Introduction

Pancreatic cancer is an aggressive disease associated with an extremely poor prognosis. It is one of the most malignant tumors, characterized by an insidious onset, late diagnosis and low survival rate (1,2). Patients with pancreatic cancer usually present with locally advanced, unresectable or metastatic diseases, and are often sensitive to the adverse effects of more intensive treatments. Only 10–15% of patients have a form of this disease that is amenable to surgical resection, and the recurrence rate remains high even with radical surgery (3). Although some regressions have been made in developing new diagnostic methods and novel targeted therapies, the overall survival rate (3). Although some progress has been made in developing new diagnostic methods and novel targeted therapies, the overall survival rate has not improved over the last decade (4). Thus, there remains a need for novel therapeutic strategies for treating pancreatic cancer.

Collagen triple helix repeat containing-1 (CTHRC1) is a soluble protein reported to be involved in vascular remodeling, bone formation and morphogenesis. CTHRC1 has been shown to be expressed in human cancers such as breast cancer and melanoma. In this study, we show that CTHRC1 is highly expressed in human pancreatic cancer tissues and plays a role in the progression and metastasis of the disease. CTHRC1 promoted primary tumor growth and metastatic spread of cancer cells to distant organs in orthotopic xenograft tumor mouse models. Overexpression of CTHRC1 in cancer cells resulted in increased motility and adhesiveness, whereas these cellular activities were diminished by down-regulation of the protein. CTHRC1 activated several key signaling molecules, including Src, focal adhesion kinase, paxillin, mitogen-activated protein kinase kinase (MEK), extracellular signal-regulated kinase and Rac1. Treatment with chemical inhibitors of Src, MEK or Rac1 and expression of dominant-negative Rac1 attenuated CTHRC1-induced cell migration and adhesion. Collectively, our results suggest that CTHRC1 has a role in pancreatic cancer progression and metastasis by regulating migration and adhesion activities of cancer cells.

Materials and methods

Cell lines and materials

Human pancreatic cancer cell lines (Panc-1, MiaPaCa-2 and BxPC-3) were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured at 37°C in a humidified 5% CO₂ incubator in Dulbecco’s modified Eagle medium containing 10% fetal bovine serum (FBS; Panc-1); Dulbecco’s modified Eagle medium containing 10% FBS and 2.5% horse serum (MiaPaCa-2) or RPMI medium containing 10% FBS (BxPC-3). Cell lines overexpressing CTHRC1 were generated by amplifying the coding region of CTHRC1 with PCR using the primers 5′-TGG TAC CGG CCA TCA GGG CCA TGC GAC CCC-3′ and 5′-CCT TTC TAG ATG GGC CAG CCA GGC CTT-3′. The resulting PCR product was cloned into the KpnI and Xhol sites of pcDNA3.1(+)-Myc-His (Invitrogen, Carlsbad, CA) to produce the CTHRC1 expression plasmid pLF238. MiaPaCa-2 cells were transfected with pLF238 or pcDNA3.1(+)-Myc-His using Lipofectamine (Invitrogen). Stably transfected clones were selected using G418. CTHRC1 knockdown cell lines were generated by cloning CTHRC1-specific short hairpin RNA (shRNA; 5′-CAG GTG TGG TAT TCC ACA T3′) into the lentiviral vector shLenti2.4G (Macrogen, Seoul, Korea) to produce shLenti2.4G-CTHRC1. A control lentiviral vector, shLenti2.4G-Ctrl, containing a scrambled shRNA (5′-AAT CGC ATA GGG TAT GCC CTT-3′) was also created. After introducing shLenti2.4G-CTHRC1 or shLenti2.4G-Ctrl into cells, stable shRNA-expressing clones were selected using puromycin. At least three independent clones were analyzed for each stable cell line. PPI and NSC23766 were purchased from Calbiochem (San Diego, CA) and U0126 was from Cell Signaling (Beverly, MA). Laminin, vitronectin, fibronectin and collagen type I were from Sigma (St Louis, MO). Antibodies against Src, p-Src, focal adhesion kinase (FAK), p-FAK, paxillin, p-paxillin, mitogen-activated protein kinase kinase (MEK)-1/2, p-MEK1/2, extracellular signal-regulated kinase (Erk) and p-Erk were from Cell Signaling. The anti-paxillin antibody for immunofluorescence staining was from

Abbreviations: CTHRC1, collagen triple helix repeat containing-1; DN-Rac1, dominant-negative Rac1; Erk, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FBS, fetal bovine serum; GTP, guanosine triphosphate; MEK, mitogen-activated protein kinase kinase; PDAC, pancreatic ductal adenocarcinoma; RT–PCR, reverse transcription–PCR; shRNA, short hairpin RNA; TGF-β, transforming growth factor-β.

These authors contributed equally to this work.
CTHRC1 promotes pancreatic cancer cell migration and adhesion

Abcam (Cambridge, MA). The anti-CTHRC1 antibody was from Abcam and Abgent (San Diego, CA). The anti-Fc antibody was from Sigma. The anti-β-actin antibody was from Santa Cruz (Santa Cruz, CA). The EZ-Detect Rac1 activation kit was from Pierce (Rockford, IL).

Genome-wide expression analysis

Microarray expression analyses of normal pancreas (n = 13) and pancreatic adenocarcinomas (n = 28) were performed using the HG-U133 GeneChip from Affymetrix (West Sacramento, CA) as described previously (15). Briefly, clinical tissue samples were harvested within 10 min of surgical resection, snap frozen in liquid nitrogen and stored at −80°C until further processing. All samples were subjected to review by pathologists to confirm diagnosis and assess overall tumor content. Normal pancreatic tissues were obtained from patients who underwent pancreatic resection for other diseases. These tissues were histologically normal tissues with no visible dysplastic changes in the ducts. The ductal adenocarcinoma samples analyzed contained 25–70% of tumor cells. RNA preparation and hybridization to the HG-U133 were performed according to the manufacturer’s directions. Expression values of all gene fragments were normalized and analyzed using Microarray Suite 5.0 (Affymetrix). Outliers were detected by principal component analyses using the MatLab program (The MathWorks, Natick, MA) and were excluded from the clustering analysis. Confidence limits were set at a P-value < 0.05 for each differentially expressed gene fragment. The ethical committee and institutional review board of Yonsei University College of Medicine approved the protocol of tissue acquisition from surgical specimens, and written informed consent was obtained from each patient.

Reverse transcription–PCR

Total RNA was prepared from clinical tissue specimens using an RNeasy kit (Qiagen, Valencia, CA). Total RNA from matched pancreas tissues was purchased from Ambion (Carlsbad, CA). Reverse transcription–PCR (RT–PCR) was performed using PreMix RT–PCR (Bioneer, Daejeon, Korea). A 573 bp fragment of the CTHRC1 gene was amplified with the primers 5′-AGC GCC TCT GAG TTC CCC AA-3′ and 5′-TGA ACA AGT GCC AACCCA GA-3′. A 555 bp fragment of the glyceraldehyde-3-phosphate dehydrogenase gene, used as an internal reference, was amplified with the primers 5′-TGA TGA CAT CA A GA GGT GGT GAA-3′ and 5′-TCC TTG GAC ATG TGG GCC AAT-3′.

Immunohistochemistry and evaluation

Surgically resected pancreatic cancers from 30 patients were studied by immunohistochemistry. All tumors were adenocarcinomas and were defined as primary pancreatic adenocarcinoma arising from pancreatic ducts. Specimens were fixed in formalin, embedded in paraffin and analyzed immunohistochemically using the EnVision-horseradish peroxidase detection system from Dako (Carpinteria, CA) according to the protocol suggested by the supplier. Isotype control excluding the primary antibody was used as a negative control.

Preparation of recombinant CTHRC1

Recombinant CTHRC1 (rCTHRC1) was generated by inserting the coding region of CTHRC1, a thrombin cleavage sequence and a constant region of fibronectin or collagen type 1, the lower compartment was filled with medium containing 10% FBS. The Transwell system was incubated for 20 h in 5% CO₂ at 37°C. Chemicals, antibodies or recombinant proteins, as needed, were added in the upper chamber. Migrated cells were quantified as described previously (16). Cell adhesiveness was assayed using 96-well plates coated with 1 μg/ml of laminin, vitronectin, fibronectin or collagen type 1. Cells were seeded at a density of 1 × 10⁵ cells per well in 100 μl of serum-containing medium and then incubated at 37°C. Unbound cells were removed by washing twice with phosphate-buffered saline. Adhered cells were quantified as described previously (17). Western blot analysis

Cells lysates were prepared in radiomununoprecipitation assay (RIPA) buffer and culture supernatants were concentrated using Microcon centrifugal filters (Millipore, Billerica, MA). Immunoblot analyses were performed as described previously (16).

Results

CTHRC1 expression is up-regulated in pancreatic ductal adenocarcinomas

Comparison of genome expression data between pancreatic ductal adenocarcinomas (PDAC) and normal pancreas revealed a significant increase (~8.0-fold) in CTHRC1 transcripts in cancer tissues samples (Figure 1A). Consistent with this, CTHRC1 messenger RNA was also up-regulated in PDAC, as confirmed by RT–PCR (Figure 1B). A comparison of matched samples in another tissue subset also indicated up-regulation of CTHRC1 expression in cancerous tissue compared with adjacent non-cancerous tissue (Figure 1C). CTHRC1 expression was further evaluated by immunohistochemical analysis of 25 pancreatic cancer tissue specimens from patients. CTHRC1 expression was very weakly detected in normal pancreatic ductal epithelium, but was strongly detected in adenocarcinoma cells (Figure 1D(a)). The results showed that 23% of pancreatic tumors (7 out of 30) showed weak or no staining (Figure 1D(b)), whereas 77% (23 out of 30) showed moderate or strong staining in cytoplasm and cell membrane (Figure 1D(c) and d)). Some metastatic tumor cells showed strong expression (Figure 1D(e)). There was no correlation between CTHRC1 expression and histological grade, age or sex (Supplementary Table 1, available at Carcinogenesis Online). CTHRC1 was expressed in pancreatic cancer cell lines, whereas no expression was detected in non-cancerous cell lines, including normal pancreatic ductal epithelial cells (Supplementary Figure S1, available at Carcinogenesis Online). Collectively, these data indicate that CTHRC1 is expressed in PDAC but not in normal pancreas.

CTHRC1 enhances tumor progression and metastasis in vivo

To explore the effects of CTHRC1 expression, we established a set of human pancreatic cancer cell lines in which CTHRC1 was stably up- or down-regulated. The MiaPaCa-2 cell line, exhibiting little CTHRC1 expression, was stably transfected with a CTHRC1 expression plasmid, generating the CTHRC1-overexpressing cell line, MiaPaCa-2-CTHRC1. A control cell line, MiaPaCa-2-Mock, containing an empty vector was also generated. For CTHRC1 knockdown studies, the BxPC-3 and Panc-1 cell lines, which express high levels of CTHRC1, were stably transfected with a CTHRC1-specific shRNA, generating the cell lines BxPC-3-shCTHRC1 and Panc-1-shCTHRC1, respectively. The corresponding control shRNA cell lines were designated BxPC-3-shCtrl and Panc-1-shCtrl. Establishment of overexpression and knockdown stable cell lines was confirmed by western blot analysis (Supplementary Figure S2, available at Carcinogenesis Online). As a final step, we stably introduced the firefly luciferase gene into the indicated cell lines by lentiviral gene transfer. Cell lines were then injected into mice.
and the effects of CTHRC1 on the progression of implanted tumors was evaluated using in vivo bioluminescence imaging analysis. An examination of tumor progression in this orthotopic human pancreatic tumor model revealed that the intensity of luminescence signals was markedly higher in MiaPaCa-2-CTHRC1-injected mice compared with control mice injected with MiaPaCa-2-Mock, with evidence of metastatic spread to several secondary organs, including spleen, liver, colon and stomach (Figure 2A). In contrast, BxPC-3-shCTHRC1-injected mice showed decreased primary tumor progression and metastatic spread to distant organs compared with control mice injected with BxPC-3-shCtrl (Figure 2B). These in vivo data suggest a role for CTHRC1 in the metastatic spread of pancreatic cancer cells.

CTHRC1 enhances tumor cell migration and adhesion in vitro

On the basis of our finding that CTHRC1-induced metastasis of pancreatic cancer cells in vivo, we investigated whether CTHRC1 influenced the motility and adhesiveness of pancreatic cancer cells in vitro. Compared with the corresponding control cells, MiaPaCa-2-CTHRC1 cells showed significantly increased migration ability when tested on a filter coated with extracellular matrix components (laminin, vitronectin, fibronectin or collagen type I) (Figure 3A). The adhesiveness of MiaPaCa-2 cells was also increased by CTHRC1 overexpression (Figure 3B). CTHRC1 overexpression in Panc-1 cells also enhanced cell migration and adhesion (Supplementary Figure S5B and C, available at Carcinogenesis Online). Knockdown of CTHRC1 in BxPC-3 and Panc-1 cells significantly decreased motility and adhesiveness of pancreatic cancer cells (Figure 3C and D and Supplementary Figure S5, available at Carcinogenesis Online). Neither CTHRC1 overexpression nor knockdown altered the proliferation rate of pancreatic cancer cells (data not shown). Because CTHRC1 is a secreted protein, we sought to determine whether CTHRC1 could regulate cell migration and adhesion in an autocrine manner. To this end, we prepared recombinant CTHRC1 protein (rCTHRC1) using the CHO cell line (Supplementary Figure S4A, available at Carcinogenesis Online). The motility and adhesiveness of MiaPaCa-2 cells were increased by rCTHRC1 in a concentration-dependent manner (Supplementary Figure S4B and C, available at Carcinogenesis Online), a result that is in good agreement with the results obtained with CTHRC1 overexpression. In addition, the enhancement in the motility and adhesiveness of pancreatic cancer cells by rCTHRC1 largely disappeared upon pretreatment of cells with an anti-CTHRC1 antibody, but not a control antibody, confirming the specificity of the rCTHRC1 effects (Figure 3E and F). CTHRC1 overexpression in pancreatic cancer cells induced reorganization of the actin cytoskeleton and formation of lamellipodia (Supplementary Figure S5A, available at Carcinogenesis Online). We also found that CTHRC1-overexpressing cells exhibited increased numbers of focal adhesion complexes with a smaller average size (Supplementary Figure S5B, available at Carcinogenesis Online), indicating increased turnover of focal adhesions (19). These in vitro findings indicate that CTHRC1 has a significant role in the motility and adhesiveness of pancreatic cancer cells.

CTHRC1 activates Src-FAK signaling

In order to elucidate the molecular mechanisms responsible for the induction of cell adhesion and migration by CTHRC1, we examined the activation of FAK, which is fully activated after recruiting Src and formation of a Src-FAK complex (20,21). CTHRC1 overexpression increased the levels of phosphorylated Src and FAK, whereas knockdown of CTHRC1 expression decreased their levels (Figure 4A and Supplementary Figure S5A, available at Carcinogenesis Online). Phosphorylation of Paxillin, a focal-adhesion adaptor molecule, was also increased by CTHRC1 overexpression, whereas CTHRC1
CTHRC1 promotes pancreatic cancer cell migration and adhesion knockdown led to reduced levels of activated paxillin. Because paxillin also serves as a scaffold for the organization and activation of the MAPK proteins Raf, MEK and Erk (22,23), we determined whether CTHRC1 activated these paxillin-associated molecules. Indeed, the levels of phosphorylated MEK and Erk were increased by CTHRC1 overexpression and decreased by CTHRC1 knockdown (Figure 4A and Supplementary Figure S5A, available at *Carcinogenesis* Online). Consistent with this, treatment of MiaPaCa-2 cells with rCTHRC1 induced the rapid, transient activation of Src, FAK, paxillin, MEK and Erk (Figure 4B). CTHRC1-induced migration and adhesion activities were significantly attenuated by the Src inhibitor PP2 and the MEK inhibitor U0126 (Figure 4C–H), which also efficiently blocked the CTHRC1-mediated activation of their respective targets (Supplementary Figure S7A and B, available at *Carcinogenesis* Online). Moreover, PP2 attenuated the CTHRC1-induced increases in actin cytoskeleton reorganization, lamellipodia formation and focal adhesion turnover (Supplementary Figure S6, available at *Carcinogenesis* Online). Thus, CTHRC1 activates Src and Erk.

![Graph showing total flux from bioluminescence imaging](image_url)
signaling pathways, both of which contribute to the motility and adhesiveness of pancreatic cancer cells.

**CTHRC1 induces Rac1 activation**

We next examined whether CTHRC1 induces activation of Rac1—a critical factor for the migration of most cell types that is stimulated by paxillin signaling (24, 25)—in pancreatic cancer cells. We found that GTP-Rac1 levels were increased in MiaPaCa-2-CTHRC1 cells and diminished in Panc-1-shCTHRC1 cells compared with their respective controls (Figure 5A). Rac1 activation was also induced by rCTHRC1, peaking 5min after stimulation (Figure 5B). Conversely, the Rac1 inhibitor NSC23766 blocked the rCTHRC1-mediated activation of GTP-Rac1 (Supplementary Figure S7C, available at Carcinogenesis Online). Furthermore, transfection of dominant-negative (DN)-Rac1 into MiaPaCa-2-CTHRC1 cells blocked activation of Rac1 by CTHRC1 (Supplementary Figure S7D, available at Carcinogenesis Online). The CTHRC1-induced increase in the motility and adhesiveness of MiaPaCa-2-CTHRC1 cells was significantly decreased by treatment with NSC23766 (Figure 5C and E) or overexpression of DN-Rac1 (Figure 5D and F). NSC23766 also attenuated the CTHRC1-induced increases in actin cytoskeleton

---

**Fig. 3.** CTHRC1 enhances migration and adhesion activities of pancreatic cancer cells in vitro. (A and C) Migration activity of MiaPaCa-2-CTHRC1 (A) and BxPC-3-shCTHRC1 (C) cells was determined using Transwell chambers coated with laminin, vitronectin, fibronectin or collagen type I. Cells that had migrated to the lower surface of the Transwell membrane after 20h incubation were stained with hematoxylin. (B and D) Adhesion activity of MiaPaCa-2-CTHRC1 (B) and BxPC-3-shCTHRC1 (D) cells was determined using 96-well plates coated with laminin, vitronectin, fibronectin or collagen type I. After 20min of incubation, adhered cells were stained and counted. (E and F) Migration (E) and adhesion (F) activities of MiaPaCa-2 cells on surfaces coated with collagen type I were measured after treatment with 100nM of rCTHRC1 in the presence of 10 μg/ml of anti-CTHRC1 antibody or control IgG. Migration and adhesion results were from three independent experiments with triplicate samples in each. *P < 0.05; **P < 0.01.
CTHRC1 promotes pancreatic cancer cell migration and adhesion

reorganization, lamellipodia formation and focal adhesion turnover (Supplementary Figure S6, available at Carcinogenesis Online). These data suggest that Rac1 is required for the effects of CTHRC1 on the motility and adhesiveness of pancreatic cancer cells.

Discussion

In this study, we describe the aberrant expression of CTHRC1 in pancreatic cancer and investigate the potential role of CTHRC1 in the progression and metastasis of the disease. In previous reports, CTHRC1 expression appeared to correlate with cancer progression. Immunohistochemical analysis has revealed that CTHRC1 is highly overexpressed in metastatic melanoma (11), dermatofibrosarcoma protuberans (a locally aggressive neoplasm that frequently recurs and metastasize (13)) and breast cancer compared with normal tissue. Kaplan-Meier survival analyses have also shown that the time to relapse (bone metastasis) in breast cancer patients with high levels of periostin, a critical regulator of bone formation and maintenance, is shorter in patients with high CTHRC1

Fig. 4. CTHRC1 facilitates Src-FAK signaling. (A) Lysates prepared from MiaPaCa-2-CTHRC1, MiaPaCa-2-Mock, Panc-1-shCTHRC1 and Panc-1-shCtrl cells were used for western blot analysis with antibodies against p-Src, Src, p-FAK, FAK, p-paxillin, paxillin, p-MEK, MEK, p-Erk or Erk. β-Actin was used as a loading control. (B) MiaPaCa-2 cells were serum starved for 16 h and then treated with 100 nM of rCTHRC1 for 0, 5, 10, 20, 30, 120 or 720 min. Cell lysates were prepared and used for western blot analysis as in A. (C and D) Serum-starved MiaPaCa-2 cells were treated with 100 nM of rCTHRC1 in the presence or absence of with 10 μM of PP2 (C) or 10 μM of U0126 (D). Cell lysates were prepared and used for western blot analysis as in A. (E–H) Migration (E and F) and adhesion (G and H) activities of MiaPaCa-2-Mock and MiaPaCa-2-CTHRC1 cells on surfaces coated with collagen type I were measured in the presence of PP2 (E and G) or U0126 (F and H). Western signals were quantified by ImageJ and presented as the ratio of band intensities relative to that of the control sample. Migration and adhesion results were from three independent experiments with triplicate samples in each. *P < 0.05; **P < 0.01.
expression (14). Consistent with these observations, our results provide strong preclinical and experimental evidence that CTHRC1 is a critical factor that facilitates pancreatic cancer progression and metastasis. Mice injected with CTHRC1-overexpressing cells showed increased tumor progression and metastasis into secondary organs, such as spleen, liver, colon, stomach and diaphragm. These results indicate that, although pancreatic tumor formation and metastasis may not absolutely require CTHRC1 expression, CTHRC1 plays an essential role in enhancing the aggressiveness of pancreatic cancer, including the metastatic properties of the disease.

Because cancer cell metastasis is dependent on increased migratory and adhesive properties, we tested the possibility that the mechanism underlying the effects of CTHRC1 overexpression in pancreatic cancers and cell lines involved the promotion of migration and adhesiveness.

Both gain of function and loss of function approaches were used to establish the critical role of CTHRC1 in the migration and adhesiveness of pancreatic cancer cells. Overexpression of CTHRC1 in MiaPaCa-2 cells increased the migratory and adhesive properties of these cells. Conversely, shRNA-mediated knockdown of CTHRC1 expression in BxPC-3 or Panc-1 cells reduced cell migration and adhesiveness. Our studies using an orthotopic xenograft model and pancreatic cancer cell lines indicate that CTHRC1 expression led to more aggressive behavior, including metastasis, and increased tumor cell motility and adhesiveness.

However, how CTHRC1 regulates cancer cell migration and adhesin is not understood. To further explore the molecular mechanism by which CTHRC1 overexpression affects the migration of pancreatic cancer cells, we investigated its effects on the Src-FAK signaling cascade, which plays a crucial role in regulating the formation of protein complexes at focal adhesions in the migration and metastasis of cancer cells (26). FAK promotes cancer cell migration by regulating focal adhesion formation and turnover, which involves activation of Src and paxillin (27). Overexpression of CTHRC1 and treatment with rCTHRC1 led to significant increase in the levels of activated p-Src and p-FAK. Furthermore, we found that treatment with PP2, an inhibitor of Src, diminished the migration of pancreatic cancer cells and Src-FAK signaling induced by rCTHRC1 treatment, suggesting that autocrine and paracrine activation of the CTHRC1 signaling pathway is critical for cell motility in pancreatic cancer cells. In addition, we found that CTHRC1-induced activation of Rac, a key regulator of migration that localizes in its active form at the leading edge of motile cells (28).
CTHRC1 promotes pancreatic cancer cell migration and adhesion

The ability of CTHRC1 to activate the Src-FAK signaling cascade appears to account for the effect of CTHRC1 on cell motility. One possible mechanism by which CTHRC1 might enhance cell migration is through interaction with Wnt5a, which has been implicated in the oncogenic effects of CTHRC1. CTHRC1 has been shown to stabilize the Wnt receptor complex by enhancing Wnt-receptor interaction, thereby enhancing the activity of the non-canonical Wnt/planar cell polarity pathway, which regulates actin polymerization through activation of small GTPases, c-Jun N-terminal kinase and Rho kinase (29). Our preliminary studies suggested that Wnt5a and its associated molecules, Ror2 (receptor tyrosine kinase-like orphan receptor 2) and Fzd2 (frizzled homolog 2), were up-regulated in pancreatic cancer tissue and CTHRC1 could bind Wnt5a in pancreatic cancer cells (Supplementary Figure S8A and B, available at Carcinogenesis Online). Wnt5a indeed induced the activation of Src and FAK in pancreatic cancer cells (Supplementary Figure S8D, available at Carcinogenesis Online). Stabilization of the Wnt5a complex by overexpressed CTHRC1 might induce activation of Src and FAK and turnover of paxillin, as has been shown in gastric cancer (30). Moreover, we found that phosphorylation of Erk, which is a well-known target of Src-FAK signaling whose activation correlates with epithelial cell migration (31), was induced by CTHRC1 overexpression in pancreatic cancer cells. On the basis of these results and the observation that CTHRC1 most effectively enhances cell adhesion through binding to the monomeric form of collagen type I, we speculate that activation of the Wnt5a signaling cascade by CTHRC1 also contributes to the regulation of integrin family proteins. Specifically, CTHRC1 might alter expression of integrin members, especially α1β1 and α2β1. Therefore, it is conceivable that CTHRC1 is a multifunctional protein that promotes cell migration through activation of the Wnt5a signaling cascade.

In addition to implicating CTHRC1 as an important positive regulator of Src-FAK signaling in pancreatic cancer, our findings demonstrate that CTHRC1 is overexpressed in pancreatic cancer tissue and cell lines and plays a critical role in cell migration, adhesiveness and tumorigenesis. These results have several potential significant implications. First, CTHRC1 overexpression might serve as a novel biomarker for pancreatic cancer, as has been shown for breast and colon cancers, suggesting that a comprehensive examination of CTHRC1 expression in a large number of pancreatic cancers with respect to its prevalence and relation to clinical outcome and response to various treatments is warranted. Second, our results establish a novel and solid foundation for future studies on the molecular mechanisms governing the role of CTHRC1 in pancreatic cancer cell migration, adhesiveness and metastasis, and its interplay with other proteins involved in the development, progression and metastasis of pancreatic cancers. Finally, CTHRC1 might offer a novel target in the treatment of pancreatic cancers; because CTHRC1 expression induces metastasis, targeting CTHRC1 could be a potential strategy for interfering with multiple pathways involved in cancer metastasis, but this hypothesis need to be explored carefully and will be the subject of future investigation.

Supplementary material
Supplementary materials and methods, Table 1 and Figures S1–8 can be found at http://carcin.oxfordjournals.org/.

Funding
National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology, (5789M3G6F8008) and the Biomedical Fusion Technology Development Project of the Korea Research Institute of Bioscience and Biotechnology (KGM1231211).

Acknowledgements
We are grateful to Ming-Sound Tsao for supplying the HPDE cell line. We thank Semi Kim for providing the DN-Rac1 plasmid, Yangsoon Lee and Kyung Sook Yu for rCTHRC1 production, and Hye-jin Min, Jung-Whoi Lee, Donggwang Lee and Hyunho Yoon for technical assistance.

Conflict of Interest Statement: The authors declare no conflicts of interest.

References

Received June 26, 2012; revised November 6, 2012; accepted November 22, 2012