Integrated analysis of microfibrillar-associated proteins reveals *MFAP4* as a novel biomarker in human cancers

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Aim: The potential functions and underlying mechanism of microfibrillar-associated proteins (*MFAPs*) are explored in human cancers. **Materials & methods:** Here, we examined the expression profiles, prognostic values, epigenetic and genetic alterations of *MFAPs* in human cancers from public omics repository. **Results:** Among *MFAPs* family, *MFAP4* was frequently downregulated in the most human cancers and high mRNA expression of *MFAP4* significantly correlated with better overall survival in breast cancer. DNA hypermethylation in the promoter of *MFAP4* decreased its mRNA expression. *MFAP4* strongly associated with pathway in impairment and alteration of the elastic fibers. **Conclusion:** This integrated analysis provides new insights into *MFAPs* in human cancers and indicates that *MFAP4* could be used as novel biomarker for developing therapies against human cancers.

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Cancer is a highly complex trait that threatens billions of people worldwide. It is now clear that cancer is a consequence of the interaction of multiple acquired genetic and epigenetic lesions influenced by heredity and environment. Cancer cells can survive and proliferate under extremely adverse microenvironment, induce and maintain angiogenesis, migrate and invade into the surrounding tissues. Any molecule contributing to these processes is worthy of particular interest.

Accumulated studies demonstrate that microfibrils provide structural integrity of specific organ systems, but they can also provide a scaffold for elastogenesis in elastic tissues [1]. Interestingly, an elastin plays a role in cell adhesion, cell migration, survival and differentiation and has the ability to participate in cell signaling [2,3]. The microfibrillar-associated proteins (*MFAPs*) are located to microfibrils and can interact with fibrillin to influence microfibril function. Those evidence indicate *MFAPs* may play an important role in the phenotype of cancer cells. So far, five *MFAPs* family members have been identified, *MFAP1*, *MFAP2*, *MFAP3*, *MFAP4* and *MFAP5*. *MFAP2* and *MFAP5* encode protein MAGP-1 and MAGP-2, respectively. *MFAP1*, *MFAP3* and *MFAP4* cannot encode MAGP proteins but they were grouped together with MAGPs due to their small size and localization to microfibrils. Among them, *MFAP2*, *MFAP4* and *MFAP5* encode extracellular matrix proteins that play important biological roles in multiple organs, including bone, blood vessels, hemostasis and the immune system [4,5]. Recent

findings show that MFAP1 associates with spliceosome function and is required for pre-mRNA processing [6–8]. MFAP3 functions as a nuclear kinase [9]. However, the prognostic significance, potential functions and molecular mechanism of *MFAPs* in tumorigenesis and tumor development remains largely unknown.

With the fast development and reduced costs of high throughput technologies, extensive public efforts to annotate of cancer genomes and seek for susceptibility loci facilitate researchers to illuminate molecular mechanism of human tumors as well as develop new strategies for precision therapy. For example, the Cancer Genome Atlas (TCGA) project has generated comprehensive, multidimensional maps of the key genomic changes in 33 types of cancer. By profiling gene expression, epigenetic modifications, copy-number variation and somatic mutations of more than 11,000 patients, this project accelerates the understanding of the molecular basis of cancer and improves our ability to diagnose, treat and prevent human cancers.

In this study, we performed a deep analysis of gene mRNA expression, genetic mutation and methylation modifications of *MFAPs* in breast carcinoma and other human cancers based on TCGA data. We also explored the potential prognostic values and gene regulatory networks of *MFAPs* involved in carcinogenesis and cancer progression.

Materials & methods

MFAPs-mRNA expression analysis

MFAPs-mRNA expressions in different human cancers were analyzed using Oncomine gene expression array datasets [10]. The cut-off of p-value and fold change were defined as 0.01 and 2, respectively. UALCAN is a web portal to perform in-depth analyses of gene expression in various tumor subgroups based on individual clinicopathologic features from the TCGA data. *MFAPs*-mRNA expression level in different cancer subtypes and grades/stages was evaluated using UALCAN [11].

Kaplan–Meier survival analysis

The correlation between the *MFAPs* expression and prognosis was analyzed using Kaplan–Meier plotter (KM plotter) [12]. We chose ‘overall survival’ (OS), ‘auto-select best cut-off’ and ‘only JetSet best probe set’ to calculate and plot Kaplan–Meier survival curves. Best cut-off values were generated through computing all possible cut-off values between the lower and upper quartiles and the best performing threshold was used as a cut-off (Supplementary Table 1). Statistical significance was determined by log-rank p-value and hazard ratios (HRs) with 95% CIs are displayed. The Affymetrix probeset IDs of *MFAPs* in breast, lung and stomach cancers are shown as follows: *MFAP1*: 203406_at; *MFAP2*: 203417_at; *MFAP3*: 213123_at; *MFAP4*: 212713_at and *MFAP5*: 213764_at. RNA-Seq ID of *MFAP4* in liver cancer is 4239. GSE62254 datasets were excluded when KM plots were generated for *MFAP4* in stomach cancer because GSE62254 has markedly different characteristics (longer survivals, shifted expression) than the other datasets.

Mutations & copy-number alterations analysis

The cBioPortal (www.cbioportal.org) is an open access resource for interactive exploration of multidimensional cancer genomics datasets [13]. Breast invasive carcinoma (TCGA, Provisional, 1105 samples) was selected to analyze mutations and copy-number alterations of *MFAP4*. GISTIC 2.0 were used to identify significant somatic copy-number alterations, such as amplifications and deletions [14].

Methylation modifications analysis

To plot the relationship between DNA methylation and *MFAP4* mRNA expression, 871 breast invasive carcinoma samples in MEXPRESS datasets were analyzed [15]. Then we used MethHC which included 839 breast invasive carcinoma samples to check the correlation of DNA methylation in the promoter and *MFAP4*-mRNA expression [16].

Gene regulatory networks analysis

Functional protein association network for *MFAP4* was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) with a confidence score of 0.4 [17]. Metascape was used for gene ontology and pathway enrichment analysis of the *MFAP4*-related genes [18].

Figure 1. The mRNA levels of MFAPs in different cancer types. The data were collected from Oncomine cancer profiling database (www.oncomine.org/) in cancer tissues compared with normal tissues. The gene rank was analyzed by percentile of target gene in the top of all genes measured in each research. Red and blue color scales determined by the best gene rank percentile indicate gene expression up-regulation and down-regulation, respectively. The cut-off of p-value, fold change and gene ranking were defined as 0.01, 2 and 10% respectively.

Results

MFAPs-mRNA expression in breast cancer

To investigate the roles of MFAPs in human carcinogenesis, we analyzed the mRNA expression of MFAPs in a wide variety of cancer types represented in the Oncomine databases. The mRNA expression levels of MFAP2, MFAP4 and MFAP5 were abnormally expressed in most human cancers, among which breast cancer has the most data available (Figure 1). Compared with normal tissues, MFAP2 were significantly upregulated in breast cancer (21 datasets). Meanwhile, MFAP4 and MFAP5 were obviously downregulated in breast cancer tissues compared with those in normal tissues. However, in Finak's datasets, MFAP5 was significantly increased in invasive breast carcinoma with a 3.746-fold change [19]. Oncomine data also revealed an increase of MFAP1 and MFAP3-mRNA in breast cancer compared with those in normal tissues ($p < 0.01$) (Table 1).

Next, we confirmed the expression of MFAPs using UALCAN. We found that MFAP1, MFAP2, MFAP4 and MFAP5 were significantly dysregulated in breast cancer. Among them, MFAP2 was upregulated 4.80-fold ($p = 1.62e-12$) (Figure 2B), while MFAP4 and MFAP5 were greatly downregulated 8.59-fold and 3.18-fold, respectively ($p < 1.00e-12$ and $p = 3.46e-10$) (Figure 2D & 2E). The mRNA expression of MFAP1 was just slightly increased 1.04-fold in breast cancer ($p = 0.0024$) (Figure 2A). MFAP3 was upregulated 1.14-fold and there was no significant difference of MFAP3 between breast cancer tissues and normal tissues ($p = 0.1532$) (Figure 2C).

Figure 2. Box-whisker plots showing the mRNA levels of MFAPs in breast invasive carcinoma samples based on major cancer stages from UALCAN database. (A) MFAP1; (B) MFAP2; (C) MFAP3; (D) MFAP4; (E) MFAP5.

**p < 0.01.

Table 1. The significant changes of microfibrillar-associated proteins-mRNA expression between different types of breast cancer and breast tissues in Oncomine database.

Gene name	Types of breast cancer vs normal breast tissues	Fold change	p-value	t-test	Ref.
<i>MFAP1</i>	Tubular breast carcinoma	1.527	7.63E-31	15.791	[20]
	Mucinous breast carcinoma	1.557	4.76E-18	12.072	
<i>MFAP2</i>	Invasive ductal breast carcinoma	4.265	6.80E-05	4.571	[21]
<i>MFAP3</i>	Ductal breast carcinoma <i>in situ</i>	1.252	4.10E-05	5.754	[20]
	Benign breast neoplasm	1.290	0.007	5.883	
	Invasive ductal and invasive lobular breast carcinoma	1.311	3.51E-19	10.061	
<i>MFAP4</i>	Breast carcinoma	1.208	3.90E-4	4.120	TCGA
	Mucinous breast carcinoma	-16.406	1.07E-14	-23.455	
	Invasive ductal breast carcinoma	-8.969	8.59E-36	-20.036	
	Invasive breast carcinoma	-3.752	4.11E-18	-9.952	
	Invasive lobular breast carcinoma	-2.971	3.21E-9	-6.649	
	Intraductal cribriform breast adenocarcinoma	-6.842	0.008	-6.184	
<i>MFAP5</i>	Invasive breast carcinoma	3.746	9.66E-23	17.24	[19]
	Invasive breast carcinoma	-1.718	4.30E-5	-6.146	

Prognostic values of *MFAPs* in all breast cancer patients

Analysis of 1402 breast cancer in KM plotter showed that high mRNA levels of *MFAP3* and *MFAP4* were significantly associated with better prognosis (R: 0.64 [0.49–0.83] [Figure 3C & 3D]; $p = 0.00081$ and HR: 0.52 [0.41–0.67]; $p = 1.4e-07$). In addition, high mRNA levels of *MFAP1* and *MFAP2* were modestly associated with increased OS in breast cancer (HR: 0.81 [0.64–1.03]; $p = 0.081$ and HR: 0.81 [0.65–1.01]; $p = 0.059$) (Figure 3A & 3B). However, *MFAP5* (HR: 1.18 [0.93–1.49]; $p = 0.17$) (Figure 3E) was not correlated with OS despite its significantly decreased mRNA expression. Considering their mRNA expression and association with OS, *MFAP4* might provide higher prognostic values in clinical practice for breast cancer patients compared with other *MFAPs* family members.

Prognostic values of *MFAP4* in breast cancer patients with different intrinsic subtypes & cancer grades

To further examine the prognostic values of *MFAP4*, we first checked mRNA expression of *MFAP4* in different major subclasses of TCGA breast cancer samples using UALCAN database. As shown in Figure 4A, mRNA levels of *MFAP4* were significantly reduced in luminal, *HER2* positive and triple negative breast cancers compared with normal tissues ($p = 1.62e-12$; $p = 1.62e-07$ and $p < 1.00e-12$; respectively). Then we assessed prognostic values of *MFAP4* in different intrinsic subtypes using KM plotter. The results showed high mRNA expression of *MFAP4* in all four intrinsic subtypes significantly correlated with better prognosis (Basal; HR: 0.53 [0.32–0.90]; $p = 0.016$; Luminal A; HR: 0.42 [0.28–0.64] [Figure 4B–E]; $p = 2.2e-05$; Luminal B; HR: 0.66 (0.44–0.99); $p = 0.045$; *HER2*+, HR: 0.43 (0.22–0.85); $p = 0.012$, respectively).

We also investigated prognostic values of *MFAP4* in breast cancer patients with different cancer grades. UALCAN database analysis showed *MFAP4* was frequently downregulated in all individual cancer stages (all $p < 0.01$) (Figure 4F). Further analysis revealed high mRNA expression of *MFAP4* was correlated with better prognosis in grade II (HR: 0.45 [0.29–0.68]; $p = 0.00014$) (Figure 4H). Interestingly, high mRNA expression of *MFAP4* was related to poor prognosis in grade III (HR: 1.6 [1.15–2.22]; $p = 0.0052$) (Figure 4I). However, there was no significant correlation of high mRNA expression of *MFAP4* in grade I and OS (HR: 0.49 [0.2–1.19]; $p = 0.11$) (Figure 4G). Taken together, these findings indicate a high prognostic value of *MFAP4* for breast cancer patients.

DNA hypermethylation in the promoter of *MFAP4* negatively correlates with *MFAP4*-mRNA expression in breast cancer

DNA methylation plays a critical role in the epigenetic regulation of chromatin structure and gene transcription and high levels of DNA methylation in promoter regions could directly lead to stable epigenetic repression of gene expression [23]. To assess whether abnormal expression of *MFAP4* is associated with DNA methylation status, MEXPRESS analysis was used in this study. As shown in Figure 5A, the samples are ordered by their expression level.

Figure 3. Prognostic values of MFAPs for OS in all breast cancer patients. The Kaplan–Meier plots were generated by analyzing 1105 breast invasive carcinoma patients (TCGA, Provisional, 1105 samples). p-values were calculated by log-rank test. HRs with 95% CIs are displayed. (A) MFAP1; (B) MFAP2; (C) MFAP3; (D) MFAP4; (E) MFAP5.

The expression of *MFAP4* in breast cancer was negatively correlated with its DNA methylation in the promoter, which was confirmed by the Pearson correlation coefficients on the right (Figure 5A). MEXPRESS analysis also revealed that *MFAP4*-mRNA expression level was much lower in breast cancer samples than those in normal samples and correlated with *PR* status ($p = 5.7\text{e-}07$) and *ER* status ($p = 5.62\text{e-}09$). Data from the MethHC database confirmed DNA hypermethylation in the promoter of *MFAP4* in breast cancer patients compared with normal samples ($p < 0.01$) (Figure 5B). These results demonstrate that DNA hypermethylation in the promoter of *MFAP4* may result in a downregulation of its mRNA expression.

We also determined whether decreased mRNA expression of *MFAP4* was associated with genetic alterations, the cBioPortal was used to analyze mutations, copy-number alterations and mRNA expression changes of *MFAP4*. The alterations of *MFAP4* were mutually exclusive, occurred in 57 (6%) of 960 patients with breast invasive carcinoma, 17 samples of whom occurred DNA amplification, one sample was inframe mutated, eight samples were observed to have deep deletion and only 31 samples were mRNA upregulated (Figure 5C). Despite its DNA copy-number alteration, there was almost no elevation in the mRNA expression of *MFAP4*, but slightly decreased (Figure 5D).

MFAP4-regulated biomolecular network

To investigate how *MFAP4* exerts its regulatory effect in the tumorigenesis and tumor progression, we searched its potential regulated genes using STRING. Functional interaction network analysis revealed that *MFAP4* was closely associated with 35 genes, including *FBLN1*, *FBLN5*, *EFEMP1*, *EFEMP2*, *MFAP2*, *MFAP5*, *PMP22* and *COTL1* (Figure 6A). Then we performed gene ontology and pathway enrichment analysis for those genes using

Figure 4. Prognostic values of MFAP4 in breast cancer patients with different intrinsic subtypes and cancer grades. (A) mRNA expression of MFAP4 in breast cancer patients with different intrinsic subtypes, including luminal, HER2 positive and triple negative breast cancer patients. Then MFAP4 was plotted for different intrinsic subtypes of breast cancer. (B) Basal breast cancer; (C) Luminal A breast cancer; (D) Luminal B breast cancer; (E) HER2+ breast cancer; (F) mRNA expression of MFAP4 in breast cancer patients with different cancer stages. Then prognostic values of MFAP4 were plotted for different cancer grades of breast cancer; (G) Stage 1; (H) Stage 2; (I) Stage 3. **p < 0.01.

Figure 4. Prognostic values of *MFAP4* in breast cancer patients with different intrinsic subtypes and cancer grades (cont.). (A) mRNA expression of *MFAP4* in breast cancer patients with different intrinsic subtypes, including luminal, *HER2* positive and triple negative breast cancer patients. Then *MFAP4* was plotted for different intrinsic subtypes of breast cancer. (B) Basal breast cancer; (C) Luminal A breast cancer; (D) Luminal B breast cancer; (E) *HER2*+ breast cancer; (F) mRNA expression of *MFAP4* in breast cancer patients with different cancer stages. Then prognostic values of *MFAP4* were plotted for different cancer grades of breast cancer; (G) Stage 1; (H) Stage 2; (I) Stage 3. **p < 0.01.

Metascape and the most significantly enriched gene sets were molecules associated with elastic fibres (*FBLN1*, *FBLN2*, *FBN1*, *EFEMP1*, *MFAP2*, *MFAP5*, *FBLN5*, *EFEMP2*, *DCN*) and nucleotide-excision repair (NER), DNA damage recognition (*GPS1*, *UBB*, *COPS3*, *COPS2*, *COPS8*, *SHMT2*, *GJB6*, *FBN1*, *DCN* and *NEURL1*) (Figure 6B & Supplementary Table 2). In addition, signaling by *NOTCH1* HD domain mutants in cancer (*UBB*, *NEURL1*, *NEURL1B*, *EFEMP1*, *OCA2*, *SLC26A8* and *DCN*), PID *Notch* pathway (*MFAP2*, *MFAP5*, *NEURL1*) and negative regulation of cell proliferation (*FBLN1*, *PMP22*, *SFTPD*, *NEURL1*, *GJB6*, *COPS8* and *FBN1*) were also significantly enriched, which have been reported as pivotal biological process in tumor progression. These results indicated that downregulation of *MFAP4* in breast cancer may play an important role in cell proliferation and impairment and alteration of the elastic fibers structural appearance.

Prognostic values of *MFAP4* in other human cancers

According to the latest cancer situation and trend from the Chinese National Cancer Center, lung cancer, liver cancer and stomach cancer are top three cancers with the highest morbidity and mortality in China. We decided to test

Figure 5. Impact of epigenetic and genetic alterations on *MFAP4* mRNA expression in breast cancer. (A) Visualization of the TCGA data for *MFAP4* mRNA expression in breast cancer using MEXPRESS. 871 breast invasive carcinoma samples were included. The methylation data for each probe was determined using Infinium HumanMethylation 450 microarray. Gene expression data was derived from IlluminaHiSeq.RNASeqV2. The samples are ordered by their expression level. The significance of the relation (correlation coefficient or p-value) between each row of data (clinical, expression or methylation) was shown in the right side. **(B)** The promoter methylation levels of *MFAP4* in 839 breast invasive carcinoma samples were analyzed by MethHC database. **(C)** OncoPrint of *MFAP4* alterations in breast cancer. Tumor samples are shown in columns. Genomic alterations of *MFAP4* are mutually exclusive. **(D)** Correlation plot for putative copy-number alterations and *MFAP4* mRNA expression in breast cancer. Putative copy-number calls on 1080 breast cancer cases determined using GISTIC 2.0. Values: -2 = homozygous deletion; -1 = hemizygous deletion; 0 = neutral/no change; 1 = gain; 2 = high level amplification. *p < 0.05; **p < 0.01; ***p < 0.001.

the prognostic values of *MFAP4* in these human cancers. UALCAN analysis showed a significantly downregulated mRNA level of *MFAP4* in cancer tissues compared with normal tissues (Figure 7A–C). KM plotter database revealed high mRNA expression of *MFAP4* were significantly correlated with better outcome in all lung cancer patients (HR: 0.58 [0.51–0.67]; p = 5.4e-15) (Figure 7D), liver cancer patients (HR: 0.63 [0.42–0.93]; p = 0.02) (Figure 7E) and stomach cancer patients (HR: 0.72 [0.59–0.88]; p = 0.0013) (Figure 7F). We also investigated its prognostic values in lung, liver and stomach cancer patients with different stages. As shown in Table 2 and Supplementary Figure 1, *MFAP4* presented a good prognostic values for these three cancer patients in most cases.

Discussion

Seeking for useful biomarkers and novel therapeutic targets represent an important milestone toward precision oncology for diagnosis and treatment of human cancers. The present study systematically profiled the global landscape of mRNA expression, prognostic values, epigenetic and genetic alterations and regulatory network for *MFAP4* in human cancers. Using breast cancer as a study model, we identified a frequent downregulation of *MFAP4* in most human cancers and its high mRNA level predicts a better prognosis for breast cancer patients with different intrinsic subtypes and cancer grades, except for stage III. Consistent with that of breast cancer, high mRNA levels of *MFAP4* in lung, liver and stomach cancers are also significantly associated with better outcome in early stages. Compared with established prognostic biomarker for breast cancer, such as cancer antigen 15-3 (*CA 15-3*), *CA 125*, carcinoembryonic antigen, *ER*, *PR* and *HER2*, *MFAP4* seems to be a common prognostic biomarker in most types of human cancers. These findings may contribute to the development of precise diagnosis and prognosis based on *MFAP4* in human cancers. Increased DNA copy number always means an increase of gene mRNA expression level. But several biological factors like mutation, DNA methylation, histone modifications and microRNAs could significantly affect gene mRNA expression beyond copy number itself [24]. In this study, hypermethylation in

Figure 5. Impact of epigenetic and genetic alterations on *MFAP4* mRNA expression in breast cancer (cont.).

(A) Visualization of the TCGA data for *MFAP4* mRNA expression in breast cancer using MEXPRESS. 871 breast invasive carcinoma samples were included. The methylation data for each probe was determined using Infinium HumanMethylation 450 microarray. Gene expression data was derived from IlluminaHiSeq_RNASeqV2. The samples are ordered by their expression level. The significance of the relation (correlation coefficient or p-value) between each row of data (clinical, expression or methylation) was shown in the right side. **(B)** The promoter methylation levels of *MFAP4* in 839 breast invasive carcinoma samples were analyzed by MethHC database. **(C)** OncoPrint of *MFAP4* alterations in breast cancer. Tumor samples are shown in columns. Genomic alterations of *MFAP4* are mutually exclusive. **(D)** Correlation plot for putative copy-number alterations and *MFAP4* mRNA expression in breast cancer. Putative copy-number calls on 1080 breast cancer cases determined using GISTIC 2.0. Values: -2 = homozygous deletion; -1 = hemizygous deletion; 0 = neutral/no change; 1 = gain; 2 = high level amplification.

*p < 0.05; **p < 0.01; ***p < 0.001.

Figure 6. MFAP4-regulated biomolecular network. (A) Protein-protein interaction networks were analyzed by STRING database with a confidence score of 0.4 and 35 genes interacted with *MFAP4* were identified. Line thickness indicates the strength of data support. (B) Top 9 clusters from Metascape pathway enrichment analysis of 35 *MFAP4*-associated genes carried out with the following ontology sources: KEGG Pathway, GO Biological Processes, Reactome Gene Sets, Canonical Pathways and CORUM. Length of bars represent log₁₀(p-value) based on the best-scoring term within each cluster.

Figure 7. Prognostic values of *MFAP4* in other human cancers. *MFAP4* mRNA expression was analyzed in 574 lung cancer cases, 421 liver cancer cases and 449 stomach cancer cases from UALCAN database. (A) Expression of *MFAP4* in lung cancer. (B) Expression of *MFAP4* in liver cancer. (C) Expression of *MFAP4* in stomach cancer. Then we analyzed prognostic values of *MFAP4* in these cancers using KM plotter. (D) *MFAP4* in all lung cancer patients. (E) *MFAP4* in all liver cancer patients. (F) *MFAP4* in all stomach cancer patients. p-values were calculated by log-rank test. HRs with 95% CIs are displayed. **p < 0.01.

the promoter may be a key drive for repression of *MFAP4* transcription and mRNA expression. Meanwhile, the mutations and copy-number alterations have little impact on gene transcription for *MFAP4*.

MFAP4, a human homolog to *MAGP-36*, is expressed in both elastic and nonelastic tissues [25–27]. *MFAP4* was reported to be involved in immune response, skin disease, lung function and liver fibrosis [28–31]. Several studies have also indicated that *MFAP4* may play potential roles in human cancers. For example, *MFAP4* was downregulated and may function as tumor suppressor in prostate cancer and urinary bladder cancer [32,33]. Consistent with our results, Muraoka *et al.* found that *MFAP4* protein level was higher in low-risk breast cancer patients than those of high-risk patients, but the function of *MFAP4* in breast cancer was not clarified [34]. In neuroblastoma, however, Zhao *et al.* reported that miR-449a inhibited cell proliferation and induced cell-cycle arrest by targeting *MFAP4*, *PKP4* and *TSEN15* and high level of *MFAP4* was correlated with poor survival of neuroblastoma patients [35]. Similar to neuroblastoma, here we also observed that high level of *MFAP4* predicted a poor prognosis for patients with advanced stages of lung and stomach cancer as well as breast cancer. This apparent shift of *MFAP4* in prognostic ability probably results from a dual role of *MFAP4*. In early stage cancer, *MFAP4* facilitates inflammatory cell recruitment and assists immunological cancer surveillance to restrain cancer-cell survival [28]. In late stage cancer, alteration of tumor microenvironment results in decreased immune function of lymphocytes and *MFAP4* predominantly promotes cancer cell proliferation and migration and therefore, accelerates tumor exacerbation [36]. As the physiological and pathophysiological roles of *MFAP4* are still largely unknown, further

Table 2. Prognostic values of *MFAP4* in lung cancer, liver cancer and stomach cancer patients with different stages.

Cancer type	Patients number at risk	<i>MFAP4</i> expression level	Stage	HR	95% CI	Prognostic outcome	p-value
Lung cancer	363	Low	1	0.28	0.19–0.4	Good	4.4e-13
	214	High					
	144	Low	2	0.65	0.44–0.95	Good	0.026
	100	High					
	27	Low	3	1.48	0.84–2.62	Worse	0.17
	43	High					
	4	–	4	–	–	–	–
	42	Low	1	0.45	0.23–0.89	Good	0.019
	128	High					
	27	Low	2	0.53	0.24–1.16	Good	0.11
Liver cancer	56	High					
	41	Low	3	0.66	0.36–1.19	Good	0.16
	42	High					
	4	–	4	–	–	–	–
	16	Low	1	0.52	0.17–1.6	Good	0.25
	23	High					
Stomach cancer	11	Low	2	2.72	0.64–11.66	Worse	0.16
	38	High					
	62	Low	3	1.39	0.96–2.03	Worse	0.082
	155	High					
	26	Low	4	1.37	0.75–2.49	Worse	0.3
	48	High					

studies are needed to determine the protein levels of *MFAP4* in different breast cancer stages, which is important for us to explain the obvious distinction of *MFAP4* in prognostic ability in late stage cancer.

By browsing networks for genes of interest, especially rare reported genes, we can predict its potential functions and identify a number of previously unappreciated biological processes and pathways associated with human diseases. Functional enrichment analysis of 35 *MFAP4*-related genes resulted in the identification of remarkable pathways which may play key roles in cancer initiation and progression. As expected, genes that associate with elastic fibres were significantly enriched. More importantly, newly identified *MFAP4*-related pathways, NER and negative regulation of cell proliferation will encourage future investigations which may contribute to the discovery of novel mechanisms governing carcinogenesis and tumor progression.

The accumulated DNA damage is an important driving force for cancer initiation. Endogenously metabolic products (such as reactive oxygen species) and environmental chemicals (tobacco) and radiation (ultraviolet) can generate single nucleotide mutations and DNA strand breaks [37–39]. These damages may activate oncogenes or inactivate tumor suppressor genes and cause abnormal cell growth, invasion and metastasis. NER is the most versatile system to eliminate diverse DNA lesions [40]. A significant correlation has been reported between polymorphisms in NER genes and a group of cancers such as breast, ovarian, lung and non-small-cell lung cancers [41–44]. Based on protein–protein interaction network in STRING database, *MFAP4* might participate in NER process by direct or indirect interaction with NER proteins through predicted proteins such as *SHMT2*, *COPS3* and *COPS8*, most of which have not yet been studied. In fact, these genes play important roles in diverse biological processes in human cancers. Kim *et al.* reported that *SHMT2* desuccinylation at lysine 280 by *SIRT5* increased enzymatic activity and promoted tumor cell growth [45]. *COPS3* have been shown frequently upregulated and promoted cell proliferation, metastasis and apoptosis in clear cell renal cell carcinoma, osteogenic sarcoma and lung cancer [46–48].

Another identified important pathway related to *MFAP4* was negative regulation of cell proliferation. *FBLN1* belongs to extracellular matrix protein and is epigenetically downregulated in bladder cancer. Overexpression of *FBLN1* could significantly suppress tumor growth and induce tumor cell apoptosis [49]. *PMP22* was frequently amplified and upregulated in high-grade osteosarcoma and overexpression of *PMP22* significantly promoted the proliferation, invasion, migration of osteosarcoma cells [50,51]. In this study, *Notch* signaling pathway was also

enriched which is critical for cell proliferation, differentiation, development and homeostasis [52]. Dysregulation of the *Notch* signaling pathway has been implicated in a variety of hematologic and solid malignancies [53]. The study identified three genes (*MFAP2*, *MFAP5* and *NEURL1*) associated with this pathway. *MFAP2* was reported a crucial role in ECM function, cell adhesion, hemostasis, thrombosis and metabolic stress [54–56]. *MFAP5* is a protein that can be secreted by cancer cells and promotes angiogenesis in cholangiocarcinoma and ovarian cancer [57,58]. *MFAP2* and *MFAP5* were also reported to possess the ability to interact with the EGF-like repeats of Notchs and subsequently activate *Notch* signaling pathway [59]. *NEURL1* can function as an E3 ubiquitin ligase and decrease Notch ligand, Jagged1 and the target genes, *HES1* and *HEY1* in medulloblastoma [60,61]. These results indicated a potential role of *MFAP4* in the development and progression of human cancers.

One limitation of this study is that all our analysis was based on mRNA level from publicly available datasets and further investigations should be performed to validate the consistence with protein level by Western blot and immunohistochemistry staining. Although our work emphasizes the prognostic value of *MFAP4* in human cancers and predicts several pathways involved in its function, experimental data are warranted to clarify the specific roles and underlying mechanisms through which *MFAP4* influences cancer phenotypes such as cell growth, metastasis and apoptosis. Besides *MFAP4* is an oligomeric glycoprotein containing several disulphide bridges [62]. That kind of structure is usually not found within cells, only in the extracellular milieu. It is of great interest to find how *MFAP4* directly or indirectly participates in intracellular excision repair and DNS damage recognition.

Conclusion

In summary, our work for the first time systematically investigated the potential function and molecular mechanism of *MFAPs* in human cancers based on TCGA data. This integrated study revealed a profound impact of genetic and epigenetic alterations on gene expression in cancers. Our findings suggested *MFAP4* may function as a candidate tumor suppressor gene and its potential roles in carcinogenesis is worthy of further study. Furthermore, *MFAP4* could be used as a promising prognostic biomarker and potential novel therapeutic target against human cancers.

Summary points

- We investigated the expression profiles of *MFAPs* in different cancer types using Oncomine and UALCAN databases and found *MFAP4* was frequently down-regulated.
- We found that high mRNA expression of *MFAP4* was identified to significantly correlate with better overall survival in breast cancer.
- Epigenetic and genetic analysis revealed DNA hypermethylation in the promoter of *MFAP4* decreased its mRNA expression in breast cancer.
- We demonstrated that *MFAP4* strongly associated with pathways in impairment and alteration of the elastic fibers, nucleotide-excision repair and negative regulation of cell proliferation.
- *MFAP4* was also confirmed high prognostic values in lung, liver and stomach cancers.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at:
<https://www.futuremedicine.com/doi/suppl/10.2217/epi-2018-0080>

Author's contributions

Y Li and X Hao conceived and designed the study. J Yang, H Song, L Chen and Y Zhang obtained data. J Yang, H Song and K Cao performed the data analysis. J Yang and H Song drafted the manuscript. All authors reviewed the manuscript before submission. All the authors approved the final version of the manuscript.

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