SPSS Inc., Chicago, IL) Data are expressed as means $\pm$ SD. P values of <0.05 were considered to indicate statistical significance.

4. RESULTS

4.1. Tumor and stromal cell CD147 expression increases during colon cancer xenograft growth

To analyze the potential involvement of extracellular matrix (ECM)-modifying enzymes in the tumor microenvironment in colon cancer growth, we first profiled stromal cell MMP gene expression by using a mouse MMP array. In line with the increased CD147 expression found in colon cancer patients (21), gene profiling arrays indicated elevated transcript levels of mouse CD147/Basigin in SW620 colon cancer xenografts in nude mice on day 22 following cancer cell injection compared with SW620 cells cultured *in vitro* (data not shown). Next, we examined the mRNA and protein expression by real-time RT-PCR and Western blotting, respectively, to confirm and further examine the increased expression of cancer cell-derived and stromal cell-derived CD147 during the growth of tumors from days 8 to 30. Analysis of CD147 protein expression in tumor tissue from SW620 xenografts by Western blotting revealed significant increases in mouse and human CD147 levels during tumor growth (Figure 1A). Real time RT-PCR studies showed that both human (tumor cell-derived) CD147/EMMPRIN mRNA and also mouse stroma-derived CD147/Basigin mRNA were significantly upregulated during tumor growth (Figure 1B).

4.2. Blockade of host CD147/Basigin but not tumor cell-expressed CD147/EMMPRIN suppresses colon cancer growth

Small interfering RNAs (siRNAs) specifically directed against human CD147/EMMPRIN and mouse CD147/Basigin down-regulated target gene expression in human SW620 colon cancer cells and mouse S3T3 fibroblasts in a species-specific manner *in vitro* (Figure 2A). To analyze the role of CD147 colon cancer growth *in vivo*, we then treated mice bearing human colon cancer xenografts with intratumoral injections of siRNA against human CD147/EMMPRIN, mouse CD147/Basigin, combined human CD147/EMMPRIN and mouse CD147/Basigin or scrambled siRNA controls. Mouse CD147/Basigin siRNA (993 ± 83 mg; $P<0.05$) treatment markedly reduced tumor weights by 26% compared to untreated controls (1343 ± 139 mg) whereas tumor weights were not influenced by human CD147/EMMPRIN siRNA

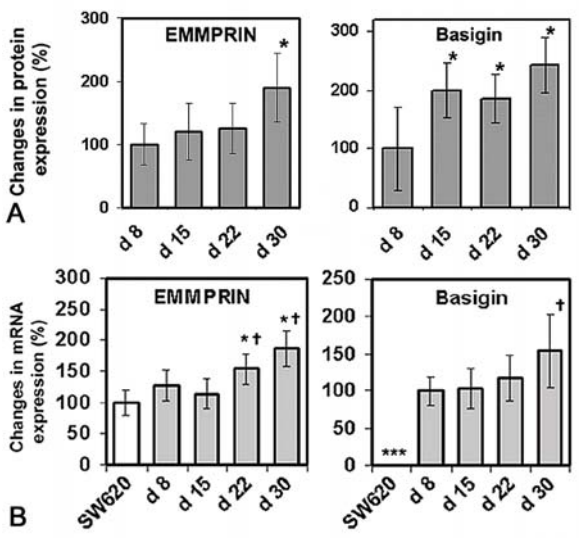

Figure 1. A, Expression of CD147/EMMPRIN (left panel) and CD147/Basigin protein expression in tumor tissue by Western blotting and B, mRNA expression of cancer cell-derived CD147/EMMPRIN (left panel) and host CD147/Basigin by real-time RT-PCR. Protein and gene expression of human colon cancer xenografts in nude mice was measured at 8, 15, 22 and 30 days (d) following tumor cell engraftment. mRNA levels were compared with SW620 cells cultured *in vitro* (white column; *** not detectable). Results are expressed as mean ± SD. *Protein expression significantly different compared to day 8. mRNA expression: *P<0.05 compared to expression in SW620 colon cancer cells; † significantly different compared to expression on day 8.

treatment (1433 ± 238 mg) (Figure 2B). Combined EMMPRIN/Basigin treatment markedly reduced tumor weights (960 ± 208 mg; P<0.05) by 28.5%, which was comparable to CD147/Basigin treatment alone (Figure 2B).

Although cancer cell CD147/EMMPRIN tissue mRNA significantly declined, host (mouse) CD147/Basigin and MMP-2 tissue expression increased following human CD147/EMMPRIN blockade. In contrast, treatment with mouse CD147/Basigin or combined EMMPRIN/Basigin siRNA down-regulated host (mouse) CD147/Basigin and MMP-2 tissue mRNA expression (Figure 3A). Expression of host (mouse) VEGF-A, however, was neither affected by CD147/EMMPRIN nor by CD147/Basigin blockade (Figure 3A). In addition, mouse CD147/Basigin siRNA treatment alone reduced human CD147/EMMPRIN mRNA levels. Interestingly, protein expression analysis revealed a significant upregulation of MMP-9 following treatment with human CD147/EMMPRIN siRNA, while MMP-2, MMP-12 and MT1-MMP protein levels were unchanged (Figure 3B).

These experiments indicated that blockade of host CD147/Basigin downregulates host MMP-2 and significantly suppresses the growth of colon cancer xenografts in mice. In contrast, treatment with human CD147/EMMPRIN siRNA was associated with upregulated MMP-9 levels and did not affect tumor growth.

4.3. Cell-type specific effects of CD147 on gene expression in tumor-stromal cell interactions

The interaction of SW620 colon cancer cells with mouse fibroblasts and macrophages was then analyzed in co-cultures treated with siRNA against human CD147/EMMPRIN and mouse CD147/Basigin Both, human CD147/EMMPRIN and mouse CD147/Basigin inhibition downregulated fibroblast MMP-2 expression in co-cultures of colon cancer cells with fibroblasts, while not affecting VEGF-A expression. Interestingly, inhibition of human CD147/EMMPRIN resulted in a significant increase in mouse CD147/Basigin gene expression, but not vice versa (Figure 4A). In contrast, CD147/Basigin inhibition did not change VEGF-A or MMP-2 expression in co-cultured macrophages, although human CD147/EMMPRIN expression was decreased following this treatment (Figure 4B). These experiments suggest that CD147 regulates MMP-2 expression in fibroblasts but not macrophages in colon cancer. In addition, stromal cell VEGF-A expression is independent of CD147 in co-cultured cells.

4.4. CD147 glycoform variability in colon cancer cells and stromal cells

Based on the above findings, we investigated whether the glycosylation pattern of CD147 may differ between tumor cells and stromal cells, which could explain the cell type specific effects of CD147. We used anti-CD147-siRNA treated SW620 colon cancer cells, mouse macrophages (CRL-2740) and mouse S3T3 fibroblasts to examine the expression of CD147 in cell lysates with Western blotting using anti-CD147/EMMPRIN and anti-CD147/Basigin polyclonal antibodies (Figure 5). We observed a substantial variability in CD147 glycosylation (26) as illustrated in Figure 5. Interestingly, the low glycosylated (LG) form (~35 kDa) is the major variant relative to the highly glycosylated (HG) form (50-65 kDa) in macrophages. In contrast, the LG form is the minor variant in SW620 colon cancer cells and fibroblasts. Treatment of cells with anti-CD147-siRNA affected both LG and HG forms in all cells. The LG form almost completely disappeared in macrophages and the level of the HG form (~50 kDa) was greatly diminished in all cell types (Figure 5).

5. DISCUSSION

One promising approach for future cancer therapy resides in the ability to focus on and exploit relationships between cancer cells and the host environment. Tumor tissue is composed of cancer cells and stromal elements, including fibroblasts and macrophages, which are active players in the process of tumor progression and invasion (27, 28). Tumor-associated fibroblasts play a pivotal role in regulating ECM turnover by not only secreting ECM components that promote cellular migration but also by secreting and stimulating the production of ECM-degrading MMPs from accessory cells. Similarly, tumor-associated macrophages can also produce enzymes and inhibitors that regulate the digestion of the

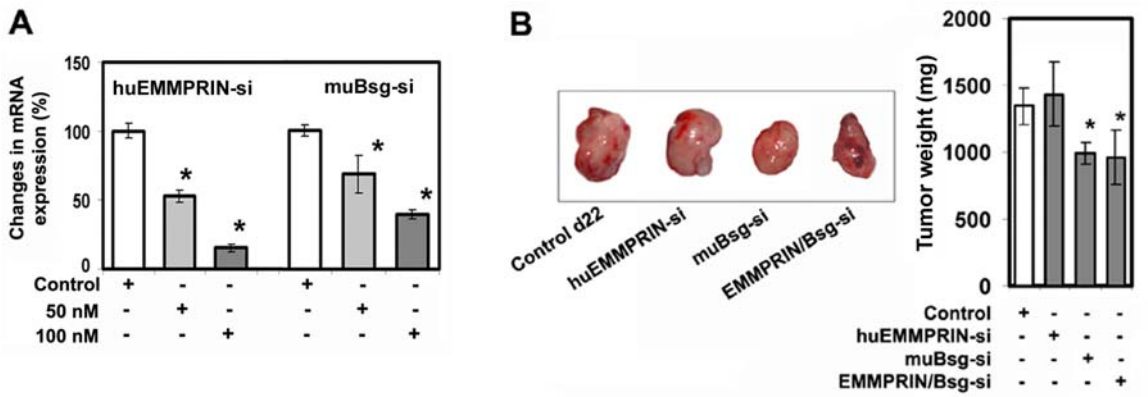

Figure 2. A, siRNAs (si) directed against human CD147/EMMPRIN (huEMMPRIN-si) and mouse CD147/Basigin (muBsg-si) specifically down-regulate target mRNA expression. Real-time RT-PCR amplifications from human SW620 and mouse S3T3 cells show a concentration-dependent downregulation of target mRNAs after treatment with 50 and 100 nM siRNA. Results are shown as percentage expression units normalized to β-2 microglobulin levels and are expressed as mean ± SD. * significantly different from control (P<0.05). siRNA (si) against mouse CD147/Basigin suppresses colon cancer growth in mice. Representative tumor images and mean colon cancer xenograft tumor weights on day 22. Animals received huEMMPRIN-si, muBsg-si or combined huEMMPRIN-si/Bsg-si treatment that was initiated on day 8. The control group received scrambled siRNA. Treatment against mouse CD147/Basigin significantly reduced tumor weight. * significantly different from scrambled siRNA (control) on day 22 ($P < 0.05$).

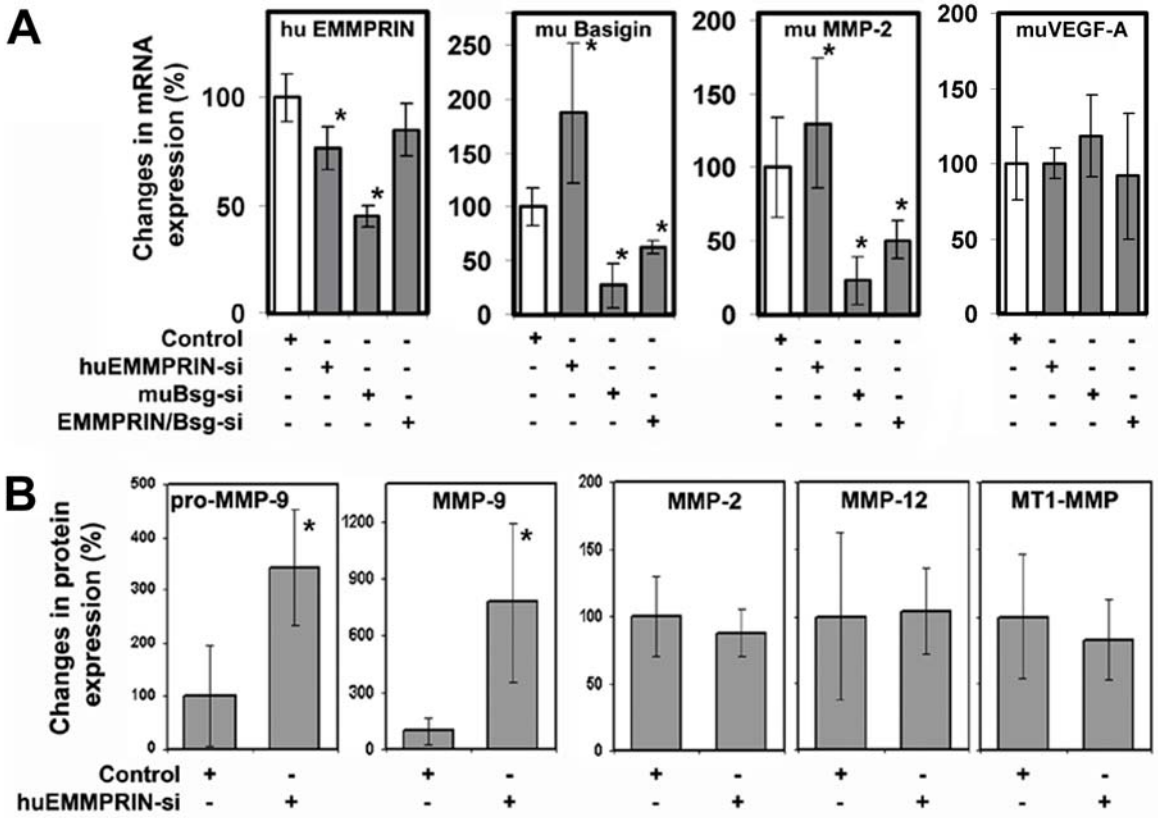

Figure 3. A, Real time RT-PCR measurements of human and mouse CD147, host MMP-2, and host VEGF-A in tumor lysates. Human EMMPRIN/CD147 siRNA reduced mRNA expression *in vivo* whereas Basigin/CD147 siRNA reduced both mouse basisin/CD147 and human EMMPRIN/CD147 *in vivo*. Mouse Basigin/CD147 siRNA-treated animals had reduced tumor levels of mouse MMP-2 mRNA. Results are expressed as mean ± SD. * significantly different from scrambled siRNA (control) ($P < 0.05$). B, Western blot analysis of pro-MMP-9, MMP-9, MMP-2, MMP-12 and MT1-MMP in tumor lysates. Pro-MMP-9 and MMP-9 protein levels were increased in CD147/EMMPRIN siRNA-treated animals. Results are expressed as mean ± SD. * significantly different from scrambled siRNA (control) ($P<0.05$)

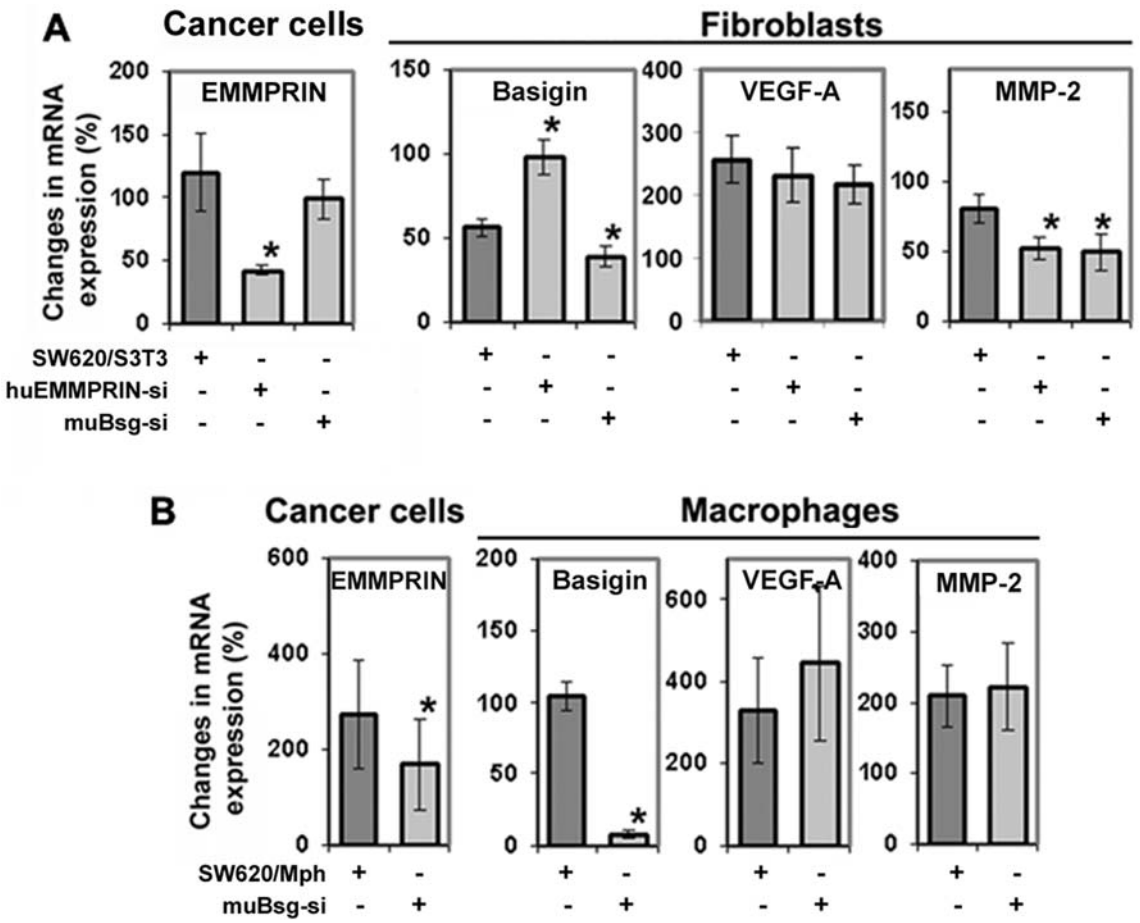

Figure 4. Quantification of human CD147/EMMPRIN, and mouse CD147/Basigin, VEGF-A and MMP-2 mRNA expression in co-cultured SW620 cancer cells and S3T3 fibroblasts or macrophages (Mph) treated with siRNA against human CD147/EMMPRIN (huEMMPRIN-si) or mouse CD147/Basigin (muBsg-si). A, Inhibition of human CD147/EMMPRIN downregulated fibroblast MMP-2 and induced mouse CD147/Basigin expression. B, CD147/Basigin inhibition did not affect macrophage VEGF-A or MMP-2 expression. Results are shown as percentage expression units normalized to β-2 microglobulin levels and are expressed as means ± SD. * significantly different from co-cultured SW620 cells and fibroblasts or macrophages, respectively ($P<0.05$).

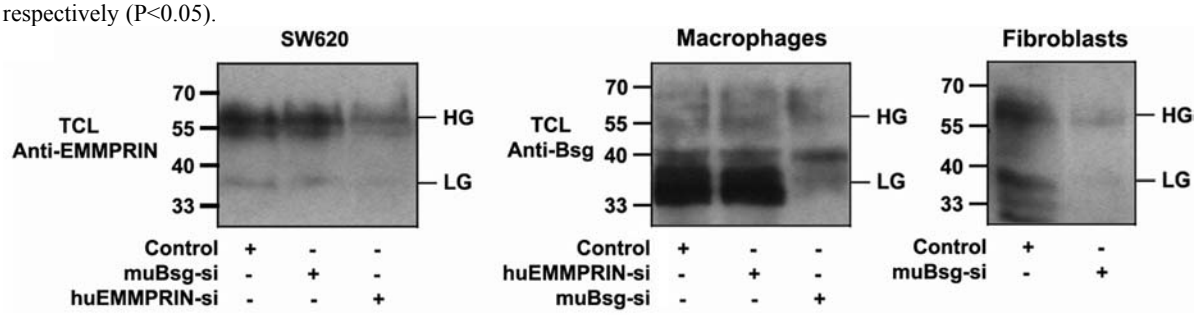

Figure 5. A, Variability in CD147 glycoform expression. SW620 cancer cells, mouse macrophages (CRL-2740) and mouse S3T3 fibroblasts were treated with 100 nM siRNA directed against human CD147/EMMPRIN and mouse CD147/Basigin for 48 h. HG and LG indicate the high and low molecular weight glycoforms of CD147. siRNA treatment downregulates the HG and LG glycoforms of CD147. Molecular weights deduced from marker proteins are indicated on the left in kDa.

ECM such as MMPs, plasmin and urokinase-type plasminogen activator (29).

Our data provide evidence that specific tumor-stromal cell interactions mediated by CD147 promote colon cancer growth. Importantly, our finding that blockade of host (mouse) CD147/Basigin expression, but not of cancer cell-derived CD147/EMMPRIN, suppresses tumor growth in human colon cancer xenografts, adds a new facet to the complex role of tumor-host interactions in tumor growth

mediated by CD147. In our studies, fibroblast MMP-2 production was downregulated following siRNA blockade of both tumor cell-associated CD147/EMMPRIN and fibroblast-associated CD147/Basigin in co-cultures of colon cancer cells with fibroblasts. These findings suggest that MMP-2 stimulation in fibroblasts is due to direct cell-cell contact between cancer cells and fibroblasts, although a contribution from soluble factors cannot be excluded. Similar results were obtained from co-cultures of laryngeal cancer cells with fibroblasts (30). In line with this, it has been shown that CD147 is enriched on the surface of tumor cells and stimulates the production of MMPs by adjacent stromal fibroblasts (15, 31). Although it has been suggested that CD147 may serve as its own counter-receptor in cancer cells, thus stimulating MMPs via a homophilic interaction (17), the receptor on fibroblasts that is responsible for CD147-mediated stimulation of MMP production remains elusive. Thus, it might be possible that in xenograft models, Basigin expressed by fibroblasts could also serve as a counter-receptor for tumor cell-associated CD147 in stimulating MMP expression. Previously we have shown that SW620 colon cancer cells induced the up-regulation of host (mouse) MMP-2 expression. Our findings presented here suggest that fibroblasts contribute to elevated host MMP-2 levels mediated by CD147-induced fibroblast-colon cancer cell interactions. This finding further supports the importance of interactions between fibroblasts and tumor cells for tumor growth. In contrast, blockade of mouse CD147/Basigin expression in macrophages co-cultured with SW620 cells did not affect the MMP-2 level in macrophages.

To this end, a previously published study from our group already indicated that the interplay between colon cancer cells and stromal cells is essential in colon cancer growth by showing that MMP-2, VEGF-A and CSF-1 production by stromal cells is enhanced by CSF-1 negative SW620 colon cancer cells (22). Moreover, in a recent study we provide evidence that tumor necrosis factor α (TNF-α) derived from colon cancer cells, increases macrophage migration and stimulates CSF-1 production by stromal macrophages and possibly other cell types. CSF-1 in turn, induces VEGF-A and MMP-2 over-expression in macrophages in an autocrine manner, thereby modulating angiogenesis and colon cancer growth (32). Fibroblasts, however, play a different role in tumorigenesis, as indicated by our finding that the expression of MMP-2 and VEGF-A in fibroblasts co-cultured with SW620 cells is independent of TNF-α (data not shown). These findings fit to the obtained MMP-2 data in our studies, indicating cell type-specific MMP-2 regulation in stromal cells, i.e. CD147-dependent MMP-2 regulation in fibroblasts and TNF-α/CSF-1-dependent MMP-2 regulation in macrophages (32) in colon cancer.

Interestingly, the differential activities of CD147/Basigin in macrophages and fibroblasts observed in our *in vitro* experiments may be due to the prevalence of different CD147 glycoforms in these cell types which exert different functions. CD147 can be composed of multiple, high (HG) and low (LG) glycosylated forms and the HG forms of CD147 are active in the induction of MMPs (17, 33). In macrophages, however, we observed reduced levels of the HG forms of CD147 as compared to fibroblasts and cancer cells, leading to a downward shift in the HG/LG ratio. This might be an explanation for the lack of CD147-induced MMP-2 induction in macrophages. Consequently, this role of CD147 in macrophages in the context of colon cancer remains unclear.

Of note, inhibition of CD147 in the SW620 colon cancer cells led to a significant increase in fibroblast Basigin expression in co-culture experiments, but not *vice versa*. Accordingly, blockade of human EMMPRIN/CD147 by siRNA in colon cancer bearing mice resulted in an upregulation of host CD147/Basigin expression associated with increased MMP-2 levels. Moreover, inhibition of cancer cell-derived CD147 also led to increased MMP-9 levels *in vivo*. Up-regulation of host CD147/Basigin, MMP-2 and MMP-9 might be due to a compensatory reaction, which might explain the failure of CD147/EMMPRIN siRNA treatment to suppress tumor growth. This assumption is supported by the fact that both MMP-2 and MMP-9 have been implicated in colon cancer progression and metastasis in animal models and patients (34).

Recently it has been shown that in MDA-MB-231 human breast cancer cells, CD147 can stimulate VEGF secretion in both tumor and stromal compartments to promote angiogenesis and tumor progression (35) and that CD147 stimulates VEGF production in both MDA-MB-231 breast cancer cells and fibroblasts via the phosphoinositide 3-kinase (PI3K)-Akt pathway (36). Similar, Chen *et al* showed that VEGF expression positively correlated with CD147 expression in melanoma cells, although the inhibitory effect of CD147 knock down by siRNA on VEGF production was limited (37). By contrast, our data show that in colon cancer, both human CD147/EMMPRIN and mouse CD147/Basigin inhibition do not affect stromal VEGF-A expression, neither in fibroblasts and macrophages co-cultured with colon cancer cells, nor *in vivo*. Thus, VEGF production by stromal cells seems to be regulated by different mechanisms in colon cancer. In favor of this assumption are our previous results indicating that VEGF production by macrophages is CSF-1 dependent in our colon cancer model (32). In addition, the ECM itself can act as a source of VEGF. Recent studies have shown that MMP-9 and, to a lesser extent MMP-2, are required for the mobilization of sequestered VEGF from the ECM and the initiation of tumor angiogenesis (38). Consequently, it is tempting to speculate that increased pro-angiogenic VEGF levels liberated from the ECM by upregulated MMP-9 might counteract suppression of tumor growth following blockade of cancer cell-derived CD147 in SW620 colon cancer xenografts. These data indicate that the signaling events downstream of CD147-mediated tumor-host interactions differ in different types of tumors. Further studies are necessary to elucidate CD147 mediated regulation of signaling pathways in colon cancer cells and surrounding stromal cells.

In this study we show that stromal cell CD147-targeting siRNA could significantly down-regulate host

CD147 mRNA levels in mice bearing human colon cancer xenografts and that it suppresses tumor growth in colon cancer, suggesting that CD147 highly expressed on the surface of stromal cells plays an important role in the growth of colon cancer. We provide evidence that CD147 functions in a cell-type specific manner leading to CD147-dependent MMP-2 production in fibroblasts but not macrophages, regulated by direct cancer cell-stromal cell contact. Our findings provide new insights into tumor-host interactions for the development of novel siRNA-based therapeutic strategies to treat patients with colon cancer.

6. ACKNOWLEDGEMENTS

This study was supported by the Austrian Research Foundation (FWF) grant S9412-B11 and the European Commission ''Anti-tumor-targeting'' grant LSHC-CT-2005-518178 to S. Aharinejad.

7. REFERENCES

1. Elenbaas B & R. A. Weinberg: Heterotypic signaling between epithelial tumor cells and fibroblasts in carcinoma formation. *Exp Cell Res* 264, 169-184 (2001)
2. Bissell M J & D. Radisky: Putting tumours in context. *Nat Rev Cancer* 1, 46-54 (2001)
3. Wiseman B S & Z. Werb: Stromal effects on mammary gland development and breast cancer. *Science* 296, 1046-1049 (2002)
4. Farber E: The multistep nature of cancer development. *Cancer Res* 44, 4217-4223 (1984)
5. Weinberg R A: Oncogenes, antioncogenes, and the molecular bases of multistep carcinogenesis. *Cancer Res* 49, 3713-3721 (1989)
6. Prescott J, N. Troccoli & C. Biswas: Coordinate increase in collagenase mRNA and enzyme levels in human fibroblasts treated with the tumor cell factor, TCSF. *Biochem Int* 19, 257-266 (1989)
7. Brown P D, A. T. Levy, I. M. Margulies, L. A. Liotta & W. G.Stetler-Stevenson: Independent expression and cellular processing of Mr 72,000 type IV collagenase and interstitial collagenase in human tumorigenic cell lines. *Cancer Res* 50, 6184-6191 (1990)
8. Overall C M, J. L. Wrana & J. Sodek: Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor-beta 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression. *J Biol Chem* 266, 14064-14071 (1991)
9. Biswas C, Y. Zhang, R. DeCastro, H. Guo, T. Nakamura, H. Kataoka & K. Nabeshima: The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. *Cancer Res* 55, 434-439 (1995)
10. Kanekura T, X. Chen & T. Kanzaki: Basigin (CD147) is expressed on melanoma cells and induces tumor cell invasion by stimulating production of matrix metalloproteinases by fibroblasts. *Int J Cancer* 99, 520-528 (2002)
11. Yoshida S, M. Shibata, S. Yamamoto, M. Hagihara, N. Asai, M. Takahashi, S. Mizutani, T. Muramatsu & K. Kadomatsu: Homo-oligomer formation by basigin, an immunoglobulin superfamily member, via its N-terminal immunoglobulin domain. Eur *J Biochem* 267, 4372-4380 (2000)
12. Polette M, C. Gilles, V. Marchand, M. Lorenzato, B. Toole, J. M. Tournier, S. Zucker, & P. Birembaut: Tumor collagenase stimulatory factor (TCSF) expression and localization in human lung and breast cancers. *J Histochem Cytochem* 45, 703-709 (1997)
13. Davidson B, I. Goldberg, A. Berner, G. B. Kristensen & R. Reich: EMMPRIN (extracellular matrix metalloproteinase inducer) is a novel marker of poor outcome in serous ovarian carcinoma. *Clin Exp Metastasis* 20, 161-169 (2003)
14. Sameshima T, K. Nabeshima, B. P. Toole, K. Yokogami, Y. Okada, T. Goya, M. Koono & S. Wakisaka: Glioma cell extracellular matrix metalloproteinase inducer (EMMPRIN) (CD147) stimulates production of membrane-type matrix metalloproteinases and activated gelatinase A in co-cultures with brain-derived fibroblasts. *Cancer Lett* 157, 177-184 (2000)
15. Li R, L. Huang, H. Guo & B. P. Toole: Basigin (murine EMMPRIN) stimulates matrix metalloproteinase production by fibroblasts. *J Cell Physiol* 186, 371-379 (2001)
16. Dalberg K, E. Eriksson, U. Enberg, M. Kjellman & M. Backdahl: Gelatinase A, membrane type 1 matrix metalloproteinase, and extracellular matrix metalloproteinase inducer mRNA expression: correlation with invasive growth of breast cancer. *World J Surg* 24, 334-340 (2000)
17. Sun J & M. E. Hemler: Regulation of MMP-1 and MMP-2 production through CD147/extracellular matrix metalloproteinase inducer interactions. *Cancer Res* 61, 2276-2281 (2001)
18. Zucker S, M. Hymowitz, E. E. Rollo, R. Mann, C. E. Conner, J. Cao, H. D. Foda, D. C. Tompkins & B. P. Toole: Tumorigenic potential of extracellular matrix metalloproteinase inducer. *Am J Pathol* 158, 1921-1928 (2001)
19. Tang Y, P. Kesavan, M. T. Nakada & L. Yan: Tumor-stroma interaction: positive feedback regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) expression and matrix metalloproteinase-dependent generation of soluble EMMPRIN. *Mol Cancer Res* 2, 73-80 (2004)
20. Sidhu S S, A. T. Mengistab, A. N. Tauscher, J. LaVail & C. Basbaum: The microvesicle as a vehicle for EMMPRIN in tumor-stromal interactions. *Oncogene* 23, 956-963 (2004)
21. Jin J S, C. Y. Wu, Y. F. Lin, J. Y. Wang, C. P. Yu, L. F. Sheu, H. Chiang, W. C. Tsai & W. H. Lee: Higher expression of epidermal growth factor receptor is associated with extracellular matrix metalloprotease inducer in colorectal adenocarcinoma: tissue microarray analysis of immunostaining score with clinicopathological parameters. *Dis Markers* 22, 309-316 (2006)
22. Aharinejad S, D. Abraham, P. Paulus, H. Abri, M. Hofmann, K. Grossschmidt, R. Schafer, E. R. Stanley & R. Hofbauer: Colony-stimulating factor-1 antisense treatment suppresses growth of human tumor xenografts in mice. *Cancer Res* 62, 5317-5324 (2002)

23. Aharinejad S, P. Paulus, M. Sioud, M. Hofmann, K. Zins, R. Schafer, E. R. Stanley & D. Abraham: Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of human mammary tumor xenografts in mice. *Cancer Res* 64, 5378-5384 (2004)
24. Stanley E R: The macrophage colony-stimulating factor, CSF-1. *Methods Enzymol* 116, 564-587 (1985)
25. Paulus P, E. R. Stanley, R. Schafer, D. Abraham & S. Aharinejad: Colony-stimulating factor-1 antibody reverses chemoresistance in human MCF-7 breast cancer xenografts. *Cancer Res* 66, 4349-4356 (2006)
26. Tang W, S. B. Chang & M. E. Hemler: Links between CD147 function, glycosylation, and caveolin-1. *Mol Biol Cell* 15, 4043-4050 (2004)
27. Pollard J W: Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 4, 71-78 (2004)
28. Mueller M M & N. E. Fusenig: Friends or foes - bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer* 4, 839-849 (2004)
29. Sica A, T. Schioppa, A. Mantovani & P. Allavena: Tumour-associated macrophages are a distinct M2 polarised population promoting tumour progression: potential targets of anti-cancer therapy. *Eur J Cancer* 42, 717-727 (2006)
30. Suzuki S, M. Sato, H. Senoo & K. Ishikawa: Direct cell-cell interaction enhances pro-MMP-2 production and activation in co-culture of laryngeal cancer cells and fibroblasts: involvement of EMMPRIN and MT1-MMP. *Exp Cell Res* 293, 259-266 (2004)
31. Kataoka H, R. DeCastro, S. Zucker & C. Biswas: Tumor cell-derived collagenase-stimulatory factor increases expression of interstitial collagenase, stromelysin, and 72-kDa gelatinase. *Cancer Res* 53, 3154-3158 (1993)
32. Zins K, D. Abraham, M. Sioud & S. Aharinejad: Colon Cancer Cell-Derived Tumor Necrosis Factor-{alpha} Mediates the Tumor Growth-Promoting Response in Macrophages by Up-regulating the Colony-Stimulating Factor-1 Pathway. *Cancer Res* 67, 1038-1045 (2007)
33. Guo H, S. Zucker, M. K. Gordon, B. P. Toole & C. Biswas: Stimulation of matrix metalloproteinase production by recombinant extracellular matrix metalloproteinase inducer from transfected Chinese hamster ovary cells. *J Biol Chem* 272, 24-27 (1997)
34. Mook O R, W. M. Frederiks & C. J. Van Noorden: The role of gelatinases in colorectal cancer progression and metastasis. *Biochim Biophys Acta* 1705, 69-89 (2004)
35. Tang Y, M. T. Nakada, P. Kesavan, F. McCabe, H. Millar, P. Rafferty, P. Bugelski & L. Yan: Extracellular matrix metalloproteinase inducer stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor and matrix metalloproteinases. *Cancer Res* 65, 3193-3199 (2005)
36. Tang Y, M. T. Nakada, P. Rafferty, J. Laraio, F. L. McCabe, H. Millar, M. Cunningham, L. A. Snyder, P. Bugelski & L.Yan: Regulation of vascular endothelial growth factor expression by EMMPRIN via the PI3K-Akt signaling pathway. *Mol Cancer Res* 4, 371-377 (2006)
37. Chen X, J. Lin, T. Kanekura, J. Su, W. Lin, H. Xie, Y. Wu, J. Li, M. Chen & J. Chang: A small interfering CD147-targeting RNA inhibited the proliferation, invasiveness, and metastatic activity of malignant melanoma. *Cancer Res* 66, 11323-11330 (2006)
38. Kalluri R: Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat Rev Cancer* 3, 422-433 (2003)

Abbreviations: CSF-1: colony-stimulating factor-1; ECM: extracellular matrix; EMMPRIN: extracellular matrix metalloproteinase inducer; HG: highly glycosylated form; LG: low glycosylated form; MMP: matrix metalloproteinase; MT1-MMP: membrane type 1-MMP; siRNA: small interfering RNA; TNF-α: tumor necrosis factor alpha; VEGF: vascular endothelial growth factor;

Key Words: Colon cancer model, Tumor-stromal cell interaction, Emmprin, CD147, Macrophage, Fibroblast

Send correspondence to: Seyedhossein Aharinejad, Laboratory for Cardiovascular Research, Center for Anatomy and Cell Biology, Vienna Medical University, Waehringerstrasse 13, A-1090 Vienna, Austria, Tel: 4314277-61119, Fax: 4314277-61120, E-mail: seyedhossein.aharinejad@meduniwien.ac.at

http://www.bioscience.org/current/vol13.htm