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Tissue factor-factor VIIa regulates interleukin-8, tissue factor and caspase-7 expression in SW620 cells through protease-activated receptor-2 activation

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Abstract. The tissue factor-factor VIIa (TF/VIIa) complex is believed to activate protease-activated receptor-2 (PAR2) and to trigger the malignant behavior of various types of cancer cells. In our previous study, it was demonstrated that TF and PAR2 were overexpressed in high metastatic potential colon cancer cells (SW620). Both PAR2 agonist (SLIGKV-NH2, PAR2-AP) and factor VIIa facilitated SW620 cell proliferation and migration. In the present study, the molecular mechanisms of TF/VIIa-induced SW620 cell proliferation and migration were investigated. It was found that factor VIIa (10 nM) significantly increased interleukin-8 (IL-8) expression at the mRNA and protein levels, enhanced TF mRNA expression and TF activity, and decreased caspase-7 expression at the mRNA and protein levels in the SW620 cells. These effects of factor VIIa were similar to those of PAR2-AP. All effects of factor VIIa were blocked by anti-TF and anti-PAR2 antibodies, but not by an isotype control antibody. Furthermore, both PAR2-AP and factor VIIa decreased the phosphorylation of p38 mitogen-activated protein kinase (MAPK). The results of this study suggest that the TF/VIIa complex regulates IL-8, TF and caspase-7 expression in SW620 cells via PAR2 activation, thereby promoting colon cancer cell proliferation and migration. The p38MAPK signal transduction pathway may negatively regulate these processes.

Introduction

Tissue factor (TF), a 47-kDa transmembrane glycoprotein, is the cellular receptor for factor VII/VIIa. Binding of plasma factor VII/VIIa to TF triggers the blood coagulation cascade leading to thrombin generation, which subsequently stimulates platelet activation and cleaves fibrinogen (1). Besides its role in coagulation, it has recently come to be believed that TF has many non-clotting functions, including roles in inflammation, angiogenesis, metastasis and cell migration (2,3). It was reported that TF is expressed in a wide variety of malignancies and is responsible for the malignant behavior of cancer cells (4). The TF/VIIa complex was found to initiate the signaling pathway in tumor cells and to accelerate tumor growth by two means: one dependent on the TF cytoplasmic domain and the other dependent on the proteolytic activity of VIIa. The proteolytic activity of VIIa is mostly related to the activation of protease-activated receptor-2 (PAR2) (5). The TF/VIIa complex is believed to activate PAR2, and may trigger the malignant behavior of various types of cancer cells.

PARs are members of a group of seven transmembrane G protein-coupled receptors activated by the proteolytic cleavage of their amino terminal domain (6). To date, four PARs have been discovered and characterized. PAR1, 3 and 4 were the first identified targets for thrombin, but can also be activated by trypsin or cathepsin-G. By contrast, PAR2 is resistant to thrombin but can be activated by trypsin, mast cell tryptase, leukocyte proteinase-3, bacteria-derived enzymes, factor Xa and the TF/VIIa complex (7). Experimentally, PARs can also be activated by synthetic peptides (agonist peptides; APs) that mimic the neo-amino terminus of the cleaved receptors. Specific PAR-APs are important probes for investigating the role of PAR activation (8). The activation of PAR2 induced by the TF/VIIa complex has recently attracted attention in regards to several pathogenetic processes such as angiogenesis, cell migration and invasion in various types of cancer (9).

In our previous study, we observed that TF and PAR2 were highly expressed in the colon cancer cell line SW620. PAR2 agonist (SLIGKV-NH2, PAR2-AP) and factor VIIa enhanced SW620 cell proliferation and migration. It was found that the stimulating effects of factor VIIa were inhibited by anti-TF and anti-PAR2 antibodies (10). These data suggest that the activity of the TF/VIIa complex in colonic cancer invasion and metastasis is carried out via the PAR2 pathway. However, the molecular mechanisms of SW620 cell proliferation and migration and the involvement of the relationship between TF/VIIa and PAR2 are unclear. As is well known, the progression of the malignant behavior of

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cancer can be driven by a large number of factors, including various angiogenic growth factors, chemotactic factors and certain antiapoptotic molecules (11). Of these, interleukin-8 (IL-8) is a key chemokine that has recently been shown to be expressed in many types of tumor cells and to contribute to human cancer progression (12). Caspase-7, an executioner caspase, also plays important roles in regulating cell apoptosis (13). Aberrant regulation of the apoptotic pathways, resulting from mutations or excessive activation of the antiapoptotic pathways, is intrinsic to the oncogenic process in general and to the early stages of metastasis in particular (14).

It is therefore strongly hypothesized by our group that the TF/VIIa complex activates PAR2 in SW620 cells, thereby regulating the expression of various key molecules that contribute to the proliferation and migration of colonic cancer cells. In this study, we investigated whether the expression of IL-8, TF and caspase-7, as well as the phosphorylation of p38 mitogen-activated protein kinase (MAPK), is regulated via the activation of PAR2 by the TF/VIIa complex. The data indicate that IL-8 and TF were significantly up-regulated, while caspase-7 and p38MAPK were down-regulated, via PAR2 activation by the TF/VIIa complex. The TF/VIIa/PAR2 axis is a potential therapeutic target for colon cancer.

Materials and methods

Materials. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin-ethylenediamine tetraacetic acid (EDTA) and penicillin-streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). Recombinant human VIIa was obtained from Novo Nordisk (Maaloev, Denmark). Monoclonal anti-PAR2 (α-PAR2) and anti-TF (α-TF) antibodies were purchased from Zymed Laboratories (South San Francisco, CA, USA). Polyclonal anti-caspase-7, anti-phospho-p38MAPK and anti-p38MAPK antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). PAR2 agonist (SLIGKV-NH2, PAR2-AP) was synthesized by Proteintech Group Inc. (Wuhan, China). IL-8 ELISA and TF activity assay kits were purchased from American Diagnostica Inc. (Greenwich, CT, USA). TRIzol and RT-PCR reagents were purchased from Invitrogen (Carlsbad, CA, USA). All other chemicals and reagents used were of the best commercial quality available.

Cell line and cell culture. The SW620 human colon cancer cell line was obtained from Shanghai Institutes Biological Sciences (Shanghai, China). Cells were maintained in DMEM with low-glucose medium supplemented with 1% glutamine, 1% penicillin/streptomycin and 10% FBS. The cells were cultured at 37˚C in 5% CO_2 in a humidified incubator to near confluence, then were deprived of serum for 16 h before being assayed.

Quantitative real-time polymerase chain reaction. The SW620 cells were seeded at $1x10^6$ cells/ml on a 6-well plate and serum-starved for 16 h prior to stimulation with PAR2-AP (100 μM) or VIIa (10 nM) for different time periods. As needed, cells in certain wells were pre-treated with 10 μg/ml antibodies for 1 h. Subsequently, the total cellular RNA was isolated, and first-strand cDNA was synthesized using SuperStrip II reverse transcriptase (Life Technologies). Finally, quantitative real-time polymerase chain reaction (Q-PCR) was performed in the Rotor-Gene 2000 (Corbett Research, Australia) using the following primer pairs: IL-8 forward: 5'-AACATGACTTCCAAGCTGGCCG-3', reverse: 5'-CAGTTTTCCTTGGGGTCCAGAC-3'; TF forward: 5'-TCA GGTGATCCACCCACCTT-3', reverse: 5'-GCACCCAAT TTCCTTCCATTT-3'; caspase-7 forward: 5'-TGACCTATC CTGCCCTCA-3', reverse: 5'-TCTCCTGCCTCACTGTCC-3; GAPDH (as a control housekeeping gene) forward: 5'-GGA TTTGGTCGTATTGGG-3', reverse: 5'-GGAAGATGGTGA TGGGATT-3'. Each primer pair was shown to produce only one product. Q-PCR parameters consisted of an initial hold at 95˚C for 5 min followed by 35 cycles at 95˚C for 30 sec and 60˚C (IL-8, TF), 54.5˚C (caspase-7) or 56˚C (GAPDH) for 30 sec, then 72˚C for 30 sec. After each Q-PCR run, a melting curve was performed to ensure that only a single amplification was generated. IL-8, TF and caspase-7 mRNA levels were normalized to the control values of GAPDH (%).

IL-8 protein detection. The SW620 cells were seeded at $5x10^5$ cells/ml on a 24-well plate and serum-starved for 16 h prior to stimulation with PAR2-AP (100 μM) or VIIa (10 nM) for different time periods. According to requirements, cells in certain wells were pre-treated with 10 μg/ml antibodies for 1 h. IL-8 protein secreted into the cell supernatants was measured using IL-8 ELISA assay kits following the manufacturer's instructions. The IL-8 protein level was expressed in pg/ml.

Measurement of TF activity. The SW620 cells were seeded at $1x10^6$ cells/ml on a 6-well plate and treated similarly to the above-described conditions. The cells were then briefly trypsinized, suspended in DMEM/10% FBS, pelleted and resuspended in Tris-buffered saline (TBS) at a density of 10^6 cells/ml, pelleted again and lysed with 1 ml of lysate buffer (TBS/1% Triton X-100/1 mM PMSF). The TF activity of the lysates was measured as factor X activation by the factor TF/VIIa complex, and the generated factor Xa was determined using a chromogenic assay (Actichrome® TF provided by ADI).

Western blot analysis. The conditioned cells were collected, and the cell lysates produced following the above-described method. The cell lysates (30 μg) were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes (Bio-Rad, CA, USA). The membranes were subsequently blocked with 5% milk-TBS buffer containing 0.1% Tween-20 (TBS/Tween) for 1 h at room temperature, and then probed with the anti-caspase-7 or anti-phospho-p38MAPK (p-p38MAPK) antibody. To detect total p38MAPK, membranes were stripped and reprobed with an anti-p38MAPK antibody. Immunoblots were developed, imaged and quantitated using a Bio-Rad Fluor-S MultiImager (Typhoon 9400, Amersham, Sweden).

Statistical analysis. Data are shown as the mean ± standard error of the mean (SEM) of triplicate determinations, and were calculated using SPSS software (version 10.0). A p-value of <0.05 was considered statistically significant.

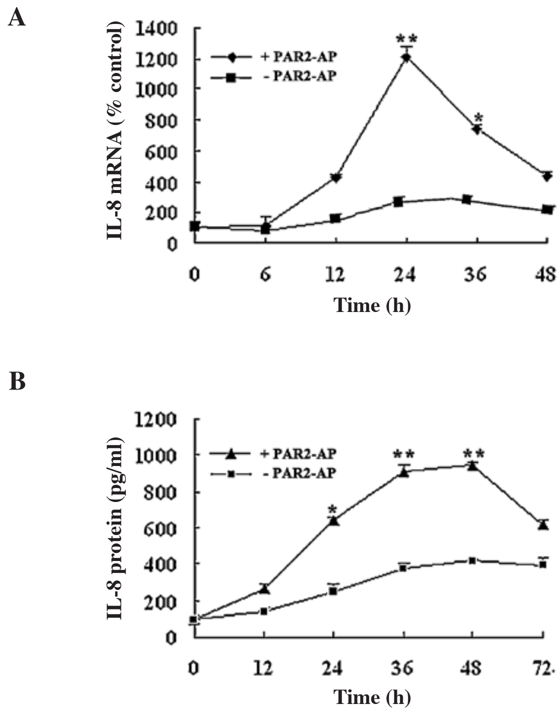

Figure 1. Effects of PAR2-AP on IL-8 expression in SW620 cells. Cells were cultured in the absence or presence of PAR2-AP (100 μM) for different time periods. The IL-8 mRNA level from whole cells was determined by Q-PCR as described in Materials and methods and normalized to GAPDH expression levels (%) (A). The IL-8 protein level in media was detected using the IL-8 ELISA kit and expressed in pg/ml (B). Data shown are the mean ± SEM of triplicate determinations. *p<0.05 and **p<0.01 vs. control.

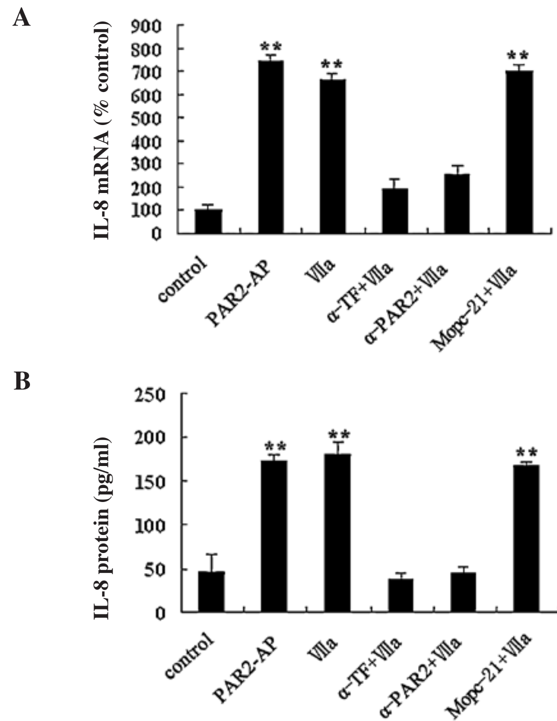

Figure 2. Factor VIIa induced IL-8 expression in SW620 cells via PAR2. The cells were pre-treated for 1 h with or without monoclonal antibodies against PAR2 (α-PAR2, 10 μg/ml) or TF (α-TF, 10 μg/ml), or with an isotype control antibody (Mopc-21, 10 μg/ml), and then incubated with PAR2-AP (100 μM) or factor VIIa (10 nM) for 24 h. The IL-8 mRNA levels of cells (A) and the protein concentration in culture supernatants (B) were determined by Q-PCR and ELISA, respectively. Data shown are the mean ± SEM of triplicate determinations. **p<0.01 vs. control.

Results

TF/VIIa increased IL-8 release in SW620 cells via PAR2 activation. IL-8 expression in SW620 cells with different stimulants was investigated. First, PAR2-AP at a concentration of 100 μM (selected based on our previous assay) was used in the experiments as a positive control of PAR2 activation. The time course of the PAR2-AP effects was also evaluated. As shown in Fig. 1, PAR2-AP (100 μM) increased IL-8 release at both the mRNA and protein levels in a time-dependent manner. The IL-8 mRNA level reached a peak at 24 h of stimulation with PAR2-AP (Fig. 1A), while its protein expression reached the maximal level between 36 and 48 h of stimulation (Fig. 1B).

Upon confirming the effects of PAR2-AP on PAR2 activation, the stimulating effects of TF/VIIa on PAR2 were tested. The data show that in the SW620 cells, factor VIIa (10 nM) increased the IL-8 mRNA level by at least 6-fold (Fig. 2A) and IL-8 protein concentration by ~4-fold (Fig. 2B) compared to untreated cells. The effects of VIIa were dramatically attenuated by pre-treatment with anti-TF or anti-PAR2 antibodies, but not by the isotype control antibody Mopc-21. These results demonstrate that the effects of VIIa on IL-8 expression in SW620 cells were VIIa/TF complex-mediated, and were also related to PAR2 activation.

TF/VIIa enhanced TF expression in SW620 cells via PAR2 activation. TF is originally expressed in SW620 cells and is closely associated with cell proliferation and migration (10). However, whether the expression of TF is further increased in SW620 cells through PAR2 activation is unclear. The present data showed that PAR2-AP (100 μM) markedly enhanced both TF expression at the mRNA level (Fig. 3A) and TF activity (Fig. 3B). Similar to the effects of PAR2-AP, factor VIIa (10 nM) also significantly increased the TF mRNA level as well as TF activity in SW620 cells. Compared to the media control, PAR2-AP and factor VIIa elevated TF mRNA and TF activity by 7- to 8- and 16- to 18-fold, respectively. Furthermore, the effects of VIIa were significantly inhibited by anti-TF or anti-PAR2 antibodies, but not by the control isotype antibody Mopc-21. These results further demonstrate that the induction of TF overexpression by factor VIIa in SW620 cells is dependent on the TF/VIIa complex, as well as on the activation of PAR2.

TF/VIIa decreased caspase-7 expression in SW620 cells via PAR2 activation. Caspase-7 is a key effector caspase that induces cell apoptosis. Whether the growth and proliferation of SW620 cells induced by PAR2-AP or VIIa (reported in our previous study) is related to caspase-7 expression is unclear. Therefore, caspase-7 mRNA and protein levels were evaluated under different conditions. As shown in Fig. 4A, after 2 h of treatment, PAR2-AP (100 μM) decreased the caspase-7 mRNA level to ~38% of that of untreated control cells. Caspase-7 mRNA expression was also down-regulated by 2 h of treatment with factor VIIa. Similarly, the effects of VIIa on the regulation of caspase-7 were attenuated by anti-TF or anti-

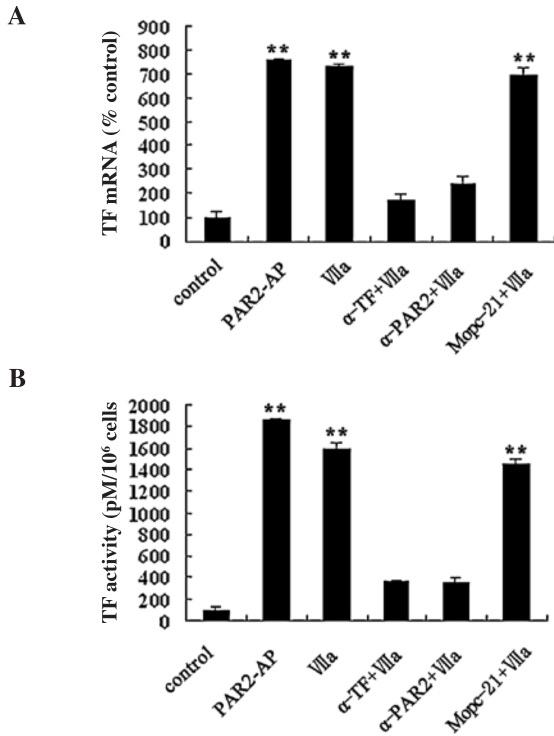

Figure 3. Effects of factor VIIa on TF expression in SW620 cells via PAR2. Cells were pre-treated for 1 h with or without monoclonal antibodies against PAR2 (α-PAR2, 10 μg/ml) or TF (α-TF, 10 μg/ml), or with an isotype control antibody (Mopc-21, 10 μg/ml), and then incubated with PAR2-AP (100 μM) or factor VIIa (10 nM) for 24 h. TF mRNA (A) and activity (B) in cells were investigated as described in Materials and methods. Data shown are the mean ± SEM of triplicate determinations. **p<0.01 vs. control.

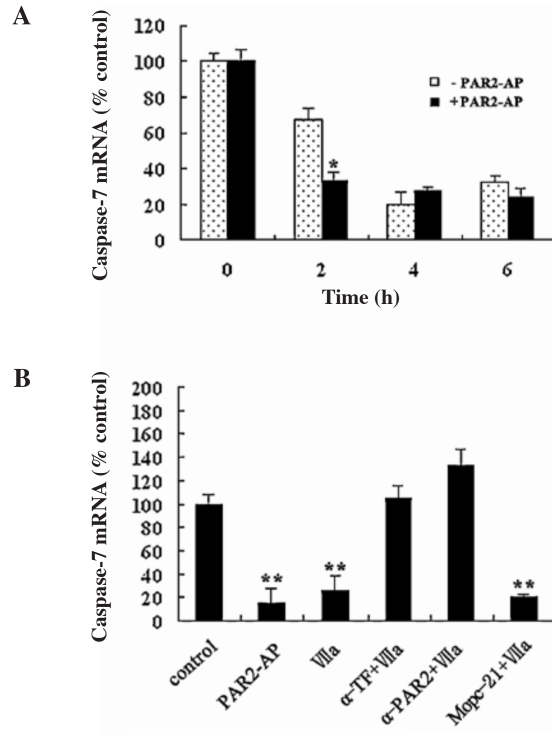

Figure 4. Effects of PAR2-AP and factor VIIa on caspase-7 mRNA expression in SW620 cells. (A) Caspase-7 mRNA expression in the absence or presence of PAR2-AP (100 μM). (B) Caspase-7 mRNA expression in cells treated with VIIa (10 nM) or pre-treated for 1 h with monoclonal antibodies against PAR2 (10 μg/ml) or TF (10 μg/ml), or with an isotype control antibody (Mopc-21, 10 μg/ml), prior to treatment with VIIa (10 nM) for 2 h. Data shown are the mean ± SEM of triplicate determinations. *p<0.05 and **p<0.01 vs. control.

PAR2 antibodies, but not by the control antibody (Fig. 4B). Concurrently with changes in caspase-7 mRNA, the caspase-7 protein level was decreased by the stimulation of PAR2-AP as well as that of VIIa (Fig. 5).

TF/VIIa suppressed the phosphorylation of p38MAPK via PAR2 activation. PAR2 activation affects cellular functions through several different signal transduction pathways. This study investigated whether the phosphorylation of p38MAPK is also regulated through PAR2 activation. As shown in Fig. 6A, the p-p38MAPK level was significantly decreased after treatment with PAR2-AP (100 μM) for 30-60 min, and recovered to the original level at 2 h of treatment. Similar to the effect of PAR2-AP, factor VIIa (10 nM) also decreased p38MAPK phosphorylation in SW620 cells after a 30-min exposure (Fig. 6B).

Discussion

There is increasing evidence that PARs are overexpressed in several types of malignant cancer, transmit signals in response to tumor-generated proteases and promote tumor growth, invasion and metastasis (15). PAR2, the only PAR not activated by thrombin, is an important mediator of tumor progression. It is generally recognized that PAR2 can be activated by trypsin, mast cell tryptase, factor VIIa and Xa (7). Multiple lines of evidence support the contribution of TF/VIIa signaling to tumor progression through PAR2. It has

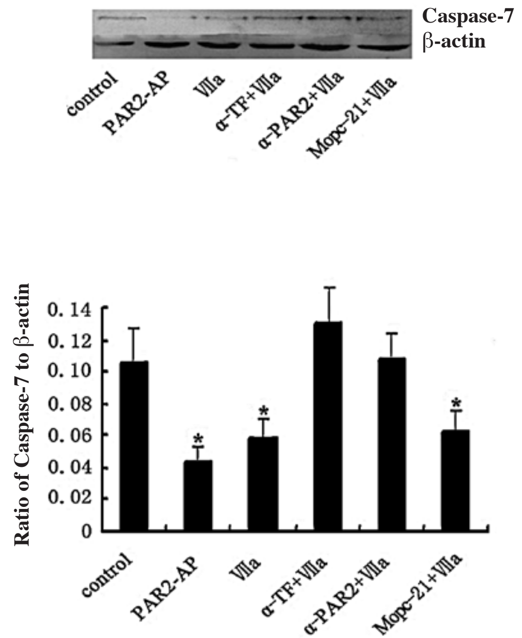

Figure 5. Factor VIIa suppressed caspase-7 protein expression in SW620 cells via PAR2. Cells were treated with VIIa (10 nM) or pre-treated for 1 h with monoclonal antibodies against PAR2 (10 μg/ml) or TF (10 μg/ml), or with an isotype control antibody (Mopc-21, 10 μg/ml), prior to treatment with VIIa (10 nM) for 24 h. The caspase-7 protein level in cell lysates was analyzed by Western blotting as described in Materials and methods and normalized to β-actin. Data shown are the mean ± SEM of triplicate determinations. *p<0.05 vs. control.

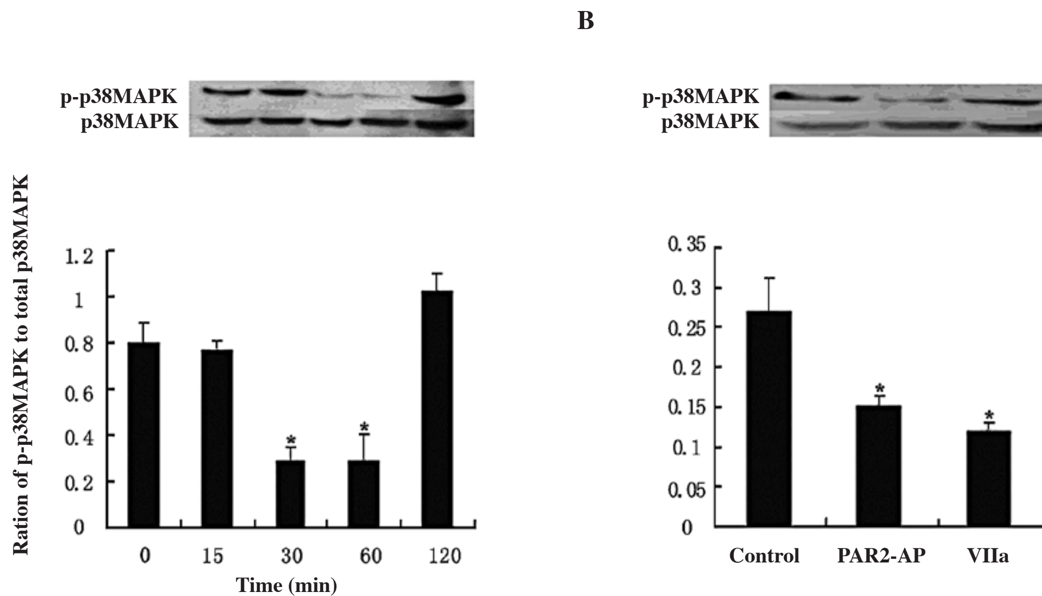

Figure 6. PAR2-AP and factor VIIa decreased phosphorylation of p38MAPK in SW620 cells. The cells were treated with PAR2-AP (100 μM) for the indicated times and p-p38MAPK was determined by Western blotting as described in Materials and methods, and normalized to total p38MAPK (A). The p-p38MAPK level was measured in cells treated with factor VIIa (10 nM) for 30 min (B). Data shown are the mean ± SEM of triplicate determinations. *p<0.05 vs. control.

been reported that TF/VIIa stimulates breast carcinoma cell migration and invasion through the activation of PAR2 (16,17). On the other hand, TF has been linked to cancer progression (4,18). In our previous study, we found that both TF and PAR2 were overexpressed in the high metastatic potential colon cancer cell line SW620, and that TF/VIIa carries out its role in colonic cancer invasion and metastasis via the PAR2 pathway (10). However, the molecular mechanisms and the relationship between TF/VIIa and PAR2 activation as well as the signaling involved in SW620 cell proliferation and migration have not been clearly defined.

In the present study, we first demonstrated that both PAR2-AP and factor VIIa significantly up-regulate IL-8 secretion (Figs. 1 and 2). The stimulating effects of factor VIIa were TF-dependent and occurred via PAR2 activation, verified by its inhibition by both anti-TF and anti-PAR2 antibodies, and not by an isotype control antibody. As is well known, IL-8 is capable of stimulating tumor cell migration and invasion in an autocrine fashion (16). IL-8 secreted by tumor cells was previously shown to regulate angiogenesis directly by enhancing endothelial cell survival, proliferation and matrix metalloproteinase production (19). In addition, IL-8 together with CXCR2 receptor expression on invasive tumor cells contributes to the breakdown of the endothelial barrier by enhancing tumor cell force generation and cytoskeletal remodeling dynamics (20). Notably, although many types of cancer cells express TF and PAR2, factor VIIa by itself does not induce IL-8 release in these cells (21). This suggests that other cell components may provide further specificity for the protease-induced signaling.

In addition to IL-8, the PAR2 agonist and factor VIIa also up-regulated TF expression at the mRNA level and TF activity in SW620 cells (Fig. 3). These results closely correspond to those of another study (22). It has also been reported that PAR2 activation induces TF endocytosis and mobilizes the Golgi TF pool to the cell surface in fibroblasts. It would appear that PAR2 activation is responsible for the regulation of TF expression in certain types of cells (23). A great number of reports indicate that TF expression in many types of cancer cells is linked to the genetic status of the cells, and may serve as a useful prognostic indicator of recurrence in tumor patients (24,25). In particular, the TF cytoplasmic domain is very important for signal transduction, and subsequently contributes to angiogenesis and tumor growth, invasion and metastasis. Thus, TF may serve as a key therapeutic target in malignancies (26). One recent study found that recombinant nematode anticoagulant protein c2 (rNAPc2), a specific inhibitor of the TF/VIIa complex, inhibits pulmonary metastasis in mice systemically disseminated with CT26 murine colon carcinoma cells (27). This further supports the therapeutic activity of TF in various types of cancer.

On the other hand, we found that PAR2 agonist and factor VIIa suppressed caspase-7 expression at both the mRNA and protein levels in SW620 cells (Figs. 4 and 5). It was demonstrated in another study (28) that factor VIIa inhibits cell death and caspase-3 activation induced by serum deprivation and loss of adhesion in TF-overexpressing cells, but not in non-TF expressing cells. Caspase-7 and -3 are closely related to each other, with an overall sequence identity of 56% and a sequence similarity of 73% (29). Both are key effectors for inducing cell apoptosis and have similar substrates. Several research groups have found that the inhibitor for caspase-7 activation can hinder the progress of non-small cell lung and pancreatic cancers (13). Avoidance of apoptosis is an important contributor to the survival of tumor cells, and the ability to specifically trigger tumor cell apoptosis is a major goal of cancer treatment (14).

The activation of PAR2 induced by TF/VIIa can elicit cell signaling through different signal pathways. It is well known that the MAPK signaling pathway plays a pivotal role in many essential cellular processes, such as proliferation, inflammation and differentiation. In this study, we found that both PAR2 agonist and factor VIIa suppressed p38MAPK

phosphorylation. These data are consistent with other reports (17). p38MAPK, a member of the MAPK family, is mainly activated by pathological injury and mediates cell apoptosis. It is also activated by mitogen and mediates cell differentiation and proliferation. For example, the low-serum component of the microenvironment confers increased motility and invasion in breast cancer cells by activating the Na^{+}/H^{-} exchanger isoform 1 (NHE1) and inhibiting the p38MAPK signaling pathway (30). In addition, fenretinide-induced Ewing's sarcoma cell death is accompanied by activation of p38MAPK (31). These data reveal a negative effect of p38MAPK on the growth of various tumor cells, suggesting a different role for the p38MAPK signaling pathway in cell pathobiology.

In summary, we demonstrated that the TF/VIIa complex not only induces IL-8 expression at both the mRNA and protein levels, but also enhances TF mRNA levels and TF activity in SW620 cells, while caspase-7 expression is down-regulated by the TF/VIIa complex. All the effects of factor VIIa were blocked by anti-TF and anti-PAR2 antibodies, indicating that the role of VIIa is TF-dependent and occurs through PAR2 activation. These results suggest that the TF/VIIa complex regulates IL-8, TF and caspase-7 expression in SW620 cells via PAR2 activation, thereby promoting colon cancer cell proliferation and migration. The p38MAPK signal transduction pathway may negatively regulate these processes.

Acknowledgements

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References

1. Mackman N, Tilley RE and Key NS: Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. Arterioscler Thromb Vasc Biol 27: 1687-1693, 2007.
2. Mackman N: Role of tissue factor in hemostasis, thrombosis and vascular development. Arterioscler Thromb Vasc Biol 24: 1015-1022, 2004.
3. Versteeg HH, Spek CA, Peppelenbosch MP and Richel DJ: Tissue factor and cancer metastasis: the role of intracellular and extracellular signaling pathways. Mol Med 10: 1-6, 2004.
4. Khorana AA, Ahrendt SA, Ryan CK, *et al*: Tissue factor expression, angiogenesis and thrombosis in pancreatic cancer. Clin Cancer Res 13: 2870-2875, 2007.
5. Awasthi V, Mandal SK, Papanna V, Rao LV and Pendurthi UR: Modulation of tissue factor-factor VIIa signaling by lipid rafts and caveolae. Arterioscler Thromb Vasc Biol 27: 1447-1455, 2007.
6. Macfarlane SR, Seatter MJ, Kanke T, Hunter G and Plevin R: Proteinase activated receptors. Pharmacol Rev 53: 245-282, 2001.
7. Ossovskaya VS and Bunnett NW: Protease-activated receptors: contribution to physiology and disease. Physiol Rev 84: 579-621, 2004.
8. Kirkland JG, Cottrell GS, Bunnett NW and Corvera CU: Agonists of protease-activated receptors 1 and 2 stimulate electrolyte secretion from mouse gallbladder. Am J Physiol Gastrointest Liver Physiol 293: G335-G346, 2007.
9. Morris DR, Ding Y, Ricks TK, Gullapalli A, Wolfe BL and Trejo J: Protease-activated receptor-2 is essential for factor VIIa and Xa-induced signaling, migration and invasion of breast cancer cells. Cancer Res 66: 307-314, 2006.
10. Zhou H, Hu H, Shi W, Ling S, Wang T and Wang H: The expression and the functional roles of tissue factor and protease-activated receptor-2 on SW620 cells. Oncol Rep 20: 1069-1076, 2008.
11. Yu JL, May L, Lhotak V, *et al*: Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. Blood 105: 1734-1741, 2005.
12. Yao C, Lin Y, Chua MS, *et al*: Interleukin-8 modulates growth and invasiveness of estrogen receptor-negative breast cancer cells. Int J Cancer 121: 1949-1957, 2007.
13. Lakhani SA, Masud A, Kuida K, *et al*: Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. Science 311: 847-851, 2006.
14. Ledgerwood EC and Morison IM: Targeting the apoptosome for cancer therapy. Clin Cancer Res 15: 420-424, 2009.
15. Arora P, Ricks TK and Trejo J: Protease-activated receptor signalling, endocytic sorting and dysregulation in cancer. J Cell Sci 120: 921-928, 2007.
16. Hijortoe GM, Petersen LC, Albrektsen T, *et al*: Tissue factor-factor VIIa-specific up-regulation of IL-8 expression in MDA-MB-231 cells is mediated by PAR-2 and results in increased cell migration. Blood 103: 3029-3037, 2004.
17. Liu YM and Mueller BM: Protease-activated receptor-2 regulates vascular endothelial growth factor expression in MDA-MB-231 cells via MAPK pathways. Biochem Biophys Res Commun 344: 1263-1270, 2006.
18. Belting M, Ahamed J and Ruf W: Signaling of the tissue factor coagulation pathway in angiogenesis and cancer. Arterioscler Thromb Vasc Biol 25: 1545-1550, 2005.
19. Li A, Dubey S, Varney ML, Dave BJ and Singh RK: IL-8 directly enhanced endothelial cell survival, proliferation and matrix metalloproteinase production and regulated angiogenesis. J Immunol 170: 3369-3376, 2003.
20. Mierke CT, Zitterbart DP, Kollmannsberger P, *et al*: Breakdown of the endothelial barrier function in tumor cell transmigration. Biophys J 94: 2832-2846, 2008.
21. Rao LV and Pendurthi UR: Tissue factor-factor VIIa signaling. Arterioscler Thromb Vasc Biol 25: 47-56, 2005.
22. Mandal SK, Pendurthi UR and Rao LV: Tissue factor internalization and trafficking in fibroblasts: involvement of protease activated receptor-mediated cell signaling. Blood 110: 161-170, 2007.
23. Ahamed J and Ruf W: Protease-activated receptor 2-dependent phosphorylation of the tissue factor cytoplasmic domain. J Biol Chem 279: 23038-23044, 2004.
24. Nitori N, Ino Y, Nakanishi Y, *et al*: Prognostic significance of tissue factor in pancreatic ductal adenocarcinoma. Clin Cancer Res 11: 2531-2539, 2005.
25. Kaido T, Oe H, Yoshikawa A, Mori A, Arii S and Imamura M: Tissue factor is a useful prognostic factor of recurrence in hepatocellular carcinoma in 5-year survivors. Hepatogastroenterology 52: 1383-1387, 2005.
26. Versteeg HH, Schaffner F, Kerver M, *et al*: Inhibition of tissue factor signaling suppresses tumor growth. Blood 111: 190-199, 2008.
27. Zhao J, Aguilar G, Palencia S, Newton E and Abo A: rNAPc2 inhibits colorectal cancer in mice through tissue factor. Clin Cancer Res 15: 208-216, 2009.
28. Versteeg HH, Spek CA, Richel DJ and Peppelenbosch MP: Coagulation factors VIIa and Xa inhibit apoptosis and anoikis. Oncogene 23: 410-417, 2004.
29. Walsh JG, Cullen SP, Sheridan C, Lüthi AU, Gerner C and Martin SJ: Executioner caspase-3 and caspase-7 are functionally distinct proteases. Proc Natl Acad Sci USA 105: 12815-12819, 2008.
30. Cardone RA, Bagorda A, Bellizzi, *et al*: Protein kinase A gating of a pseudopodial-located RhoA/ROCK/p38/NHE1 signal module regulates invasion in breast cancer cell lines. Mol Biol Cell 16: 3117-3127, 2005.
31. Myatt SS, Redfern CPF and Burchill SA: p38MAPK-dependent sensitivity of Ewing's sarcoma family of tumors to fenretinide-induced cell death. Clin Cancer Res 11: 3136-3148, 2005.