Promotion of Cellular Growth and Motility Is Independent of Enzymatic Activity of Fibroblast Activation Protein-α

BAOBEI LV[1*], FEI XIE[1*], PENGXIANG ZHAO[1], XUEMEI MA[1],
WEN G. JIANG[2], JING YU[3], XIAODONG ZHANG[3] and JUN JIA[3]

[1]*College of Life Science and Bioengineering, Beijing University of Technology, Beijing, P.R. China;*
[2]*Cardiff University–Peking University Joint Cancer Institute, Cardiff China Medical Research Collaborative, Cardiff University School of Medicine, Cardiff, U.K.;*
[3]*Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), The VIP-II Division of Medical Department, Peking University Cancer Hospital and Institute, Beijing, P.R. China*

Abstract. *Background: Fibroblast activation protein-alpha (FAPα) is a type-II integral membrane serine protease and is expressed in most stromal fibroblasts. Recent studies showed that FAPα is also expressed in certain cancer cells but its role is still uncertain. Materials and Methods: We analyzed the non-enzymatic activity of FAPα in breast cancer cells by introducing an enzymatic mutant FAPα (FAPS624A), in which the serine catalytic triad was destroyed. FAPα overexpression and knockdown cells were generated as controls. Results: Cellular growth and motility were markedly increased in MCF-7 cells overexpressing FAPα and enzymatic-mutant FAPS624A. This is consistent with observations in FAPα-silenced BT549 cells. Western blotting showed activation of phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) and matrix metalloproteinase (MMP) 2/9 in both wild-type FAPα-overexpressing and enzymatic-mutant FAPS624A-overexpressing cells. Conclusion: Promotion of cellular growth and motility is independent of the enzymatic activity of FAPα in breast cancer cells. The PI3K/AKT and MMP2/9 signaling pathways might be involved in such regulation.*

Human fibroblast activation protein-alpha (FAPα, also known as seprase) is a type-II integral membrane serine protease belonging to the prolyl-cleaving peptidase family with post-prolyldipeptidyl peptidase and gelatinase activity (1, 2). It is a 170-kDa homodimeric glycoprotein consisting of two *N*-glycosylated 97 kDa subunits. Studies have shown that FAPα is primarily expressed in fetal mesenchymal tissues, wounded tissues, and stromal fibroblasts of more than 90% of malignant epithelial tumors. However, benign tumors or normal adult tissues, except bone marrow mesenchymal cells, are generally FAPα-negative (3-5). Through its gelatinase activity, FAPα degrades extracellular matrix and promotes growth and invasion of cancer cells (6, 7). Elevated expression of FAPα in stromal fibroblasts is associated with poor prognosis and increased metastasis in a number of cancer types, including colonic (8), pancreatic (9), ovarian (10), lung (11), and gastric (12) cancer. This makes FAPα a novel prognostic biomarker and a potential therapeutic target in cancer.

Furthermore, an increasing number of studies have demonstrated that FAPα expression is not confined to stromal fibroblasts. The molecule was also found in many epithelia-derived cancer cells including breast (13), gastric (14), ovarian (15), and pancreatic (16) cancer. Thus, the role of cancer cell expression of FAPα is of great interest. It has been noted that cancer cell-expressed FAPα had non-enzymatic functions. Huang *et al.* found that inhibitors of prolylpeptidase did not slow the growth of tumors produced by FAPα-expressing breast cancer cells in an animal model. The study also showed that breast cancer cells expressing a catalytically inactive mutant of FAPα also produced tumors that grew rapidly (17). Yang *et al.* reported that FAPα with integrin α3β1 and the urokinase-type plasminogen activator receptor (uPAR) signaling complex mediated ovarian cancer cell migration *via* the small GTPase Ras-related C3 botulinum toxin substrate 1 (RAC1) pathway. FAPα-mediated up-regulation of phosphorylated extracellular signal-regulated kinase (ERK) occurred in a time-dependent manner (18). Wang *et al.* found that silencing FAPα inhibited

*These Authors contributed equally to this study.

Correspondence to: Dr. Jun Jia, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), The VIP-II Division of Medical Department, Peking University Cancer Hospital & Institute, Beijing, 100142, China. Tel: +86 1088196406, Fax: +86 1088196578, e-mail: vm26jun@gmail.com

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the growth and metastasis of oral squamous cell carcinoma (OSCC) cells *in vitro* and *in vivo*. In addition, knockdown of FAPα inactivated phosphatase and tensin homolog (PTEN)/phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) and rat sarcoma viral oncogene homolog (RAS)-ERK signaling pathway and resulted in down-regulation of proliferation, migration, and invasion by OSCC cells (19).

In the present study, we analyzed the role of FAPα in regulating breast cancer cell growth, adhesion, invasion and migration. To ascertain whether FAPα has non-enzymatic functions in the regulation of cell growth and motility, an enzymatic mutant of FAPα by site-directed mutagenesis at the catalytic site 624th serine (serine replaced with alanine) to destroy the gelatinase activity was set as a control. The potential signaling pathway involved in the function of FAPα in breast cancer cells was also explored.

Materials and Methods

Cell lines. Human breast cancer cells, MCF-7 and BT549 were obtained from the American Type Cell Collection (ATCC, Manassas, VA, USA). Cells were routinely cultured with Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, penicillin and streptomycin (Gibco BRC, Paisley, Scotland, UK).

FAPα expression constructs. cDNA of FAPα was generated from normal human prostate tissues by reverse transcription polymerase chain reaction (RT-PCR) using the primers: 5'-TTA GTC TGA CAA AGA GAA ACA CTG and 5'-ATG AAG ACT TGG GTA AAA ATC G. The serine at 624 site in the catalytic triad of FAPα was substituted for alanine using QuikChange® II Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA) and forward primer: 5'-GAA TAG CCA TAT GGG GCT GGT CCT ATG GAG GAT ACG TTT C, reverse primer: 5'-GAA TAG CCA TAT GGG GCT GGG CCT ATG GAG GAT ACG TTT C. Full-length wild-type FAP and mutant type FAPS624A were then cloned into a pEF6/V5-His vector (Invitrogen, Paisley, Scotland, UK). Multiple clones of *Escherichia coli* were screened and plasmids from the clones were sequenced.

Short hairpin RNA (shRNA) interference of FAPα. Four shRNA sequences targeting the FAPα gene were designed and synthesized by Shanghai Jima Pharmaceutical Technology, China. The four shRNA sequences of FAPα and the negative control sequence are listed in Table I.

Plasmid transfection. Breast cancer cells MCF-7 and BT549 were cultured in DMEM. Lipofectamine LTX and Plus Reagent (Invitrogen) were used to transfect FAPα expression constructs and shRNA into breast cancer cells according to the manufacturer's protocol. The medium containing transfection reagents was replaced with DMEM supplemented with 10% FBS at 18 h after the transfection. The cells were collected at 48 h after the transfection, processed for the following experiments, and then prepared for protein extraction. The overexpression and silencing efficiency of FAPα was tested by RT-PCR and western blot.

Table I. *Interference sequence of short hairpin RNA (shRNA) for fibroblast activation protein-alpha (FAPα). CON-sh: Control shRNA.*

shRNA	Sequence (5'-3')
shRNA288	GCC CTT CAA GAG TTC ATA ACT
shRNA506	GCT TCA AAT TAC GGC TTA TCA
shRNA672	GGT CGC CTG TTG GGA GTA AAT
shRNA839	GCT CTC TGG TGG TCT CCT AAT
CON-sh	GTT CTC CGA ACG TGT CAC GT

RT-qPCR analysis of FAPα expression. Total RNA was extracted and reverse-transcribed into cDNA using M-MLV-RTase (Promega, Madison, WI, USA). The resulting cDNA was used for PCR, which employed a SYBR-Green Master PCR Mix (Applied Biosystem, Carlsbad, CA, USA) in triplicates. Primers for qRT-PCR were as follows: Forward primer: 5'-TGT GCA TTG TCT TAC GCC CT, reverse primer: 5'-CCG ATC AGG TGA TAA GCC GT. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control to normalize FAP expression (forward primer: 5'-GGC TGC TTT TAA CTC TGG TA, reverse primer: 5'-GAC TGT GGT CAT GAG TCC TT). Differential expression of FAP was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blotting. Confluent cells were harvested using the EDTA/Trypsin method, pelleted and then lysed using a lysis buffer containing 2.4 mg/ml Tris, 4.4 mg/ml NaCl, 5 mg/ml sodium deoxycholate, 20 μg/ml sodium azide, 1.5% Triton, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml aprotinin, for 45 min at 4˚C. After lysis and centrifugation at 10,000 × *g* for 15 min to remove the insolubles, protein concentrations for each sample were measured using an improved Lowry assay (DC Protein Assay kit; Bio-Rad Laboratories, Hercules, CA, USA). The samples were adjusted to equal protein concentrations with sample buffer and then boiled at 100˚C for 5 min, before being separated on a 10% polyacrylamide gel. Following electrophoresis, these separated protein samples were transferred to phenylmethylsulfonyl fluoride membranes (Millipore, Kankakee, IL, USA) and proteins detected with appropriate primary antibodies including those against FAPα (LifeSpan BioSciences Inc., Seattle, WA, USA), GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA), AKT, p-AKT (Ser-473), PI3K, p-PI3K (Tyr-458), matrix metalloproteinase (MMP)2 and MMP9 (Cell Signaling Technology, Danvers, MA, USA) at a concentration of 1:300, and specific peroxidase-conjugated secondary antibodies at a concentration of 1:1,000. The protein signals were detected using SuperSignal West Dura Extended Duration Substrate (Pierce, Rockford, IL, USA).

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium (MTT) assay. Cells were grown in exponential phase and detached by trypsin/EDTA treatment. Viable cells (2,000 cells/ml) were plated into 96-well tissue culture plates (100 μl complete medium/well) and cultured at 37˚C in 5% CO_2. At different time points, MTT reagent was added (10 μl per well) and cells incubated at 37˚C for a further 4 h. The reaction was stopped with addition of 100 μl dimethyl sulfoxide and the optical density was determined at OD570 nm on a multi-well plate reader.

Cell-matrix adhesion assay. A 96-well plate was pre-coated with 5 μg of Matrigel (Collaborative Research Products, Bedford, MA, USA) and allowed to dry overnight. Following rehydration with serum-free medium, 20,000 cells were seeded into each well. After 40 min of incubation, non-adherent cells were washed off using balanced salt solution buffer. The remaining cells were fixed with 4% formalin and stained with 0.5% crystal violet. The number of adherent cells was then counted under microscopy.

In vitro invasion assay. Transwell inserts (Becton Dickinson Labware, Oxford, UK) with 8 μm pore size were coated with 50 μg of Matrigel and air-dried. Following rehydration with serum-free medium, cells were seeded at a density of 30,000 per insert. After 3 days' incubation, cells that had migrated through the matrix and adhered to the other side of the insert were fixed in 4% formalin, stained with 0.5% (weight/volume) crystal violet, and counted under a microscope.

Migration/wound-healing assay. Cells were seeded into a 6-well plate and allowed to reach confluence. The monolayer of cells was then scraped with a P-200 pipette tip to create a wound of approximately 200 μm. Fresh medium supplemented with 5% FBS was added, and the wound-closing procedure was observed for 12 h. Photographs were taken at 0, 6 and 12 h, respectively.

Statistical analysis. Data from all quantitative assays were expressed as mean±SD. One-way ANOVA and independent-samples t-test were used to evaluate difference between groups in all tests performed, with the resultant p-value representing a two-sided test of statistical significance. All statistical analyses were performed and visualized by GraphPad Prism 5.0. A value of $p<0.05$ was considered statistically significant.

Results

FAPα overexpression and knockdown in breast cancer cell lines. To identify the oncogenic function of FAPα in breast cancer, we utilized shRNA to silence FAPα expression in BT549 cells, an FAPα-positive human breast cancer cell line. Knockdown efficiency was evaluated by both RT-qPCR and western blotting. The results showed that FAPα expression was reduced by 90% in BT549 cells after transfection with shRNA 288 (Figure 1). In addition, FAPα and its enzymatic mutant type FAPS624A were transfected into MCF-7 breast cancer cells, an FAPα-null cell line. As shown in Figure 1, overexpression of both wild-type FAPα and mutant-type FAPS624A in MCF-7 cells was successfully established.

Effects of FAPα expression on cell growth. We first determined the effect of FAPα overexpression on cell growth *in vitro* (Figure 2A). Overexpression of FAPα in MCF-7 cells significantly increased the growth rate at day 3 (p=0.006) and day 5 (p=0.002) relative to the control. The growth rate was also raised by overexpression of FAPS624A in MCF-7 cells at day 3 (p<0.001) and day 5 (p<0.001).

The effects of FAPα expression on cell growth of MCF-7 cells were consistent with observations in BT549 cells.

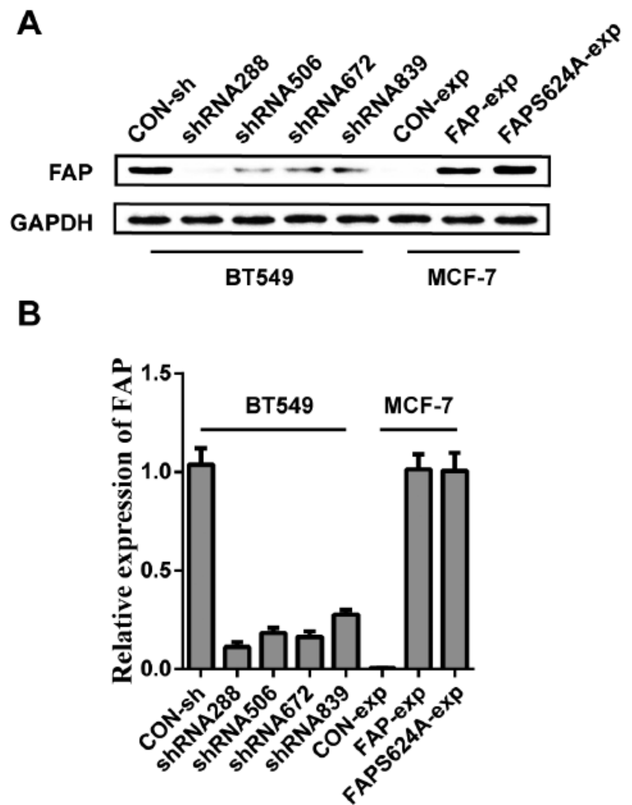

Figure 1. *Determination of the overexpression and knockdown efficiency of fibroblast activation protein-alpha (FAPα) in breast cancer cells. FAPα expression was reduced by 90% in BT549 cells after transfection with shRNA 288. Wild-type FAPα and its enzymatic mutant type FAPS624A-overexpressing MCF-7 cells were successfully established. A: FAPα expression was examined by western blotting. B: Reverse transcription qualitative real-time polymerase chain reaction was performed to determine the expression of FAPα.*

Knockdown of FAPα resulted in a dramatic reduction in cell growth rate at day 5 (p=0.004). However, the absence of FAPα did not lead to a significantly decreased growth rate at day 3.

Effects of FAPα expression on cell adhesion. The influence of FAPα on the adhesiveness of breast cancer cells to matrix proteins was also determined. As shown in Figure 2B, overexpression of both FAPα and mutant FAPS624A in MCF-7 cells significantly enhanced the adhesive properties compared with control cells (p=0.0014 and p=0.011, respectively). Conversely, knockdown of FAPα led to a significant reduction in adhesive ability (p=0.044).

Effects of FAPα expression on cell invasion. Matrigel invasion assay was used to evaluate the invasive capability of breast cancer cells. As shown in Figure 2C, MCF-7 cells

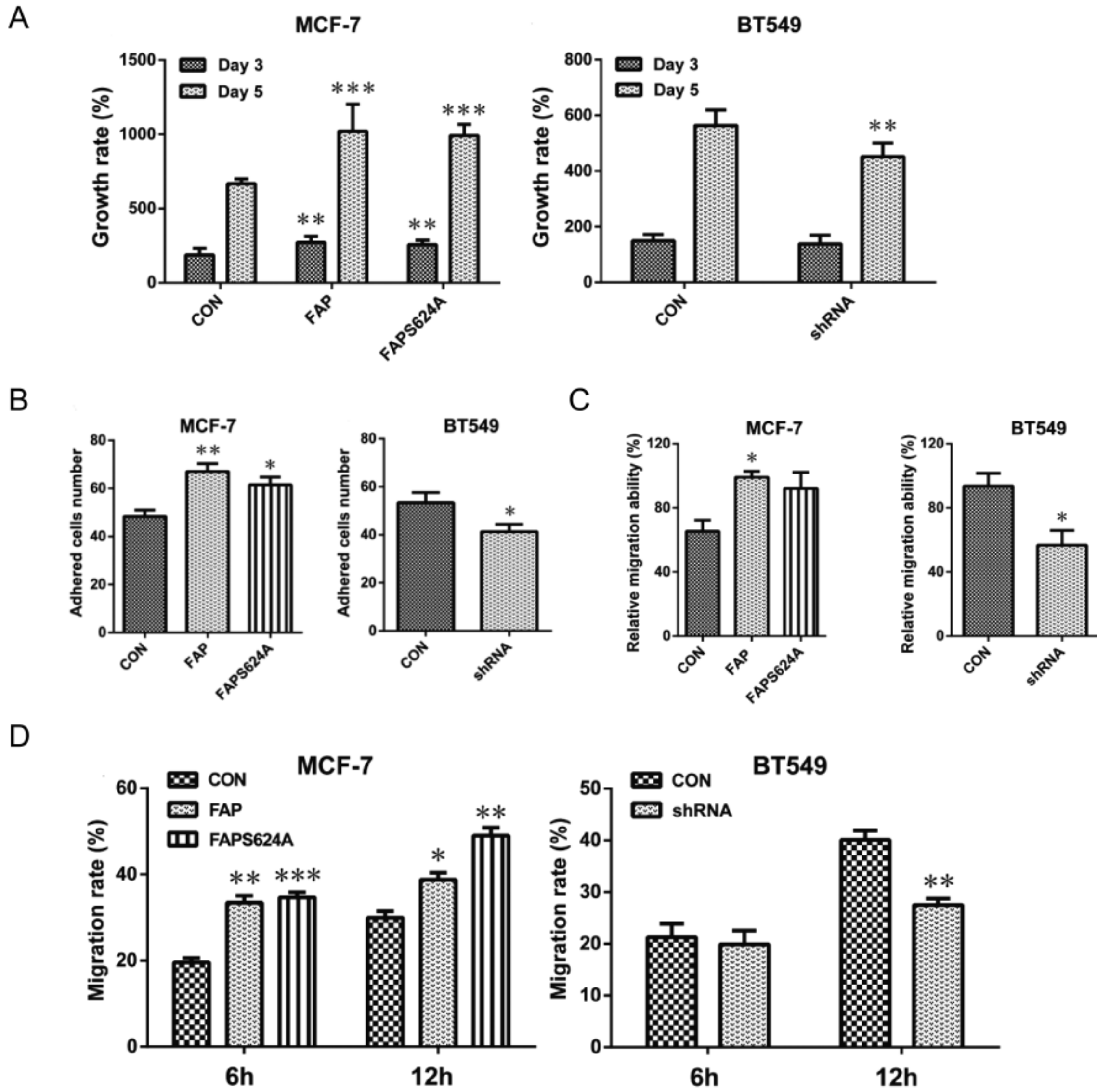

Figure 2. *Effects of fibroblast activation protein-alpha (FAPα) expression on cellular function in breast cancer cells. A: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium assay showed that overexpression of wild-type FAPα or mutant type FAPS624A increased the growth rate of MCF-7 cells at day 3 (FAP vs. CON: p=0.006. FAPS624A vs. CON: p<0.001) and day 5 (FAP vs. CON: p=0.002. FAPS624A vs. CON: p<0.001), while knockdown of FAPα dramatically reduced the growth rate of BT549 cells at day 5 (shRNA vs. CON: p=0.004). B: Cell-matrix adhesion assay revealed that overexpression of wild-type FAPα or mutant type FAPS624A in MCF-7 cells also significantly enhanced the adhesive properties (FAP vs. CON: p=0.0014. FAPS624A vs. CON: p=0.011), while knockdown of FAPα reduced the adhesive ability of BT549 cells (shRNA vs. CON: p=0.044). C: Matrigel invasion assay showed that MCF-7 cells overexpressing wild-type FAPα were significantly more invasive (FAP vs. CON: p=0.013), while absence of FAPα in BT549 cells led to a lower invasive capability (shRNA vs. CON: p=0.038). However, the presence of FAPS624A in MCF-7 cells increased the invasive capability but without statistical difference (FAPS624A vs. CON: p=0.096). D: Wound-healing assay showed that overexpression of wild-type FAPα and mutant FAPS624A markedly increased the migration capability of MCF-7 cells at 6 h (FAP vs. CON: p=0.002. FAPS624A vs. CON: p<0.001) and 12 h (FAP vs. CON: p=0.016. FAPS624A vs. CON: p=0.0014). Absence of FAPα significantly reduced the migration capability of BT-549 cells at 12 h (shRNA vs. CON: p=0.0044).*

overexpressing FAPα were significantly more invasive than the control cells (p=0.013). The presence of FAPS624A also increased the invasive capability of MCF-7 cells, but without statistical significance (p=0.096). The effect of FAPα on invasiveness was also confirmed in FAPα-knockdown BT549 cells. The absence of FAPα in BT549 cells led to a relatively lower invasive capability than that of control cells (p=0.038).

Effects of FAPα expression on cell migration. The wound-healing assay was employed to examine the influence of FAPα on cell migration. As shown in Figure 2D, overexpression of FAPα in MCF-7 cells markedly elevated the migration capability of MCF-7 cells at 6 h ($p=0.002$) and 12 h ($p=0.016$). The presence of enzymatic mutant FAPS624A also dramatically enhanced the motility of MCF-7 cells at 6 h ($p<0.001$) and 12 h ($p=0.0014$). The result was also consistent with observations in FAPα-knockdown cells. The absence of FAPα in BT549 cells significantly reduced migration capability at 12 h ($p=0.0044$). However, a marked decline in motility of BT549 cells was not seen at 6 h.

FAPα regulates cellular functions through PI3K/AKT and MMP2/9 pathway. To explore whether the effects of FAPα on the cellular functions of breast cancer cells were associated with the PI3K/AKT pathway, western blotting was performed to detect expression of total AKT and PI3K, phosphorylated AKT (p-AKT) and phosphorylated PI3K (p-PI3K) protein. As shown in Figure 3, expression of p-AKT and p-PI3K was markedly increased in MCF-7 cells expressing the wild-type FAPα and enzymatic mutant FAPS624A. However, protein levels of total AKT and PI3K were not dramatically altered. The impact of FAPα on MMP2 and MMP9 expression of MCF-7 cells was also examined. Overexpression of FAPα and FAPS624A in MCF-7 cells increased the levels of MMP2 and MMP9 compared to control cells.

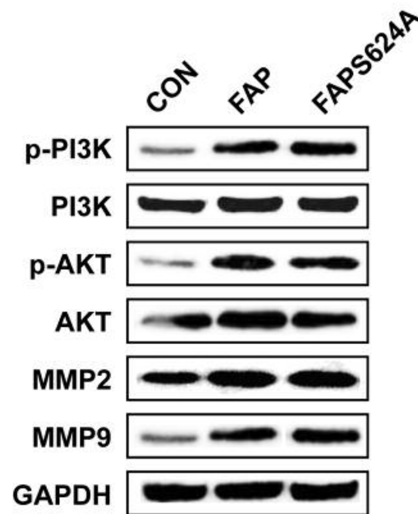

Figure 3. *Influence of fibroblast activation protein-alpha (FAPα) overexpression on phosphatidylinositol-3-kinase (PI3K)/ protein kinase B (AKT) and matrix metalloproteinase (MMP) 2/9 pathway by western blotting. Compared to control cells (CON), expression of phosphor (p)-AKT and p-PI3K was markedly increased in both wild-type FAPα and enzymatic-mutant FAPS624A-overexpressing MCF-7 cells, while expression of total AKT and PI3K protein was not dramatically altered. Expression of MMP2 and MMP9 was also increased in wild-type FAPα and enzymatic mutant FAPS624A-overexpressing MCF-7 cells.*

Discussion

Recent studies have provided evidence that FAPα may be involved in the development of over 90% of epithelial cancers and that the protein may have a potential value in both diagnosis and treatment of cancer (11, 20). Previous studies tended to analyze the localization and expression of this protease in diverse malignancies. It is noteworthy that there appears to be a clear discrepancy between the location and expression of FAPα in tissues from different tumor types (6). Some studies demonstrated that FAPα expressed by stromal fibroblast could promote cancer cell invasion and metastasis by degrading surrounding stroma and extracellular matrix though its gelatinase activity (8-12). However, there is increasing evidence showing that FAPα expressed by epithelial cancer cells in fact directly promoted cell proliferation, migration, and invasion and that this effect of FAPα was independent of its enzymatic activity (13-16).

In the current study, we clearly demonstrated that overexpression of FAPα markedly increased cell growth, adhesion, invasion and migration in MCF-7 breast cancer cells. When expression of FAPα in BT549 breast cancer cells was silenced, growth, adhesion, invasion and migration were reduced. The results were consistent with the observations in other cancer types including ovarian (18), breast (21), and oral and esophageal squamous cell carcinoma (19, 22). This suggests that cancer cell-expressed FAPα does indeed affect the biological behavior of cancer cells by acting as potential tumor stimulus.

FAPα has been shown to have two proteolytic activities: a gelatinase and an *N*-terminal post-prolyl amino peptidase that can cleave Ala-Pro- 7-amino-4-trifluoromethyl coumarin. Both the gelatinase and dipeptidyl peptidase (DPP) activities of FAPα are mediated by an active 624-site serine (23, 24). Cheng *et al*. reported that site-directed mutagenesis at the 624 catalytic site of FAPα, Ser624 to Ala624, resulted in an approximately 100,000-fold loss of FAPα protein DPP activity. The reduced enzymatic activity was associated with decreased tumor growth rates and tumorigenicity in HEK293 cells transfected with FAPS624A compared with cells transfected with wild-type FAPα (25). However, in another study, breast cancer cells expressing a catalytically inactive mutant FAPS624A produced tumors that grew rapidly in an SCID mouse model. It is also interesting to note that inhibitors of prolylpeptidases did not slow the growth of tumors (17). In the current study, as in wild-type FAPα-expressing cells, the enzymatic mutant FAPα (FAPS624A) overexpressed in MCF-7 cells also promotes cell proliferation, adhesion, and migration. Thus

our study, together with that of Huang *et al*. (17), clearly indicate that the tumorigenicity of FAPα is not confined to its enzymatic activity.

It has been recently reported that beyond its proposed enzymatic activities, FAPα expressed by epithelial cancer cells may engage with cell signaling pathways. Wang *et al*. found that the enzyme activity of FAP was not required for cell adhesion and migration. Overexpression of FAP increased the expression of MMP2 and reduced expression of integrin-β1 in 293T cells (26). Santos *et al*. found that colonic cancer cell CT26-transplanted tumors from FAPα-null mice showed an increase in phosphor focal adhesion kinase (FAK) Tyr397, phospho-ERK (p44/42) and p21^{WAF1} compared to tumors form wild-type mice. The effect of FAP depletion was fully recapitulated in the endogenous lung tumor model (27). Yang *et al*. reported that FAPα with integrin-α3β1 and the uPAR signaling complex mediated ovarian cancer cell migration *via* the small GTPase RAC1 pathway (18). In another study, knockdown of FAPα in OSCC cells inactivated PTEN/PI3K/AKT and RAS-ERK signaling pathway and resulted in down-regulation of proliferation, migration, and invasion in OSCC cells (19). Besides FAPα expressed by cancer cells, FAPα expressed by stromal cells was also reported to participate in regulation of signaling pathway such as RhoA (28), β-catenin (29), and fibroblast growth factor 1/ fibroblast growth factor receptor 3 (30).

In our study, we also investigated the potential mechanisms underlying the effects of FAPα in breast cancer. Our results showed that although the levels of total PI3K and AKT protein were not altered, p-AKT and p-PI3K were however markedly increased in both wild-type FAPα and enzymatic mutant FAPS624A overexpressing MCF-7 cells. Expression of MMP2 and MMP9 was also elevated in such MCF-7 cells. The PI3K/AKT signaling pathway plays an important role in cell proliferation and motility. The PI3K/AKT signaling pathway is up-regulated in most epithelial cancers and its activation promotes proliferation of cancer cells (31). The complex interactions of cancer cells with extracellular matrix are also crucial in regulating cell motility (32). As a critical factor mediating the interactions of cells with the extracellular matrix, the MMP family is thought to be an important determinant in cell migration. MMP2 and MMP9 are two key members of the MMP family that facilitate cell invasion and metastasis in most solid human cancer types (33-35). Together with the observations reported, it is strongly indicated that FAPα is actively involved in the activation/phosphorylation of these signaling proteins rather than in the transcriptional regulation of these signaling mediators.

The mechanism(s) by which FAPα expressed by epithelial cancer cells regulates cell signaling pathways is still uncertain. As a transmembrane protein, FAPα has similar structural homology to DPPIV (CD26). In DPPIV, the N-terminal hydrophobic sequence represents an uncleavable signal peptide that also functions as a membrane-anchoring domain (36). Indeed, it has been reported that DPPIV may serve as a receptor and participate in regulation of signaling pathways (37, 38). Thus it is highly plausible to hypothesize that FAPα has possibly a similar role, as a signaling peptide (6, 39).

Conclusion

Our study provides evidence that FAPα promotes cell growth, adhesion, invasion and migration in breast cancer. Its regulatory effects are independent of its enzymatic activity. The PI3K/AKT and MMP2/9 signaling pathway might be involved in the effects of FAPα on breast cancer cells.

Competing Interests

The Authors declare that they have no conflicts of interest.

Acknowledgements

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References

1 Aertgeerts K, Levin I, Shi L, Snell GP, Jennings A, Prasad GS, Zhang Y, Kraus ML, Salakian S, Sridhar V, Wijnands R and Tennant MG: Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein alpha. J Biol Chem *280(20)*: 19441-19444, 2005.

2 Christiansen VJ, Jackson KW, Lee KN and McKee PA: Effect of fibroblast activation protein and alpha2-antiplasmin cleaving enzyme on collagen types i, iii, and iv. Arch Biochem Biophys *457(2)*: 177-186, 2007.

3 Scanlan MJ, Raj BK, Calvo B, Garin-Chesa P, Sanz-Moncasi MP, Healey JH, Old LJ and Rettig WJ: Molecular cloning of fibroblast activation protein alpha, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. Proc Natl Acad Sci U S A *91(12)*: 5657-5661, 1994.

4 Huber MA, Kraut N, Park JE, Schubert RD, Rettig WJ, Peter RU and Garin-Chesa P: Fibroblast activation protein: Differential expression and serine protease activity in reactive stromal fibroblasts of melanocytic skin tumors. J Invest Dermatol *120(2)*: 182-188, 2003.

5 Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA and Fearon DT: Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science *330(6005)*: 827-830, 2010.

6 O'Brien P and O'Connor BF: Seprase: An overview of an important matrix serine protease. Biochim Biophys Acta *1784(9)*: 1130-1145, 2008.

7 Lai D, Ma L and Wang F: Fibroblast activation protein regulates tumor-associated fibroblasts and epithelial ovarian cancer cells. Int J Oncol *41(2)*: 541-550, 2012.

8 Henry LR, Lee HO, Lee JS, Klein-Szanto A, Watts P, Ross EA, Chen WT and Cheng JD: Clinical implications of fibroblast activation protein in patients with colon cancer. Clin Cancer Res *13(6)*: 1736-1741, 2007.

9 Cohen SJ, Alpaugh RK, Palazzo I, Meropol NJ, Rogatko A, Xu Z, Hoffman JP, Weiner LM and Cheng JD: Fibroblast activation protein and its relationship to clinical outcome in pancreatic adenocarcinoma. Pancreas *37(2)*: 154-158, 2008.

10 Kennedy A, Dong H, Chen D and Chen WT: Elevation of seprase expression and promotion of an invasive phenotype by collagenous matrices in ovarian tumor cells. Int J Cancer *124(1)*: 27-35, 2009.

11 Liao Y, Ni Y, He R, Liu W and Du J: Clinical implications of fibroblast activation protein-alpha in non-small cell lung cancer after curative resection: A new predictor for prognosis. J Cancer Res Clin Oncol *139(9)*: 1523-1528, 2013.

12 Mori Y, Kono K, Matsumoto Y, Fujii H, Yamane T, Mitsumata M and Chen WT: The expression of a type ii transmembrane serine protease (seprase) in human gastric carcinoma. Oncology *67(5-6)*: 411-419, 2004.

13 Jia J, Martin TA, Ye L and Jiang WG: Fap-alpha (fibroblast activation protein-alpha) is involved in the control of human breast cancer cell line growth and motility *via* the fak pathway. BMC Cell Biol *15*: 16, 2014.

14 Okada K, Chen WT, Iwasa S, Jin X, Yamane T, Ooi A and Mitsumata M: Seprase, a membrane-type serine protease, has different expression patterns in intestinal- and diffuse-type gastric cancer. Oncology *65(4)*: 363-370, 2003.

15 Zhang MZ, Qiao YH, Nesland JM, Trope C, Kennedy A, Chen WT and Suo ZH: Expression of seprase in effusions from patients with epithelial ovarian carcinoma. Chin Med J (Engl) *120(8)*: 663-668, 2007.

16 Shi M, Yu DH, Chen Y, Zhao CY, Zhang J, Liu QH, Ni CR and Zhu MH: Expression of fibroblast activation protein in human pancreatic adenocarcinoma and its clinicopathological significance. World J Gastroenterol *18(8)*: 840-846, 2012.

17 Huang Y, Simms AE, Mazur A, Wang S, Leon NR, Jones B, Aziz N and Kelly T: Fibroblast activation protein-alpha promotes tumor growth and invasion of breast cancer cells through non-enzymatic functions. Clin Exp Metastasis *28(6)*: 567-579, 2011.

18 Yang W, Han W, Ye S, Liu D, Wu J, Liu H, Li C and Chen H: Fibroblast activation protein-alpha promotes ovarian cancer cell proliferation and invasion *via* extracellular and intracellular signaling mechanisms. Exp Mol Pathol *95(1)*: 105-110, 2013.

19 Wang H, Wu Q, Liu Z, Luo X, Fan Y, Liu Y, Zhang Y, Hua S, Fu Q, Zhao M, Chen Y, Fang W and Lv X: Downregulation of fap suppresses cell proliferation and metastasis through pten/pi3k/akt and ras-erk signaling in oral squamous cell carcinoma. Cell Death Dis 5(e1155), 2014.

20 Yi YM, Zhang G, Zeng J, Huang SC, Li LL, Fang R, Jiang GM, Bu XZ, Cai SH and Du J: A new tumor vaccine: Faptau-mt elicits effective antitumor response by targeting indolamine2,3-dioxygenase in antigen presenting cells. Cancer Biol Ther *11(10)*: 866-873, 2011.

21 Goodman JD, Rozypal TL and Kelly T: Seprase, a membrane-bound protease, alleviates the serum growth requirement of human breast cancer cells. Clin Exp Metastasis *20(5)*: 459-470, 2003.

22 Kashyap MK, Marimuthu A, Kishore CJ, Peri S, Keerthikumar S, Prasad TS, Mahmood R, Rao S, Ranganathan P, Sanjeeviah RC, Vijayakumar M, Kumar KV, Montgomery EA, Kumar RV and Pandey A: Genomewide mrna profiling of esophageal squamous cell carcinoma for identification of cancer biomarkers. Cancer Biol Ther *8(1)*: 36-46, 2009.

23 Pineiro-Sanchez ML, Goldstein LA, Dodt J, Howard L, Yeh Y, Tran H, Argraves WS and Chen WT: Identification of the 170-kda melanoma membrane-bound gelatinase (seprase) as a serine integral membrane protease. J Biol Chem *272(12)*: 7595-7601, 1997.

24 Park JE, Lenter MC, Zimmermann RN, Garin-Chesa P, Old LJ and Rettig WJ: Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. J Biol Chem *274(51)*: 36505-36512, 1999.

25 Cheng JD, Valianou M, Canutescu AA, Jaffe EK, Lee HO, Wang H, Lai JH, Bachovchin WW and Weiner LM: Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. Mol Cancer Ther *4(3)*: 351-360, 2005.

26 Wang XM, Yu DM, McCaughan GW and Gorrell MD: Fibroblast activation protein increases apoptosis, cell adhesion, and migration by the lx-2 human stellate cell line. Hepatology *42(4)*: 935-945, 2005.

27 Santos AM, Jung J, Aziz N, Kissil JL and Pure E: Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice. J Clin Invest *119(12)*: 3613-3625, 2009.

28 Chung KM, Hsu SC, Chu YR, Lin MY, Jiaang WT, Chen RH and Chen X: Fibroblast activation protein (fap) is essential for the migration of bone marrow mesenchymal stem cells through rhoa activation. PLoS One *9(2)*: e88772, 2014.

29 Zi FM, He JS, Li Y, Wu C, Wu WJ, Yang Y, Wang LJ, He DH, Yang L, Zhao Y, Zheng GF, Han XY, Huang H, Yi Q and Cai Z: Fibroblast activation protein protects bortezomib-induced apoptosis in multiple myeloma cells through beta-catenin signaling pathway. Cancer Biol Ther *15(10)*: 1413-1422, 2014.

30 Henriksson ML, Edin S, Dahlin AM, Oldenborg PA, Oberg A, Van Guelpen B, Rutegard J, Stenling R and Palmqvist R: Colorectal cancer cells activate adjacent fibroblasts resulting in fgf1/fgfr3 signaling and increased invasion. Am J Pathol *178(3)*: 1387-1394, 2011.

31 Castaneda CA, Cortes-Funes H, Gomez HL and Ciruelos EM: The phosphatidyl inositol 3-kinase/akt signaling pathway in breast cancer. Cancer Metastasis Rev *29(4)*: 751-759, 2010.

32 Lock JG, Wehrle-Haller B and Stromblad S: Cell-matrix adhesion complexes: Master control machinery of cell migration. Semin Cancer Biol *18(1)*: 65-76, 2008.

33 Van Tubergen EA, Banerjee R, Liu M, Vander Broek R, Light E, Kuo S, Feinberg SE, Willis AL, Wolf G, Carey T, Bradford C, Prince M, Worden FP, Kirkwood KL and D'Silva NJ: Inactivation or loss of ttp promotes invasion in head and neck cancer *via* transcript stabilization and secretion of mmp9, mmp2, and il-6. Clin Cancer Res *19(5)*: 1169-1179, 2013.

34 Liu J, van Mil A, Aguor EN, Siddiqi S, Vrijsen K, Jaksani S, Metz C, Zhao J, Strijkers GJ, Doevendans PA and Sluijter JP: Mir-155 inhibits cell migration of human cardiomyocyte progenitor cells (hcmpcs) *via* targeting of mmp-16. J Cell Mol Med *16(10)*: 2379-2386, 2012.

35 Song G, Ouyang G, Mao Y, Ming Y, Bao S and Hu T: Osteopontin promotes gastric cancer metastasis by augmenting cell survival and invasion through akt-mediated hif-1alpha up-regulation and mmp9 activation. J Cell Mol Med *13(8B)*: 1706-1718, 2009.

36 Oertel M, Rosencrantz R, Chen YQ, Thota PN, Sandhu JS, Dabeva MD, Pacchia AL, Adelson ME, Dougherty JP and Shafritz DA: Repopulation of rat liver by fetal hepatoblasts and adult hepatocytes transduced *ex vivo* with lentiviral vectors. Hepatology *37(5)*: 994-1005, 2003.
37 Boonacker E and Van Noorden CJ: The multifunctional or moonlighting protein cd26/dppiv. Eur J Cell Biol *82(2)*: 53-73, 2003.
38 Wesley UV, McGroarty M and Homoyouni A: Dipeptidyl peptidase inhibits malignant phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway. Cancer Res *65(4)*: 1325-1334, 2005.
39 Kelly T: Fibroblast activation protein-alpha and dipeptidyl peptidase iv (cd26): Cell-surface proteases that activate cell signaling and are potential targets for cancer therapy. Drug Resist Updat *8(1-2)*: 51-58, 2005.

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