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Metastin and its variant forms suppress migration of pancreatic cancer cells

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Abstract

Metastin, a post-translationally modified variant of KiSS1, was recently identified as an endogenous peptide agonist for a novel G-protein coupled receptor, hOT7T175 (AXOR12, GPR54). In this study, we analyzed the role of KiSS1 and hOT7T175 in both pancreatic cancer tissues and pancreatic cancer cell lines. Furthermore, we synthesized novel short variant forms of metastin and tested the inhibitory effect of those variants on in vitro cell functions that are relevant to metastasis. Pancreatic cancer tissues showed significantly lower expression of KiSS1 mRNA than normal tissues ($p = 0.018$), while cancer tissues showed significantly higher expression of hOT7T175 mRNA than normal pancreatic tissues ($p = 0.027$). In human pancreatic cancer cell lines, KiSS1 mRNA was highly expressed in 2 out of 6 pancreatic cancer cell lines, while hOT7T175 mRNA was expressed in all cell lines at various degrees. PANC-1 cells showed the highest expression of hOT7T175. Exogenous metastin did not suppress cell proliferation but significantly reduced the in vitro migration of PANC-1 cells ($p < 0.01$). Metastin induced activation of ERK1 in PANC-1 and AsPC-1 cells. Finally, we synthesized 3 novel short variant forms of metastin, FM053a2TFA, FM059a2TFA, and FM052a4TFA. These metastin variants significantly suppressed the migration of PANC-1 cells and activated ERK1. These data suggest that the metastin receptor, hOT7T175, is one of the promising targets for suppression of metastasis, and that small metastin variants could be an anti-metastatic agent to pancreatic cancer.

Keywords: KiSS1; hOT7T175; Metastin; Pancreatic cancer; Migration; Metastasis

The KiSS1 peptide was originally identified as being differentially up-regulated in C8161 melanoma cells that have been rendered to have non-metastatic function by microcell mediated transfer of human chromosome 6 [1]. Transfection of *KiSS1* into human melanoma and breast carcinoma cells prevents these cells from metastasizing without an effect on cell proliferation [2]. Furthermore, the KiSS1 product has been shown to repress 92-kDa type 4 collagenase (MMP-9) expression by affecting NF-κB binding to the promoter [3]. The KiSS1 product was found to be expressed in normal human placenta, testis, brain, pancreas, and liver [4].

Recently, it was shown that the human metastasis suppressor gene *KiSS1* encodes a COOH-terminally amidated peptide with 54 amino acid residues, and that this peptide is a ligand of a novel human G-protein coupled receptor (AXOR12 and hOT7T175) which couples primarily to Gq/11 [4–7]. The receptor has a high degree of homology (81% amino acid identity) to the rat orphan heptahelical receptor, GPR54 [8], indicating that these two receptors are orthologs. The peptide ligand, named as metastin, enhances the expression and activity of focal adhesion kinase, and attenuates pulmonary metastasis of hOT7T175 transfected

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B16-BL6 melanomas in vivo [5]. In another experiment, metastin inhibits chemotaxis and invasion of hOT7T175 transfected Chinese hamster ovary cells (CHO cells) in vitro with the activation of phospholipase C, arachidonic acid release, and phosphorylation of ERK [6,7]. These indicate that metastin-hOT7T175 axis may act as an anti-metastatic system. The characteristics of inhibitory effects on cancer cell metastasis without affecting cellular growth properties of normal cells make the metastin receptor to be an attractive target for cancer therapy.

The *KiSS1* is located on human chromosome1q32–q41 [9]. However, evidences from subsequent experiments suggest that the expression of *KiSS1* is regulated by a gene(s) located in the region between 6q16.3 and q23 [1]. In pancreatic cancer, losses of 6q, 8p, 9p, 17p, and 18q are frequently observed and those alterations tend to cause lymph node and distant metastases, which suggests a suppressor gene(s) important for pancreatic cancer metastasis may exist in these regions [10–12]. Therefore, pancreatic cancer, has good reasons to downregulate KiSS1 expression. Moreover, in other cancers such as ovarian cancer, breast cancer, and thyroid papillary cancer, over-expression of hOT7T175 has been demonstrated, although KiSS1 is less frequently expressed in the tumor tissue [4,5]. Until now, however, expressions of KiSS1 and hOT7T175, and their function have not been investigated in pancreatic cancer. The purpose of the present study was to determine if KiSS1 and its receptor hOT7T175 are expressed in pancreatic cancer tissues, and to analyze the effect of exogenous metastin on pancreatic cancer cell lines with different expression levels of hOT7T175. Finally, we newly synthesized short variant forms of metastin and tested the inhibitory effect of those variants on in vitro cell functions that are relevant to metastasis.

Materials and methods

Cell culture. Pancreatic cancer cell lines, AsPC-1, BxPC-3, Capan-2, CFPAC-1, PANC-1, and SUIT-2 were purchased from the American Type Culture Collection. Cells were cultured as monolayers in the appropriate medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humid atmosphere of 5% CO_2/95% air. As for AsPC-1 and PANC-1, upon reaching 80% confluence, the medium was removed, the cells were washed in phosphate-buffered saline (PBS) and treated with various concentrations of metastin (Takeda Chemical Industries, Tsukuba, Japan) with 10% fetal bovine serum, and protein was isolated 15 min later as described below.

Patients and tumor samples. Pancreatic cancer tissues obtained from 30 patients who underwent pancreatectomy at our Department between January 1998 and June 2001 were used. Patients with other pancreatic malignancies, such as intraductal papillary mucinous adenocarcinoma, acinar cell carcinoma, and endocrine tumor, were excluded. Informed consent was obtained from each patient according to the institutional guidelines. Samples for mRNA expression were immediately frozen in liquid nitrogen at the time of surgery and stored at −80 °C.

Peptide synthesis. Fifty-four amino acid peptide, metastin, was kindly provided from Dr. T. Ohtaki, Takeda Chemical Industries [5]. We synthesized 3 short peptide variants of metastin, which were defined as FM053a2TFA, FM059a2TFA, and FM052a4TFA. The sequences of these peptides are as follows:

FM053a2TFA: Gu-Amb-Phe-Gly-Leu-Arg-Trp-NH_2,
FM059a2TFA: Ac-Trp-Asn-Arg-Phe-Gly-Leu-Arg-Trp-NH_2,
FM052a4TFA: Bis(Py)-Amb-Phe-Gly-Leu-Arg-Trp-NH_2.

These peptides were selected by screening from various truncated forms of KiSS1 peptide and modified peptides for gaining almost the same internal signals with metastin through the receptor using reporter gene assay in yeast.

All reagents for peptide synthesis were purchased from Watanabe Chemical Industries (Hiroshima, Japan), NovaBiochem (Darmstadt, Germany), Nacalai Tesque (Kyoto, Japan), and Wako Pure Chemical Industries (Osaka, Japan). Ion-spray mass spectra were obtained with a Sciex APIIIIE triple quadrupole mass spectrometer. Protected peptide-resins were manually constructed on Fmoc-NH-SAL resin by Fmoc-based solid-phase peptide synthesis. Trt for Asn and Pbf for Arg were employed for side-chain protection. In the synthesis of FM052a and FM053a, amino-group modifications were performed after coupling of 4-(aminomethyl)benzoic acid, respectively. Reductive amination using pyridine-2-aldehyde and $NaBH_3$ (OAc) provided the protected resin for FM052a. Treatment with 1H-pyrazole-1-carboxamidine hydrochloride and *N*,*N*-diisopropylethylamine gave the resin for FM053a. Deprotection/cleavage by treatment of the peptide resins with a mixture of TFA-thioanisole-*m*-cresol-H_2O-1,2-ethanedithiol-triisopropylsilane (80:5:5:5:2.5:2.5, v/v) followed by purification by reverse phase HPLC (Cosmosil 5C18-ARII column, Nacalai Tesque, Japan, 20 × 250 mm) yielded the peptides. FM059a was prepared by N-terminal acetylation of the protected octapeptide, subsequent deprotection/cleavage, and HPLC purification as well.

Quantitative RT-PCR. To monitor gene expression, we used quantitative real-time RT-PCR analysis [13–15]. Briefly, within the amplicon defined by a gene-specific PCR primer pair, an oligonucleotide probe labeled with 2 fluorescent dyes is created and designated as TaqMan probe. As long as the probe is intact, the emission of the reporter dye (6-carboxy-fluorescein, FAM) at the 5′-end is quenched by the second fluorescence dye (6-carboxy-tetramethyl-rhodamine, TAMRA) at the 3′-end. During the extension phase of PCR, the polymerase cleaves the TaqMan probe, resulting in a release of the reporter dye. The increasing amount of reporter dye emission is detected by an automated sequence detector combined with analysis software (ABI Prism 7700 Sequence Detection System; PE Applied Biosystems). The conditions of the reaction were according to the manufacturer's protocol. Five microliters of cDNA (reverse transcription mixture) with 25 μl TaqMan Universal PCR Master Mix (PE Applied Biosystems) and oligonucleotides at a final concentration of 0.3 μM for primers and 0.2 μM for the TaqMan hybridization probe were analyzed in a 50-μl volume.

The following primers and TaqMan probes were used for analysis. The KiSS1 specific primers were

5′-ACTCACTGGTTTCTTGGCAGC-3′ (upstream primer),
5′-ACCTTTTCTAATGGCTCCCCA-3′ (downstream primer), and
5′ (FAM)-ACTGCTTTCCTCTGTGCCACCCACT-(TAMRA)3′ (TaqMan probe).

The hOT7T175 specific primers were

5′-CGACTTCATGTGCAAGTTCGTC-3′ (upstream primer),
5′-CACACTCATGGCGGTCAGAG-3′ (downstream primer), and
5′ (FAM)-ACTACATCCAGCAGGTCTCGGTGCAGG-(TAMRA)3′ (TaqMan probe).

The thermal cycle parameters were 95 °C for 10 min (for heat activation of *Taq*-Polymerase), followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Assessment of GAPDH RNA for quality and normalization was done with the TaqMan GAPDH Control Reagent Kit (PE Applied Biosystems) which utilizes standard TaqMan probe chemistry.

Protein extraction and Western blotting. Cells were collected into microtubes with a cell scraper and lysed for 60 min in phosphorylation-inhibitory RIPA buffer containing 50 mM Hepes (pH 7.0), 250 mM NaCl, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 20 μg/ml gabexate mesilate, and then the lysate was sonicated for 10 s. Total extracts were cleaned by centrifugation at 12,000 rpm for 10 min at 4 °C and the supernatants were collected. Protein concentrations were measured using a protein assay kit (Tonein-TP, Otsuka Pharmaceutical, Tokyo, Japan). The lysates were resuspended in one volume of the gel loading buffer which contained 50 mM Tris–HCl (pH 6.7), 4% SDS, 0.02% bromophenol blue, 20% glycerol and 4% 2-mercaptoethanol, and then boiled at 95 °C for 90 s. The extracted protein was subjected to Western blotting, as previously described [16]. In brief, 30-μg aliquots of protein were size-fractionated to a single dimension by SDS–PAGE (12% gels) and transblotted to a 0.45-μm polyvinylidene difluoride membrane (Bio-Rad, Richmond, CA) in a semidry electroblot apparatus (Bio-Rad, Richmond, CA). The blots were then washed 3 times with TBST buffer and incubated for 2 h at RT in the first antibody solution containing anti-phospho-ERK antibody (pTEpY, Promega, Madison, WI), anti-phosphop38 MAPK (pTGpY, Promega, Madison, WI) or anti-phosphoJNK (pTPpY, Promega, Madison, WI), 0.2% I-block (Promega, Madison, WI). After 3 washings in TBST buffer, the blots were incubated for 1 h at RT with horseradish peroxidase-conjugated anti-rabbit IgG at a 1:2000 dilution with TBST buffer. After 3 washings in TBST buffer, membranes were treated with enhanced chemiluminescence reagents (Amersham Life Sciences, Amersham, UK) according to the manufacturer's protocol. Membranes were exposed to X-ray film for 50–60 s. Protein expression was measured by ATTO densito-analyzer system AE-6920M (ATTO Corporation, Tokyo Japan) and the quantity was expressed numerically. The quantity of the target protein was divided by that of β-actin and relative intensities were calculated.

Cell proliferation assay. AsPC-1 and PANC-1 cells (1×10^4 cells/3 cm diameter dish) were seeded in 10% FBS medium and incubated with increasing doses of metastin for 48 and 96 h. Cells were trypsinized and cell numbers were counted using hematocytometer.

Cell migration assay and Matrigel invasion assays. A polyvinyl-pyrrolidone-free polycarbonate framed filter (8 μm pores) was set in a chamber (Corning Coster, Cambridge, MA). Cells (2×10^6 cells in 200 μl RPMI1640 for AsPC-1 and in 200 μl DMEM for PANC-1) and designated concentrations of peptide were added to the upper chamber and incubated at 37 °C for 12 h to allow migration to the lower chamber, which contained 10% FBS/RPMI1640 for AsPC-1 or 5% FBS/DMEM for PANC-1 as a chemoattractant. After removing non-migrating cells with a cotton swab from the upper surface of the membrane, cells on the lower surface were fixed, stained with Diff-Quick (International Reagent, Kobe, Japan). For quantification, cells were counted under a microscope in 5 predetermined fields at 200×.

Cells and peptide (2×10^6 cells in 200 μl RPMI164O for AsPC-1 and in 200 μl DMEM for PANC-1) were added to a Matrigel-coated Transwell (8 μm pores, Becton–Dickinson Labware, Bedford, MA) and incubated at 37 °C for 12 h versus a lower chamber containing 10% FBS/RPMI1640 for AsPC-1 or 5% FBS/DMEM for PANC-1. After removing the Matrigel and cells from the upper surface of the membrane, cells on the lower surface were fixed, stained with Diff-Quick and the number quantified as well. Invasion index was defined as the number of invaded cells per that of migrated cells.

Statistical analyses. The comparative statistical evaluations among groups in the densitometry or in the migratory activity were first performed by a two-way analysis of variance for repeated measures, followed by a post hoc Tukey test. To compare the mRNA levels in pancreatic tissues, Wilcoxon's rank sum test was performed. All assays were performed 3 times independently. Statistical analyses were done using JMP statistical software (version 3.02). Probability value of <0.05 was considered significant.

Results

Expression of KiSS1 and hOT7T175 in pancreatic cancer tissues

First, we measured the mRNA expression levels of KiSS1 and hOT7T175 in 30 pancreatic cancer tissues and in 5 adjacent normal pancreatic tissues. All the normal pancreatic tissues (5/5) and 14/30 of pancreatic ductal carcinoma tissues expressed KiSS1 mRNA (Fig. 1A). The expression level of KiSS1 mRNA in

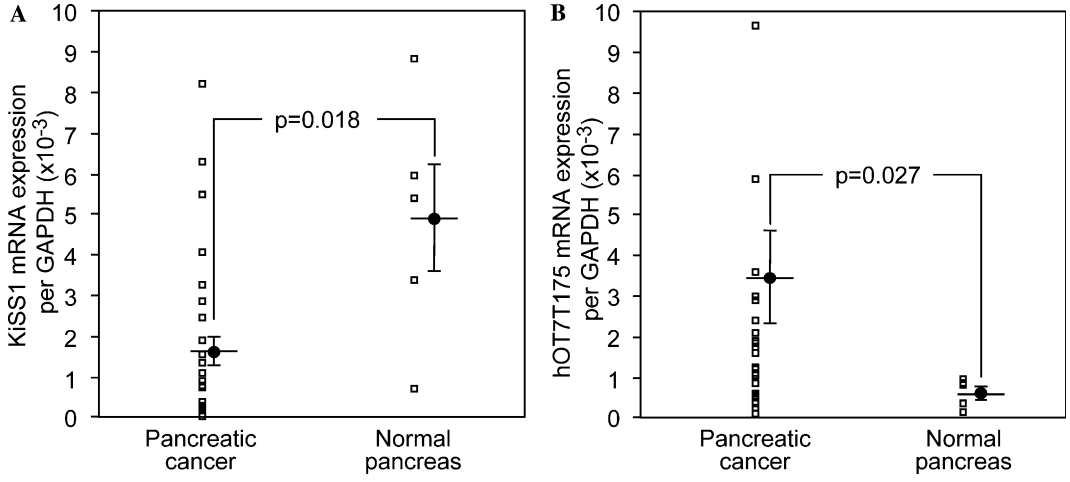

Fig. 1. Expression of KiSS1 mRNA and hOT7T175 mRNA in pancreatic cancer tissues. The KiSS1 mRNA and hOT7T175 mRNA in pancreatic cancer ($n = 30$) and normal pancreatic tissues ($n = 5$) were measured by real-time RT-PCR. The level of KiSS1 mRNA in pancreatic cancer tissues was significantly lower than that of normal pancreatic tissues ($p = 0.018$). The level of hOT7T175 mRNA in pancreatic cancer tissues was significantly higher than that of normal pancreatic tissues ($p = 0.027$).

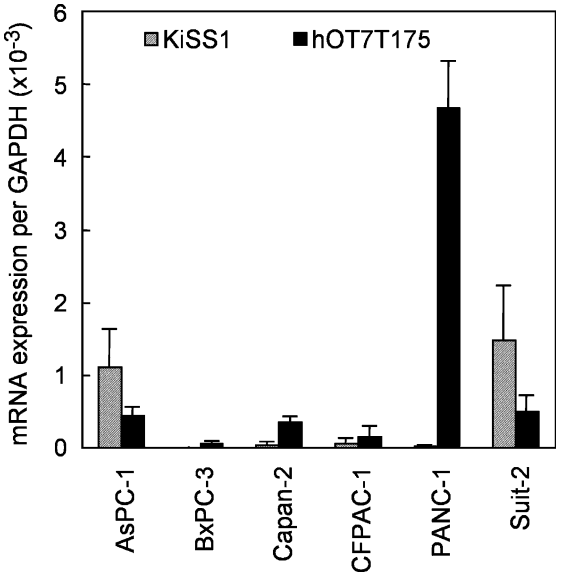

Fig. 2. Expression of KiSS1 mRNA and hOT7T175 mRNA in pancreatic cancer cell lines. The KiSS1 mRNA and hOT7T175 mRNA in pancreatic cancer cells were measured by real-time RT-PCR. AsPC-1 and SUIT-2 showed high level of metastin mRNA expression and other 4 cell lines showed very low level of expression. In contrast, all cell lines expressed hOT7T175 mRNA. PANC-1 cell most highly expressed hOT7T175 mRNA. Measurements were repeated three times and data are expressed as means ± SEM.

pancreatic cancer tissues was significantly lower than normal pancreatic tissues ($p = 0.018$). In contrast, all the pancreatic cancer tissues (30/30) and normal pancreatic tissues (5/5) expressed KiSS1 receptor hOT7T175 mRNA, and the expression level of hOT7T175 mRNA in pancreatic cancer tissues was significantly higher than that of normal tissues ($p = 0.027$) (Fig. 1B). We tested paired samples from 5 patients (cancer and normal tissues from each patient). The expression of hOT7T175 was higher in cancer tissue than the adjacent normal pancreatic tissue in all the 5 paired samples.

Expression of KiSS1 and hOT7T175 in pancreatic cancer cell lines

We next measured the expression of KiSS1 mRNA and hOT7T175 mRNA in 6 pancreatic cancer cell lines (Fig. 2). Among 6 pancreatic cancer cell lines, AsPC-1 and SUIT-2 showed high level of KiSS1 mRNA expression and the other 4 cell lines showed a very low level of expression. In contrast, all cell lines expressed hOT7T175 mRNA at various degrees. PANC-1 cell most strongly expressed hOT7T175 mRNA. According to these results, we chose AsPC-1 cell line as a representative of high KiSS1 and low hOT7T175, and chose PANC-1 cell line as that of low KiSS1 and high hOT7T175. We used these two cell lines in the following experiments.

Effects of exogenous metastin on proliferation, migration, and invasion through endogenous metastin receptor

We examined the effect of exogenous metastin on pancreatic cancer cell proliferation. Metastin peptide was added to AsPC-1 and PANC-1 cells in the phase of exponential growth at final concentrations of 0, 0.1, 1, and 10 μM for 48 and 96 h. The addition of metastin had no effects on cell proliferation of AsPC-1 and PANC-1.

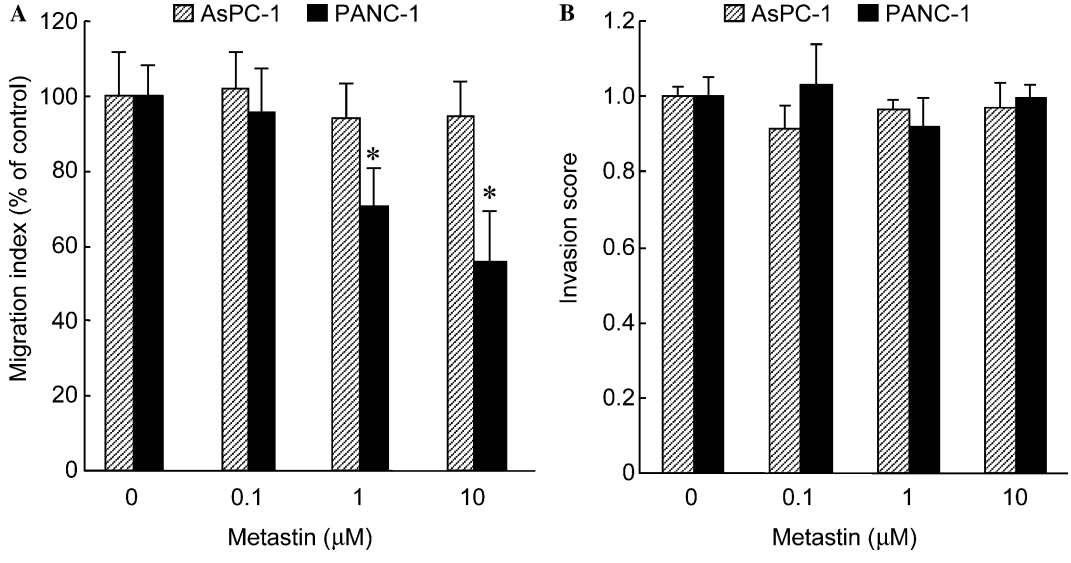

Fig. 3. Effects of exogenous metastin on migration and invasion of pancreatic cancer cells. (A) Effect of metastin on migration of pancreatic cancer cells. Cells were treated with various concentrations of metastin for 12 h. Metastin at 1 and 10 μM significantly suppressed migratory activity of PANC-1 cells. (B) Effect of metastin on invasion of pancreatic cancer cells. Cells were treated with various concentrations of metastin for 12 h. Invasion activity was not affected by tested concentrations of metastin in both AsPC-1 and PANC-1. Experiments were repeated three times and data are expressed as means ± SEM. * Represents $p < 0.05$ against control.

Next, we tested the effect of metastin on migration and invasion of these cell lines. The migration of AsPC-1 was not significantly affected by metastin, while PANC-1 was significantly inhibited by metastin at 1 and 10 μM ($p < 0.05$) (Fig. 3A). The invasion of the two cell lines was not significantly affected by metastin (Fig. 3B).

Effects of metastin on MAPK activation in AsPC-1 and PANC-1 cells

We analyzed the activation of MAPK by metastin. Cancer cells in exponential phase were incubated in serum containing medium and then transferred to 1% BSA medium with metastin as described in the section of migration and invasion assay. After incubation with metastin for 15 min, ERK1/2, p38, and JNK1/2 were investigated by immunoblotting (Fig. 4). Protein expression was measured by ATTO densito-analyzer system AE-6920M (ATTO Corporation, Tokyo Japan) and the relative intensity was expressed numerically (Fig. 5). Metastin induced a significant increase of pERK1 in AsPC-1 cells at 1 and10 μM and in PANC-1 at 0.1–10 μM (Fig. 5A). Metastin induced a significant increase of pp38 in PANC-1 cells at 10 μM (Fig. 5B).

Effects of short variant forms of metastin on proliferation and migration of hOT7T175 expressing pancreatic cancer cells

We analyzed the effects of metastin and newly synthesized short peptides, FM053a2TFA, FM059a2TFA, and FM052a4TFA, on PANC-1 cells which highly express hOT7T175. We found that the cell growth was not affected by these 3 peptides. In migration assay, metastin, FM059a2TFA, and FM052a4TFA significantly inhibited the migration of PANC-1 cells (Fig. 6). We examined the activation of ERK1/2, p38, and JNK by these variant forms and found that ERK1 and p38 were activated by metastin and all variant forms of metastin (Fig. 7).

Discussion

In this study, we have demonstrated for the first time that hOT7T175 is expressed in pancreatic cancer tissues, but KiSS1 is less expressed when compared to normal pancreatic tissues. These results are in agreement with the analysis by other investigators in ovarian cancer, breast cancer, and colon cancer [2,5]. Several reports indicated that KiSS1 and its receptor hOT7T175 are also highly expressed in placenta [4,5]. The placenta is an invasive tissue, and there are similarities in the behavior of invading cancer cells and that of invading placenta cells [17]. It is possible that KiSS1 and hOT7T175 may constitute a common mechanism in both of these processes, whereas the correlations of clinicopathological factors such as distant metastasis and invasion with KiSS1/hOT7T175 function have not been clearly proved yet.

We next demonstrated that PANC-1 cells, which express hOT7T175, showed significant suppression of cell migration with concomitant activation of ERK1 but not

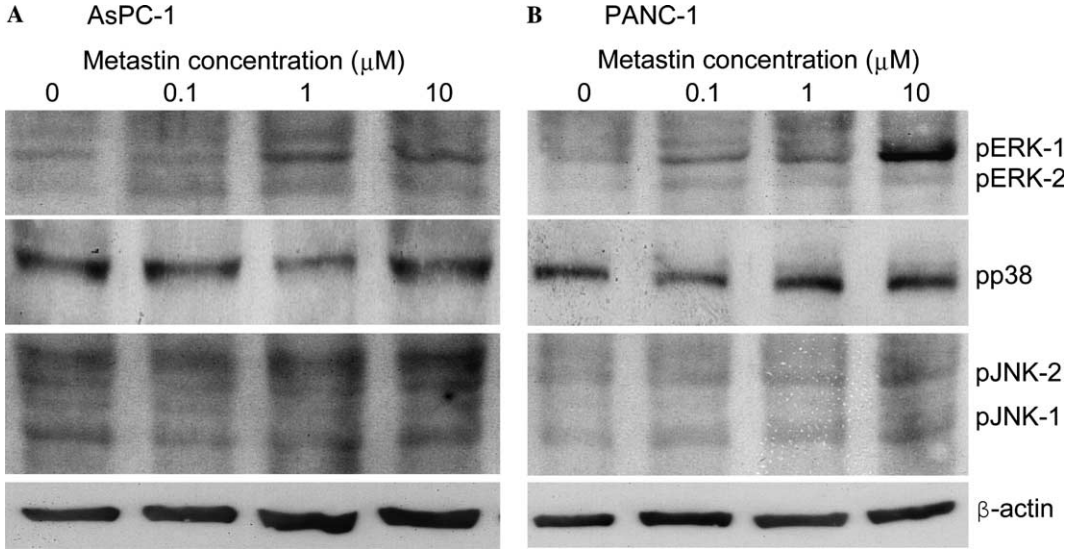

Fig. 4. Effects of metastin on MAPK activation in AsPC-1 and PANC-1 cells. AsPC-1 and PANC-1 cells were treated with various concentrations of metastin for 15 min. Western blot analysis identified double band corresponding to phosphorylated ERK1 (pERK1) and phosphorylated ERK2 (pERK2), single band of phosphorylated p38 (pp38), and double band showing phosphorylated JNK1 (pJNK1) and phosphorylated JNK2 (pJNK2). pERK1 was augmented in AsPC-1 by metastin at 1 and 10 μM and in PANC-1 by metastin at 0.1, 1, and 10 μM. p38 was augmented in PANC-1 by metastin at 10 μM.

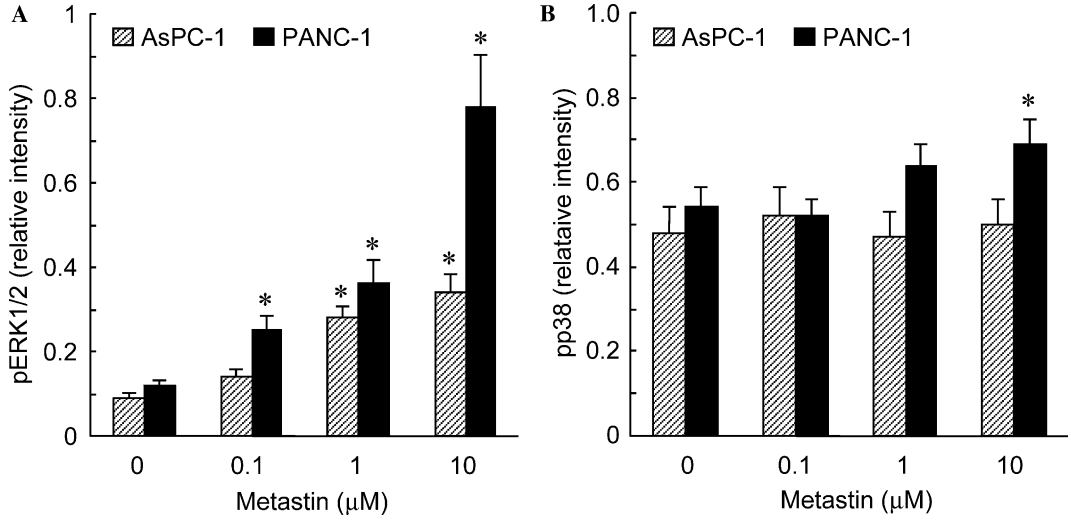

Fig. 5. Effects of metastin on pERK1/2 and of pp38 in AsPC-1 and in PANC-1 cells. AsPC-1 and PANC-1 cells were treated with various concentrations of metastin for 15 min. Protein expression of pERK1/2 or pp38 was measured by a densito-analyzer system and the quantity was expressed numerically. The quantity of the protein was divided by that of β-actin and the relative intensities were calculated. Metastin induced a significant increase of pERK1 in AsPC-1 cells at 1 and 10 μM and in PANC-1 at 0.1–10 μM (A). Metastin induced a significant increase of pp38 in PANC-1 cells at 10 μM (B). Experiments were repeated three times and data are expressed as means ± SEM. * Represents $p < 0.05$ against control.

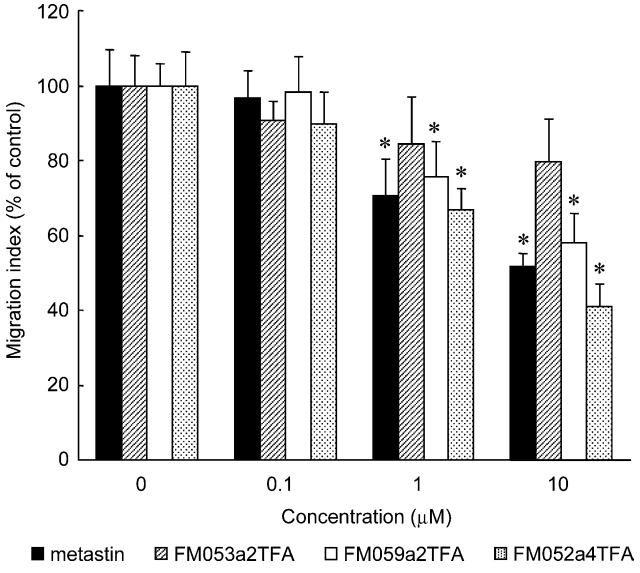

Fig. 6. Effects of short variant forms of metastin on migration of hOT7T175 expressing pancreatic cancer cells. The effect of newly synthesized short variant forms of metastin on migration of PANC-1 cells was evaluated. Metastin, FM059a2TFA, and FM052a4TFA significantly inhibited the migration activity of PANC-1 cells. Experiments were repeated three times and data are expressed as means ± SEM. * Represents $p < 0.05$ against control.

of JNK1/2 in response to exogenous metastin, while AsPC-1 cells with low expression of hOT7T175 revealed comparatively less response. Of note, the cell growth suppression was not observed both in PANC-1 and in AsPC-1, that is consistent with the previous results in melanoma and in breast cancer cells [1,2]. In contrast, in other types of cells, KiSS1 product was reported to activate ERKs and to inhibit cell proliferation [7]. These controversial results suggest that the proliferative characteristics were not a property shared by this receptor. Rather this, ERK activation may be involved in suppression of the tumor cell motility. In our study, suppression of the motility of PANC-1 cells was concomitant with the activation of ERK pathway. Moreover, as demonstrated by the newly synthesized short peptide treatment, the rate of ERK activation is in proportion to the suppression of migration.

Activation of MAP kinase and p38 has been described in hOT7T175 transfected Chinese hamster ovary cells (CHO cells) [7]. However, it has been shown that only MAP kinase but not p38 was activated with metastin treatment in anaplastic thyroid cancer cells ARO, which endogenously express hOT7T175 [18]. This discrepancy may be partly accounted by the expression level of hOT7T175 and may be by the cell specificity. In our experiment, PANC-1 cells with high expression of hOT7T175 showed activation of ERK1 and p38, while AsPC-1 cells with less hOT7T175 expression did not. These results may indicate the necessity of strong metastin-hOT7T175 signals on p38 activation in cancer cells.

We found metastin did not suppress invasion of PANC-1 and AsPC-1 cells, although several authors have reported that metastin suppresses invasion of melanoma and breast cancer cells [2,5]. The inhibitory effect on invasion could be explained by the report that showed that KiSS1 represses the invasion of HT1080 cells through decreased type 4 collagenase (MMP-9) expression and downregulation of NF-κB [3]. In pancreatic cancer tissues, we previously reported that active

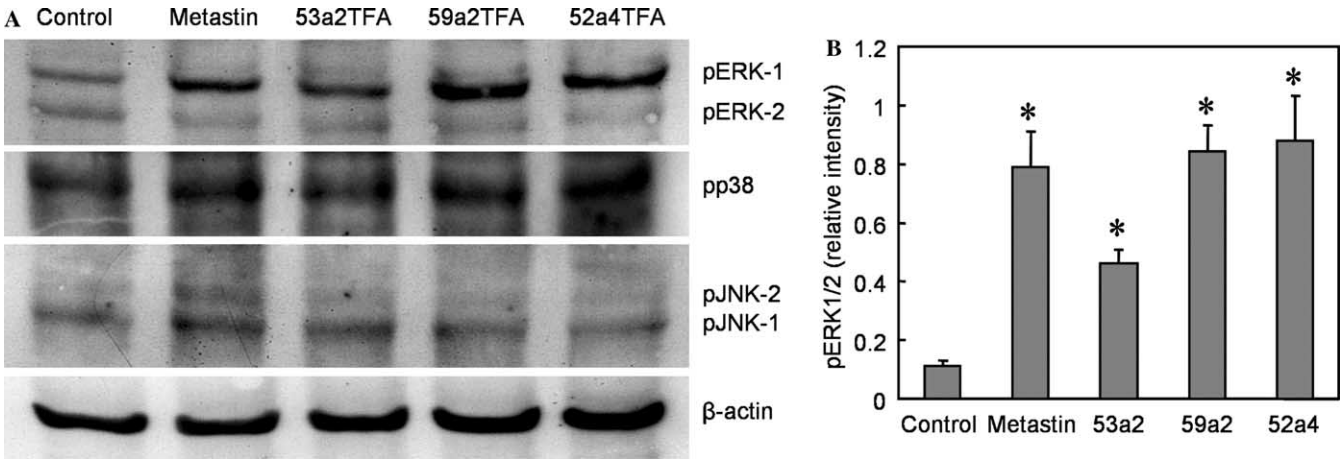

Fig. 7. Effects of short variant forms of metastin on MAPK activation in hOT7T175 expressing pancreatic cancer cells. (A) The activation of ERK1/2, p38, and JNK ERK1 activation by metastin and short variant forms of metastin was observed in PANC-1 cells. (B) pERK1 expression was measured by a densito-analyzer system, the quantity of the protein was divided by that of β-actin, and the relative intensities were calculated. In PANC-1 cells, ERK1 was significantly activated by metastin and all variant forms of metastin. Experiments were repeated three times and data are expressed as means ± SEM. * Represents $p < 0.01$ againstcontrol.

form of MMP-2 was detected in all samples; however, active form of MMP-9 was seen in only 21% of the samples [19]. Because latent forms of MMP-2 and MMP-9 were expressed in all pancreatic cancer tissues, MMP-9 may not mainly contribute to cancer invasion when compared to MMP-2. This might be one of the reasons why metastin did not affect invasion of PANC-1 and AsPC-1 cells. To our knowledge, there has been no other investigation on migration or invasion of cancer cells which endogenously express hOT7T175.

Our newly synthesized short variant forms of metastin showed significant suppression on motility of PANC-1 cells. These peptides are composed of 6–9 amino acids, suggesting that at most 10 amino acids of the C-terminus of metastin-54 will be sufficient for its binding affinity and function. However, FM053a2TFA is less effective in suppressing migration of PANC-1 cells when compared to other two compounds or metastin-54. Interestingly, the suppressive activity was in proportion to the ERK activation rates. Much remains to be understood about how effective they will block migration and how the peptides could be stably delivered to the tumors. The effective short peptide has an advantage that it would not cause immune responses if it could be given to patients orally.

In conclusion, we demonstrated that pancreatic cancer tissues express hOT7T175 and low expression of KiSS1 when compared to normal pancreatic tissues. The exogenous metastin and the variant forms of metastin suppress migration of hOT7T175-expressing pancreatic cancer cells and activate ERK1 and p38. Our results suggest that hOT7T175 may be one of the promising targets against cancer cell functions that are relevant to metastasis, and that short variant forms of metastin could be an anti-metastatic agent to pancreatic cancer.

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