FAM172A inhibits EMT in pancreatic cancer via ERK-MAPK signaling

Ying Chen*, Peihui Liu, Di Shen, Han Liu, Lepeng Xu, Jian Wang, Daguang Shen, He Sun and Hongkui Wu

ABSTRACT
FAM172A, as a newly discovered gene, is little known in cancer development, especially in pancreatic cancer (PC). We investigated the potential role and molecular mechanism of FAM172A in epithelial to mesenchymal transition (EMT) in both human clinical samples and PC cells. FAM172A was downregulated in human PC tissues compared with that in non-cancerous pancreas cells by immunohistochemistry and qRT-PCR. FAM172A expression was negatively associated with tumor size (P=0.015), T stage (P=0.006), lymph node metastasis (P=0.028) and the worst prognosis of PC patients (P=0.004). Meanwhile, a positive relationship between FAM172A and E-cadherin (E-cad) (r=0.381, P=0.002) was observed in clinical samples, which contributed to the better prognosis of PC patients (P=0.014). FAM172A silencing induced EMT in both AsPC-1 and BxPC-3 cells, including inducing the increase of Vimentin, MMP9 and pERK and the decrease of E-cad and β-catenin expression, stimulating EMT-like cell morphology and enhancing cell invasion and migration in PC cells. However, MEK1 inhibitor PD98059 reversed FAM172A silencing-enhanced EMT in PC cells. We conclude that FAM172A inhibits EMT of PC cells via ERK-MAPK signaling.

KEY WORDS: FAM172A, EMT, Pancreatic cancer, ERK-MAPK

INTRODUCTION
Pancreatic cancer (PC), as a deadly malignancy, ranks as the fourth leading cancer-related cause of death in Europe (Rawla et al., 2019). It is also ranked as the top first incidence, and second age-standardized cause of mortality in Chinese males (Chen et al., 2016). The poor prognosis for PC patients is mainly due to its aggressive biological behavior, which is boosted by epithelial-to-mesenchymal transition (EMT). EMT, as one of the most distinctive and critical features in cancer (Gaianigo et al., 2017), promotes the loss of epithelial character and the gain of invasive mesenchymal properties, and finally contributes to the highly malignant phenotype in PC (Wang et al., 2017).

Family with sequence similarity 172 member A (FAM172A), cloned from human aortic tissues, is subsequently observed in human endothelial and vascular smooth muscle cells, and macrophages (Li et al., 2010). However, its biological function in disease states is still not clear. As the imbalance of the cell cycle and apoptosis is closely related to the key characteristics of malignant tumors, FAM172A dysregulation is a potential contributing factor in cell transformation (Feng et al., 2013). Recently, FAM172A has been reported to take part in the development of several cancers, such as human liver, colorectal and papillary thyroid cancers (Qian et al., 2016; Feng et al., 2013; Li et al., 2016). However, its definite role (oncogene or tumor suppressor) in cancers remains controversial and the corresponding molecular regulation is poorly elucidated. In the last few decades, an epidemic of diabetes and obesity has been spreading worldwide, contributing to an increase in incidence of PC (Paternoster and Falasca, 2020). FAM172A was upregulated by high glucose in a concentration- and time-dependent manner (Li et al., 2010), which indicated that FAM172A may be involved in the pathogenesis of diabetic related diseases. It is well known that PC is a multi-stepped progression involving a series of genetic and epigenetic events leading to the transformation of the normal ductal cells to carcinoma. Therefore, we intend to investigate the potential role of FAM172A in PC development, which has not been reported, to our knowledge.

The ERK-MAPK signaling plays a significant role in regulating cell proliferation, differentiation, survival, migration senescence and apoptosis via transmitting signals from cell surface receptors (Sun et al., 2015). ERK-MAPK signaling acts as an oncogene in various solid tumors, such as glioma, prostate, thyroid, ovarian and non-small cell lung cancers (Montagut and Settleman, 2009; Sun et al., 2015). Here, we first found that FAM172A inhibits EMT in PC via ERK-MAPK signaling, which supplies a new gene target therapy for PC patients.

RESULTS
The clinicopathological significance of FAM172A expression in PC tissues
FAM172A was mainly expressed in cytoplasm in both PC and paired pancreas. As described previously (Sheng et al., 2017b), E-cad membrane expression in PC was identified as normal expression (Fig. 1B), while E-cad negative and cytoplasmic expression were considered as abnormal expression (Fig. 1C). Immunohistochemistry (IHC) showed FAM172A positive expression in PC samples was much lower than that in paired adjacent normal pancreas cells (26/65, 40% versus 48/65, 73.8%, P<0.01) (Fig. 1A). In most serial sections, PC tissues with FAM172A positive expression was associated with E-cad normal expression (Fig. 1B), and vice versa (Fig. 1C). Spearman’s correlation test confirmed a positive relationship between FAM172A and E-cad in 65 PC samples (r=0.381, P=0.002) (Table 1).

Chi-squared test showed that FAM172A was negatively associated with tumor size (P=0.015), T stage (P=0.006), lymph node metastasis (P=0.028), but had no relationship with age, gender, tumor location, differentiation, vascular invasion and
preoperative CA199 level in PC patients (P>0.05) (Table 2). Patients with positive FAM172A expression had a much better overall survival compared with patients with FAM172A negative expression (P=0.003) (Fig. 1D). Though E-cad had no association with the prognosis (P=0.159) (Fig. 1E), patients with the cooperative expression of FAM172A and E-cad contributed to the better overall survival of PC patients (P=0.014) (Fig. 1F). In multivariate model, FAM172A was an independent favorable prognostic indicator (P=0.020) (Table 3).

Consistent with the IHC results, FAM172A mRNA level in 16 PC tissues was much lower than that in paired adjacent pancreas by qRT-PCR (t=3.661, P=0.002) (Fig. 2A). Both FAM172A and E-cad protein and mRNA levels were much higher in AsPC-1 and BxPC-3 cells compared with that in MiaPaCa-2 and Panc-1 cells (Fig. 2B,C). The close relationship between FAM172A and E-cad (the key EMT marker) in human PC samples and cell lines drove us to further investigate the role of FAM172A in EMT in vitro.

**FAM172A silencing regulated EMT markers and ERK/MAPK signaling in PC cells**

AsPC-1 and BxPC-3 cells with high FAM172A expression were used for FAM172A silencing experiments. FAM172A protein expression in the FAM172AsiRNA group was much lower than that in the siRNAcontrol group in both cell lines (Fig. 3). FAM172A silencing downregulated EMT epithelial markers E-cad and β-catenin and upregulated mesenchymal markers Vimentin and MMP9, but had no effect in N-cad and a-SMA expression (Fig. 3). Meanwhile, FAM172A silencing upregulated pERK expression in both cell lines (Fig. 3), which indicated that ERK/MAPK signaling might regulate FAM172A-mediated EMT in vitro.

**PD98059 inhibited FAM172A silencing-enhanced EMT in PC cells**

PD98059 was identified as a highly selective inhibitor of MEK1 activation and the MAP kinase cascade (Crews et al., 1992; Cowley et al., 1994). PD98059 binds to the inactive forms of MEK1 and prevents activation by upstream activators (Rosen et al., 1994). First, we found that FAM172A silencing-induced upregulation of pERK that was significantly inhibited by PD98059 (Fig. 4). Meanwhile, PD98059 inhibited FAM172A silencing-induced downregulation of E-cad and β-catenin and upregulation of Vimentin and MMP9 in both AsPC-1 and BxPC-3 cells (Fig. 4).

In addition, FAM172AsiRNA transfected AsPC-1 and BxPC-3 cells presented EMT-like cell morphology: most cells lost their epithelial properties of tight junction, and presented a spindle-shaped and fibroblast-like morphology (Fig. 5). However, PD98059 restored FAM172A silencing-induced EMT-like cell morphology along with the little spindle-shaped and fibroblast-like morphology compared with FAM172AsiRNA groups (Fig. 5). It indicated that FAM172A silencing-induced EMT-like cell morphology in PC cells, which was mediated by ERK/MAPK signaling.

Cell invasiveness in PC is significantly driven by EMT (Wang et al., 2017). In the current study, cell invasion and migration were significantly enhanced in the FAM172AsiRNA group compared with the siRNAcontrol group in AsPC-1 and BxPC-3 cells. However, PD98059 inhibited FAM172A silencing-enhanced cell invasion and migration in vitro (Fig. 6). It indicated that FAM172A
silencing-enhanced cell invasion and migration in PC cells via regulating ERK/MAPK signaling.

**DISCUSSION**

FAM172A, as a newly discovered gene, is little known in the development of cancers. Its function was only reported in liver, colorectal and papillary thyroid cancers (Qian et al., 2016; Feng et al., 2013; Li et al., 2016). However, whether it is an oncogene or a tumor suppressor remains controversial. For example, FAM172A suppresses proliferation and invasion and promotes apoptosis and differentiation in colorectal cancer cells (Qian et al., 2016; Cui et al., 2016), but promotes the proliferation of human papillary thyroid carcinoma cells (Li et al., 2016). In the current study, FAM172A, as a tumor suppressor, inhibits EMT in PC cells via ERK-MAPK signaling, which has not been reported, to our knowledge.

We first found that FAM172A was downregulated in PC tissues and was negatively associated with tumor size, T stage, lymph node metastasis and the poor prognosis of PC patients. FAM172A was also downregulated in liver and colorectal cancer (Qian et al., 2016; Cui et al., 2016). However, FAM172A was upregulated in papillary thyroid cancer (Sheng et al. 2017b). Meanwhile, FAM172A was positively associated with TNM stage, CEA and CA19-9, lymph node involvement and poor prognosis in colorectal cancer (Liu et al., 2017a, b). It indicates that FAM172A exhibits different functions based on different cancer types. Interestingly, we found a positive relationship between FAM172A and E-cad expression in PC tissues and cell lines. Meanwhile, cooperative expression of these two proteins contributes to the better prognosis of PC patients. It is well known that E-cad decrease/loss is a hallmark of EMT in various cancers. For example, miR-151a induces partial EMT by regulating E-cad in non-small cell lung cancer cells (Daugaard et al., 2017). BCL6 induces EMT by promoting the ZEB1-mediated transcription repression of E-cad in breast cancer cells (Liu et al., 2015). Gli1 promotes TGF-β1 and EGF induced EMT in PC cells via downregulating E-cad (Yu et al., 2015). TRIM66 promotes malignant progression of liver cancer by inhibiting E-cad expression through the EMT pathway (Zhang et al., 2019). Therefore, we next investigated if FAM172A regulated EMT in PC cells.

**Table 3. Univariate and multivariate analysis of clinicopathological factors for survival in 65 postoperative PC patients**

| Parameters                        | Median survival (days) | Univariate analysis P (log rank) | Multivariate analysis hazard ratio (95% CI) | P     |
|-----------------------------------|------------------------|---------------------------------|--------------------------------------------|-------|
| Age (<65/≥65 years)               | 555/432                | 0.447                           |                                            |       |
| Gender (male/female)              | 555/425                | 0.912                           |                                            |       |
| Tumor location (head/body-tail)   | 629/480                | 0.102                           |                                            |       |
| Tumor size (<2.5/≥2.5 cm)        | 555/321                | 0.094                           |                                            |       |
| Good/poor and moderate differentiation | 555/432                | 0.096                           |                                            |       |
| T stage (T1+T2/T3+T4)            | 555/387                | 0.007                           | 1.671(0.852-3.275)                         | 0.135 |
| Lymph nodes metastasis (N0/N1)   | 585/317                | 0.004                           | 2.109(1.011-4.398)                         | 0.047 |
| Vascular permeation (absent/present) | 730/421                | 0.001                           | 2.416(1.174-4.973)                         | 0.017 |
| CA19-9 level (<37 U/ml/≥37 U/ml) | 515/418                | 0.411                           |                                            |       |
| FAM172A (positive/negative)      | 387/660                | 0.004                           | 0.412(0.195-0.868)                         | 0.020 |
| E-cad (positive/negative)        | 425/660                | 0.199                           |                                            |       |

**Fig. 2. FAM172A and E-cad expression in PC tissues and cell lines.**

(A) FAM172A mRNA expression in 16 PC and paired normal pancreas by qRT-PCR (N/C ratio). (B,C) FAM172A and E-cad protein (B) and mRNA (C) expression in PC cell lines. C, PC tissues; N, paired normal pancreas. Bars indicate ± s.e. *P<0.05, **P<0.01 compared with the control.
FAM172A silencing significantly induced EMT in both AsPC-1 and BxPC-3 cells, including inducing the increase of Vimentin, MMP9 and the decrease of E-cad and β-catenin expression, stimulating EMT-like cell morphology and enhancing cell invasion and migration. It is well known that ERK/MAPK signaling play a significant role in regulating the initiation of EMT in mammary epithelial and cancer cells. For example, activation of the ERK pathway is required for TGF-β1-induced EMT in normal murine mammary gland epithelial cells (Xie et al., 2004). ERK signaling modulates epigenome to drive epithelial to mesenchymal transition (Navandar et al., 2017). TFF3 contributes to EMT in papillary thyroid carcinoma cells via the MAPK/ERK signaling (Lin et al., 2018). Downregulation of RNF138 inhibits EMT in glioma cells via suppression of the ERK/MAPK signaling (Wu et al., 2018). Calreticulin promotes EGF-induced EMT in PC cells via Integrin/EGFR-MAPK/ERK signaling pathway (Sheng et al. 2017b). In the current study, we first found that FAM172A silencing downregulated pERK expression in vitro. Moreover, FAM172A silencing-induced change of EMT classic markers (Vimentin, MMP9, E-cad and β-catenin) was significantly reversed by MEK1 inhibitor PD98059. PD98059 also inhibited FAM172A silencing-induced EMT like cell morphology and cell invasiveness. Previous studies have shown that FAM172A promotes the proliferation of papillary thyroid carcinoma cells via p38/MAPK signaling (2013; Li et al., 2016). FAM172A modulates apoptosis and proliferation of colon cancer cells via STAT1 binding to its promoter (Qian et al., 2016). miR-27a promotes proliferation, migration and invasion of colorectal cancer by targeting FAM172A (Liu et al., 2017a,b). Our study describes a novel signaling pathway involving FAM172A regulating EMT in PC cells via ERK/MAPK signaling, which has not been reported in previous studies.

The limitation in current study is that we do not conduct the transplantation tumor experiment in vivo, which would provide strong evidence to support our current conclusion. Second, we have found the potential relationship of FAM172A and ERK/MAPK signaling in mediating the initiation of EMT in vitro. However, the detailed molecular mechanism has not been clarified. It is well known that c-Myc, as the major downstream target of ERK/MAPK pathway, is indispensable in EMT development. For example, both c-Myc and MEK1-induced ERK2 nucleus localization are required for TGF-β-induced EMT in prostate cancer (Amatangelo et al., 2012). c-Myc mediates cancer stem-like cells and EMT in triple negative breast cancers cells (Yin et al., 2017). Therefore, whether FAM172A has a specific regulatory effect on c-Myc will be investigated in our future EMT study.

In conclusion, we first found downregulation of FAM172A in PC tissues is negatively associated with advanced clinical significance and poor prognosis of PC patients. FAM172A inhibits EMT in PC via ERK-MAPK signaling, which is identified as a potential gene therapy target in PC.

**MATERIALS AND METHODS**

**Tissue samples and cell lines**

This study was approved by the academic review board from Huludao Central Hospital. Written informed consent was obtained from each patient. The study methodologies were approved by Huludao Central Hospital and all experiments conform to the Declaration of Helsinki. 65 formalin-fixed PC and paired normal pancreas were collected from postoperative patients who approved and signed consent forms at our hospital from 2005 to 2015. All PC samples were pathologically diagnosed as ductal adenocarcinoma. Additionally, 16 fresh PC tissue samples were randomly selected for late qRT-PCR assays. Human Miapaca-2, PANC-1, AsPC-1 and BxPC-3 PC cell lines were obtained from the cell bank culture collection of the Chinese Academy of Sciences (Shanghai, China), which were cultured in the recommended growth media with 10% FBS (Gibco Invitrogen, Carlsbad, CA, USA).
ICH assays
ICH was performed as described previously (Sheng et al., 2017a,b). Briefly, 4-µm sections were deparaffinized, dehydrated in ethanol, covered with 0.3% peroxyacetic acid, subjected to antigen retrieval and blocked with goat serum. Then sections were incubated with anti-FAM172A (Abcam, Cambridge, UK, ab121364) and E-cadherin (E-cad, Abcam, ab40772) overnight at 4°C. After washing three times with PBS, sections were incubated with the secondary antibody, treated with streptavidin–peroxidase reagent, visualized with DAB, stained with Hematoxylin and finally detected under microscope. Staining intensity was scored as 0/negative, 1/weak, 2/medium, and 3/strong. Staining range was scored as 0/(<5%), 1/(5–25%), 2/(26–50%), 3/(51–75%), and 4/(76–100%) according to the whole carcinoma. The intensity and extent scores were added together. Tumors with a final score ≥3 were considered to be FAM172A positive and E-cad normal expression.

Western blot
As described previously (Liu et al. 2017 a,b), whole-cell lysates of PC cells were put into 12% SDS-polyacrylamide gels, transferred to PVDF membrane (Bio-Rad, CA, USA) and incubated with primary FAM172A (Abcam, ab121364), E-cadherin (Abcam, ab40772), β-catenin (Proteintech, Chicago, IL, USA, 51067-2-AP), N-cadherin (N-cad, Abcam, ab98952), Vimentin (Proteintech, 10366-1-AP), MMP9 (Abcam, ab98952), and α-Smooth muscle actin (a-SMA, Abcam, ab7817), pERK (Cell Signaling Technology, Beverly, MA, USA, #9101), ERK (Cell Signaling Technology, #4695) and GAPDH (Proteintech, 60004-1-lg) antibodies overnight. Then membranes were incubated with secondary antibodies (Proteintech) at room temperature and were visualized with the ECL machine (Thermo Biotech Inc., USA). PC cells were pretreated with 20 µM of MEK1 inhibitor PD98059 (Cell Signaling Technology, #4695) and GAPDH (Proteintech, 60004-1-lg) antibodies overnight. Then membranes were incubated with secondary antibodies (Proteintech) at room temperature and were visualized with the ECL machine (Thermo Biotech Inc., USA). PC cells were pretreated with 20 µM of MEK1 inhibitor PD98059 (Cell Signaling Technology) for 2 h before western blotting. The experiment was repeated three times.

Real-time quantitative PC
As described previously (Fan et al., 2019), qRT-PCR was performed in a Light Cycler 2.0 with the Light Cycler kit (Takara Bio, Otsu, Japan) for the following conditions: 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 30 s. The primers: FAM172A, 5'-CGACTGCGAACACTGGAAG -3' and 5'-GAGCTCAAGGAAATAGACATCAATC -3'; E-cad, 5'-CAGCTGTTGTGACTGTGAAG -3' and 5'-AAACAGCAAGGCAGCAGAA -3'; GAPDH, 5'-CATGGAAGATGACACACGGCT -3' and 5'-AGTCCCTCCAGATACAAAGT -3'. Quality of the PCR products was monitored with post-PCR melt-curve analysis using the ΔΔCt calculation.

RNA interference
The FAM172AsiRNA sequences were: sense: 5'-GCCACTGAGGAAATAGACATCAATC -3', and antisense: 5'-GAGCTCAAGGAAATAGACATCAATC -3'. siRNA transfections (20 µM) were mixed with Oligofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for transient transfection following the manufacturer’s instructions.

EMT construction
As described previously (Fan et al., 2019), FAM172AsiRNA and siRNAcontrol transfected AsPC-1 and BxPC-3 cells were cultured with growth media containing 1% fetal calf serum (FBS) twice within 4 days to better induce EMT. The change of EMT-like cell morphology, the protein level of EMT markers and cell mobility were used for detecting the EMT construction. For EMT-like cell morphology in siRNAcontrol, FAM172AsiRNA and FAM172AsiRNA plus PD98059 groups, we pretreated PC cells with PD98059 (20 µM) for 2 h twice within 4 days. DMSO, as the dilution of PD98059, was used as the control.

Cell invasion and migration assays
Briefly, FAM172AsiRNA and siRNAcontrol transfected AsPC-1 and BxPC-3 cells (pretreated with 20 µM of PD98059 for 2 h only once) was seeded onto 8.0-µM pore size membrane inserts (Corning Inc., NY, USA) coated with matrigel (BD Biosciences, Sparks, MD, USA) in 24-well plates with FBS-free growth media. Growth media plus 10% FBS was added to the bottom wells as a chemoattractant. 24 h later, cells that moved to the underside of the inserts were stained with Crystal Violet Hydrate (Sigma-Aldrich, St Louis, MO, USA). The migratory cells were counted in five random fields per well. Results were expressed as cells migrated per field and repeated three times. The transwell assay was performed in a similar way without matrigel.
Statistical analysis
Under SPSS software 20.0 (SPSS, Chicago, IL, USA), paired nonparametric test, chi-squared test and spearman correlation test were used for IHC assays, respectively. The log-rank test and Cox’s regression was used to evaluate the postoperative survival time of PC patients. Western blotting, qRT-PCR and transwell assays \textit{in vitro} were described as means±s.e. (standard deviation) and were compared via \textit{t}-test. \textit{P}-value is presented as follow: *\textit{P}<0.05; **\textit{P}<0.01.

Competing interests
The authors declare no competing or financial interests.

Fig. 6. PD98059 inhibited FAM172A silencing-enhanced cell invasion and migration in PC cells. (A,B) Cell invasion (A) and migration (B) in siRNA\textit{control}, FAM172AsiRNA and FAM172AsiRNA plus PD98059 groups in AsPC-1 cells. (C,D) Cell invasion (C) and migration (D) in siRNA\textit{control}, FAM172AsiRNA and FAM172AsiRNA plus PD98059 groups in BxPC-3 cells. Bars indicate±s.e.*\textit{P}<0.05, **\textit{P}<0.01 compared with the control (\times200 magnification). Scale bars: 100 \textmu m.

Author contributions
Conceptualization: Y.C., H.L., J.W., H.S.; Methodology: P.L., Di Shen, L.X., Daguang Shen, H.S., H.W.; Software: Daguang Shen; Validation: P.L., Di Shen, Daguang Shen; Formal analysis: Di Shen, Daguang Shen, H.W.; Investigation: P.L., Di Shen, L.X., J.W.; Resources: Y.C.; Writing - original draft: Y.C., H.S.; Writing - review & editing: L.X.; Visualization: H.L.; Supervision: Y.C., H.L., J.W., H.W.; Project administration: H.W.

Funding
This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.
References

Amatangelo, M. D., Goodyear, S., Varma, D. and Stearns, M. E. (2012). c-Myc expression and MEK1-induced Erk2 nuclear localization are required for TGF-beta induced epithelial–mesenchymal transition and invasion in prostate cancer. Cancer Res. 72, 533–542. doi:10.1158/0008-5472.CAN-11-2286

Chen, W., Zheng, R., Baade, P. D., Zhang, S., Zeng, H., Bray, F., Siegel, R., Jemal, A., 2012. Cancer statistics in China, 2015. CA Cancer J. Clin. 66, 115-132. doi:10.3322/caac.21338

Cowley, S., Paterson, H., Kemp, P. and Marshall, C. J. (1994). Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells. Cell 77, 841-852. doi:10.1016/0092-8674(94)90133-3

Crews, C. M., Alessandrini, A. and Erikson, R. L. (1993). The primary structure of MKC, a protein kinase that phosphorylates the ERK gene product. Science 258, 478-480. doi:10.1126/science.1411546

Fan, H., Huang, S., and Sun, Y. (2018). TFF3 promotes transformation growth factor-beta1- and epidermal growth factor-induced epithelial to mesenchymal transition in colorectal cancer cells via the MAPK/ERK signaling pathway. Oncotarget 9, 353-358. doi:10.18632/oncotarget.25372

Feng, Z., Li, H., Liu, S., Cheng, J., Xiang, G. and Zhang, J. (2013). FAM172A induces S phase arrest of HepG2 cells via Notch 3. Oncol. Rep. 29, 1154-1160. doi:10.3892/or.2013.2235

Galindo, N., Melisi, D. and Carbone, C. (2017). EMT and treatment resistance in pancreatic cancer. Carcinogenesis 38, 1965-1975. doi:10.1093/carcin/bgw022

Li, L., Dong, X., Leong, M. C., Zhou, W., Yang, Z., Chen, F., Bao, Y., Jia, W. and Hu, R. (2010). Identification of the novel protein FAM172A, and its up-regulation by high glucose in human aortic smooth muscle cells. Int. J. Mol. Med. 26, 483-490. doi:10.3892/ijmm.0000489

Li, M. F., Zhang, R., Guo, M. G., Li, L. X., Lu, H. K., Lu, J. X. and Jia, W. P. (2016). FAM172A protein promotes the proliferation of human papillary thyroid carcinoma cells via the p38 mitogen-activated protein kinase pathway. Mol. Med. Rep. 13, 353-358. doi:10.3892/mmr.2015.4548

Lin, X., Zhang, H., Dai, J., Zhang, W., Zhang, J., Xue, G. and Wu, J. (2018). TFF3 Contributes to Epithelial-Mesenchymal Transition (EMT) in papillary thyroid carcinoma cells via the MAPK/ERK signaling pathway. J. Cancer 9, 4430-4439. doi:10.7150/jca.24361

Liu, Q., Sheng, W., Dong, M., Dong, Q. and Li, F. (2015). Gli1 promotes transforming growth factor-beta1- and epithelial growth factor-induced epithelial to mesenchymal transition in pancreatic cancer cells. Surgery 158, 211-224. doi:10.1016/j.surg.2015.03.016

Liu, W., Wang, S., Qian, K., Zhang, J., Zhang, Z. and Liu, H. (2017a). Expression of family with sequence similarity 172 member A and nucleotide-binding protein 1 is associated with the poor prognosis of colorectal cancer. Oncol. Lett. 14, 3587-3593. doi:10.3892/ol.2017.6985

Liu, W., Qian, K., Wei, X., Deng, H., Zhao, B., Chen, Q., Zhang, J. and Liu, H. (2017b). miR-27a promotes proliferation, migration, and invasion of colorectal cancer by targeting FAM172A and acts as a diagnostic and prognostic biomarker. Oncol. Rep. 37, 3554-3564. doi:10.3892/or.2017.5992

Montagut, C. and Settleman, J. (2009). Targeting the RAF-MEK-ERK pathway in cancer therapy. Cancer Lett. 283, 125-134. doi:10.1016/j.canlet.2009.01.022

Navandar, M., Garding, A., Sahu, S. K., Pataskar, A., Schick, S. and Tiwari, V. K. (2011). ERK signaling in cells regulates estrogen-induced epithelial to mesenchymal transition. Oncotarget 2, 29269-29281. doi:10.18632/oncotarget.16493

Paternoster, S. and Falasca, M. (2020). The intricate relationship between diabetes, obesity and pancreatic cancer. Biochim. Biophys. Acta. Rev. Cancer 1873, 188326. doi:10.1016/j.bbcar.2019.188326

Qian, K., Zhang, J., Lu, J., Liu, W., Yao, X., Chen, Q., Lu, S., Xiang, G. and Liu, H. (2016). FAM172A modulates apoptosis and proliferation of colon cancer cells via STAT1 binding to its promoter. Oncol. Rep. 35, 1273-1280. doi:10.3892/or.2015.4485

Rawla, P., Sunkara, T. and Gaduputi, V. (2019). Epidemiology of pancreatic cancer: global trends, etiology and risk factors. World J. Oncol. 10, 20-27. doi:10.14740/wjon1166

Rosen, L. B., Ginty, D. D., Weber, M. J. and Greenberg, M. E. (1994). Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. Neuron 12, 1207-1221. doi:10.1016/0896-6273(94)90438-3

Sheng, W., Dong, M., Chen, C., Wang, Z., Li, Y., Wang, K., Li, Y. and Zhou, J. (2017a). Cooperation of Musashi-2, Numb, MD2 and P53 in drug resistance and malignant biology of pancreatic cancer. FASEB J. 31, 2429-2438. doi:10.1096/fj.201601240R

Sheng, W., Chen, C., Dong, M., Wang, G., Zhou, J., Song, H., Li, Y., Zhang, J. and Ding, S. (2017b). Calreticulin promotes EGF-induced EMT in pancreatic cancer cells via Integrin/EGFR-ERK/MAPK signaling pathway. Cell Death Dis. 8, e3147. doi:10.1038/cddis.2017.547

Sun, Y., Liu, W.-Z., Liu, T., Feng, X., Yang, N. and Zhou, H.-F. (2015). Signaling pathways of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. J. Recept. Signal Transduct. Res. 35, 600-604. doi:10.3109/10799893.2015.1030412

Wang, S., Huang, S. and Sun, Y. L. (2017). Epithelial-mesenchymal transition in pancreatic cancer: a review. Biomed. Res. Int. 2017, 2646148. doi:10.1155/2017/2646148

Wu, H., Li, X., Feng, M., Yao, L., Deng, Z., Zhao, G., Zhou, Y., Chen, S. and Du, Z. (2018). Downregulation of RNF138 inhibits cellular proliferation, migration, invasion and EMT in glioma cells via suppression of the Erk signaling pathway. Oncol. Rep. 40, 3285-3296. doi:10.3892/or.2018.6744

Xie, L., Law, B. K., Chytil, A. M., Brown, K. A., Aakre, M. E. and Moses, H. L. (2004). Activation of the Erk pathway is required for TGF-beta1-induced EMT in vitro. Neoplasia 6, 603-610. doi:10.1593/neo.04241

Yin, S., Cheryan, V. T., Xu, L., Rishi, A. K. and Reddy, K. B. (2017). Myc mediates cancer stem-like cells and EMT changes in triple negative breast cancers cells. PLoS ONE 12, e0183578. doi:10.1371/journal.pone.0183578

Yu, J.-M., Sun, W., Hua, F., Xie, J., Lin, H., Zhou, D.-D. and Hu, Z.-W. (2015). BCL6 induces EMT by promoting the ZEB1-mediated transcription repression of E-cadherin in breast cancer cells. Cancer Lett. 354, 190-200. doi:10.1016/j.canlet.2015.05.029

Zhang, H. G., Pan, Y. W., Feng, J., Zeng, C. T., Zhao, X. Q., Liang, B. and Zhang, W. W. (2019). TRIM66 promotes malignant progression of hepatocellular carcinoma by inhibiting E-cadherin expression through the EMT pathway. Eur. Rev. Med. Pharmacol. Sci. 23, 2003-2012. doi:10.26355/eurrev_201903_17239

Biology Open