Fhit Regulates EMT Targets through an EGFR/Src/ERK/Slug Signaling Axis in Human Bronchial Cells

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Abstract

In many cancers, including lung carcinomas, Fragile histidine triad (Fhit) is frequently decreased or lost. Fhit status has recently been shown to be associated with elevated in vitro and in vivo invasiveness in lung cancer. Tumor cell invasion is facilitated by epithelial–mesenchymal transition (EMT), a process by which tumor cells lose their epithelial features to acquire a mesenchymal cell-like phenotype. In this study, the mechanism underlying Fhit-regulated EMT was deciphered. Using Slug knockdown, pharmacologic inhibitors PD98059, PP1, and gefitinib as well as an anti-EGFR antibody, it was demonstrated that Fhit silencing in bronchial cells induced overexpression of two primary EMT-associated targets, MMP-9 and vimentin, to regulate cell invasion dependent on an EGFR/Src/ERK/Slug signaling pathway. Moreover, ectopic expression of Fhit in Fhit-deficient lung cancer cells downregulated this pathway. Finally, an inverse correlation was observed between Fhit and phospho-EGFR levels in a cohort of human squamous cell lung carcinoma specimens. These results demonstrate a Fhit-dependent mechanism in the control of EMT-regulated EGFR signaling.

Implications: This study adds new insight into the regulatory mechanism of EMT, a process known to increase resistance to conventional and targeted therapies in lung cancer. Mol Cancer Res; 12(5); 775–83. © 2014 AACR

Introduction

Lung cancer is the leading cause of deaths worldwide. The poor survival of patients with lung cancer is mostly attributed to early metastasis and resistance to both conventional and targeted therapies. The metastatic progression of epithelial tumors is a complex process requiring tumor cell plasticity. In particular, the step of tumor cell invasion into surrounding stromal tissue is characterized by a dedifferentiation of tumor cells, largely known to involve epithelial–mesenchymal transitions (EMT). Tumor cells undergoing EMT lose epithelial features and engage in mesenchymal cell mimicry similarly as observed during embryonic development (1–3). Indeed, during tumor invasion, cell–cell adhesion complexes, especially adherens and tight junctions, are frequently reorganized in tumor cells. However, the cytoskeleton is also affected and a de novo expression of the intermediate filament vimentin is frequently observed during EMT (4–6). Tumor cells also acquire degradative properties against extracellular matrix components through their ability to produce themselves high levels of proteases such as matrix metalloproteases (MMP) among which MMP-2, MMP-9, and MMP-14 (7, 8). Specific transcription factors, such as those of the Snail, the Twist, or the ZEB family, have now been largely involved in the regulation of EMT target genes (9). Despite the difficulty to prove the occurrence of EMT in vivo because of its transient nature, several studies have reported changes of expression of EMT markers in non–small cell lung carcinomas (10, 11). There is growing evidence that EMT increases resistance to conventional chemotherapy, radiotherapy as well as targeted therapy (6). Accordingly, it has been shown that tumor cells of lung carcinomas, which have undergone an EMT, are less sensitive to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI), gefitinib and erlotinib, than epithelial tumor cells (10, 12, 13).

The Fragile histidine triad (Fhit) gene is one of the most frequently altered genes in cancers, in particular in lung carcinomas in which Fhit promoter methylation may be a prognostic marker (14, 15). The role of Fhit as a tumor suppressor gene has been well documented. Indeed, restoration of Fhit suppresses tumorigenicity in tumor cell lines and in mouse models through induction of caspase-dependent apoptosis and inhibition of proliferation of tumor cells (16–18). Besides its oncosuppressor activity, Fhit plays also a role in metastatic progression. Indeed, we...
previously showed that Fhit downregulation in non–small cell lung carcinomas is correlated with tumor invasion according to the tumor–node–metastasis (TNM) stage, particularly the lymph node status. Moreover, we demonstrated that Fhit controls invasion of lung tumor cells by regulating the expression of genes associated with EMT, including cell–cell adhesion molecules, MMPs, and vimentin (19). However, the mechanisms underlying this regulation process remain unknown.

In this study, we intended to decipher the signaling pathway engaged by Fhit to regulate lung tumor invasion. We here showed that Fhit regulates two of its main targets, MMP-9 and vimentin, in bronchial cells along with cell invasion through an EGFR/Src/ERK/Slug signaling pathway.

Materials and Methods

Cell culture

The human lung cell lines HBE4-E6/E7 and NCI-H1299 were obtained from the American Type Culture Collection. Both cell lines were tested and authenticated by short tandem repeat profiling at the DSMZ. Cells used for experiments were passaged for fewer than 6 months after resuscitation. HBE4-E6/E7 cells were cultured in Keratinocyte-SFM (Gibco; Invitrogen) supplemented with 0.2 ng/mL EGF and 25 μg/mL bovine pituitary extract (BPE). The stable transfectants NCI-H1299-mock and NCI-H1299-Fhit, generated as previously described (19), were cultured in RPMI (Gibco) containing 10% FCS.

Tumor tissue samples

Frozen tumor pieces for total protein extraction were obtained from samples of the Tumor bank belonging to the Reims University Hospital Biological Resource Collection no. DC-2008-374. Surgically resected tumors were collected after obtaining informed consent from 22 patients with squamous–cell lung carcinomas of stages I (6 cases), II (7 cases), and III (9 cases) according to the 2009 TNM classification. This study has been approved by the Institutional Review Board of the Reims University Hospital.

Pharmacologic inhibitors and neutralizing antibodies

Pharmacologic inhibitors of ERK1/2 (PD98059 and U0126), Src (PP1), p38 (SB203580), JNK (SP600125), ROCK (Y27632), PKA (KT5720), PI3K (wortmanin, U0126), Src (PP1), p38 (SB203580), JNK (SP600125), and PKC (staurosporine, G-1090) were purchased from Calbiochem, Merck) was used. Non-immune mouse IgG1 (clone 1E2.2; Millipore) served as negative control.

Transfection of siRNA

Three Fhit-specific sequences were selected in the coding sequence of Fhit (GeneBank accession number: NM_002012) to generate 21-nucleotide sense and 21-nucleotide antisense strands of the type (19N) TT (N, any nucleotide). The selected 19-nt sequences were as follow: Fhit si1, 5′-CAUCUCAUCAAGGCCUCUG-3′; Fhit si2, 5′-GGAAGGCGUGAGACUUCU-3′; and Fhit si3, 5′-GAGGACUUUCCUGCCUCU-3′ (Eurogentec). Three corresponding scrambled duplexes that do not recognize any sequence in the human genome were used as controls. A total of 100,000 HBE4-E6/E7 cells were transfected with a mix of the 3 siRNA duplexes (20 nmol/L) by calcium phosphate precipitation method as previously described (20). Two Slug-specific siRNA si1: 5′-GCUACCCAUUGGCCCUCCU-3′ and si2: 5′-UCUGGCUGCUUGUAGCACAC-3′ were also used for cotransfection experiments. After transfection, cells were 24 hours BPE/EGF starved. Cells were then submitted to an invasion assay or cultured for 24 hours in fresh BPE/EGF-free medium. For inhibition experiments, the various pharmacologic inhibitors or the EGFR neutralizing antibody were added to the cells at the adequate concentration. Supernatants and cells were then harvested for zymography and Western blot analyses.

Western blotting

Total proteins from cells or tumor samples were extracted in radioimmunoprecipitation assay buffer containing complete protease inhibitor cocktail (Roche diagnostics GmbH) and phosphatase inhibitor cocktail set II (Calbiochem, Merck). Proteins were separated on SDS-PAGE gels and transferred to a polyvinylidene difluoride membrane (NEN). The membranes were incubated with either a goat polyclonal antibody to Slug (1:1,000; G-18, Santa Cruz Biotechnology), or rabbit polyclonal antibodies to Fhit (1:250; ZR44, Zymed, Invitrogen) and ERK (1:1,000; C-16, Santa Cruz Biotechnology), or rabbit monoclonal antibodies to vimentin (1:1,000; clone SP20, Epitomics), EGFR (1:10,000; clone E235, Millipore), and phospho-Src family (Tyr416; 1:1,000; clone 100F9, Cell Signaling Technology), or mouse monoclonal antibodies to vimentin (1:1000; clone E70, Calbiochem, and phospho-EGFR (Tyr1173; 1:500; clone 9H2, Millipore). Following steps were performed as previously described (20). Subsequent detection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH; using a mouse antibody, 1:75000, clone 6C5; Chemicon, Millipore) was performed on the same membranes as a control.

Modified Boyden chamber invasion assay

The in vitro invasive properties of cells were assessed using a modified Boyden chamber assay. A total of 104 cells were placed in the upper compartment of the invasion chamber.
(BD BioCoat Matrigel Invasion Chamber; BD Biosciences).

For inhibition experiments, the various pharmacologic inhibitors or the EGFR neutralizing antibody were added to the cells in the upper compartment of the insert at the adequate concentration. The chambers were incubated for 24 hours at 37°C. The filters were then fixed in methanol and stained with hematoxylin. Quantification of the invasion assay was performed by counting the number of cells at the lower surface of the filters (23 fields at 400-fold magnification).

Gelatin zymography analysis
Supernatants of cells grown for 24 hours in BPE/EGF-free conditions were collected, centrifugated, and separated on 10% polyacrylamide SDS gel containing 0.1% (w/v) gelatin. The gel was washed for 1 hour at room temperature in a 2% (v/v) Triton X-100 solution, transferred to a 50 mmol/L Tris-HCl/10 mmol/L CaCl₂ (pH 7.6) buffer and incubated overnight at 37°C. The gel was stained in a 0.1% (w/v) Coomassie Blue (G250)/45% (v/v) methanol/10% (v/v) acetic acid solution and destained in a 10% (v/v) acetic acid/20% (v/v) methanol solution.

Immunoprecipitation and c-Src activity assay
Transfected cells treated 1 hour with DMSO or 10 μmol/L PP1 were lysed in immunoprecipitation lysis buffer [10 mmol/L Tris pH 7.4, 150 mmol/L NaCl, 5 mmol/L EDTA, 10% (v/v) glycerol, 1% (v/v) Brij 98] containing complete protease inhibitor cocktail (Roche diagnostics GmbH) and phosphatase inhibitor cocktail set II (Calbiochem). A total of 300 μg of total proteins was incubated with 2.5 μg of anti-c-Src rabbit monoclonal antibody (Cell Signaling Technology) for 1 hour at 4°C. The antigen–antibody complexes were incubated with protein G-Plus-Sepharose (Santa Cruz Biotechnology) for 1 hour at 4°C and washed 3 times in immunoprecipitation lysis buffer and twice with kinase buffer (20 mmol/L Hepes pH 7.2, 10 mmol/L MgCl₂, 10 mmol/L MnCl₂, 1 mmol/L Na₃VO₄). Pellets were resuspended in 50 μL kinase buffer supplemented with 5 μg denatured enolase (c-Src substrate; Sigma-Aldrich), 100 μmol/L ATP, and 10 μCi [γ-³²P]-ATP (Perkin-Elmer). The reaction was performed for 30 minutes at 30°C and stopped by adding Laemmli sample buffer. Samples were resolved on a 10% SDS-PAGE, gels were dried and bands were visualized by autoradiography.

Vimentin promoter-reporter assay
The 1.5-kb vimentin promoter firefly luciferase reporter vector has been characterized previously (21). Transient transfections in NCI-H1299-mock and NCI-H1299 cells and luciferase activity assay were performed as previously described (20). The firefly luciferase activity was normalized to the activity of the Renilla luciferase used as internal control. The data are expressed as fold induction relative to the empty vector–transfected NCI-H1299-mock cells.

Src transduction
HBE4-E6/E7 cells were transduced with ready-to-use lentiviral particles expressing mouse Src (CMV-mSRC-RB, LVP350) or negative control lentiviral expression particles (CMV-Null-RB) at a multiplicity of infection of 10 (Amsbio). Stable transfectants were generated using blasticidin at a concentration of 3 μg/mL.

Statistical analysis
For in vitro experiments, data are expressed as a mean of fold induction ± SEM and the one-sample t test was used for statistical analyses. In vivo association between Fhit and pEGFR was tested with the use of nonparametric Spearman rank-correlation analysis (analysis based on the ranking of the samples). Because Fhit and pEGFR levels were not normally distributed, the nonparametric Mann–Whitney U test was used for statistical analyses.
distributed, they were logarithmically transformed for graphic representation. \( P < 0.05 \) was considered significant.

**Results**

**Cell invasion induced by Fhit inhibition is Slug dependent**

Previously, we reported that Fhit inhibition is able to increase cell invasion by regulating expression of genes associated with EMT, in particular vimentin and MMP-9 (19). We thus here searched for a potential regulation of transcription factors known to modulate EMT. We found that Slug is upregulated concomitantly with vimentin and MMP-9 after Fhit silencing in bronchial HBE4-E6/E7 cells as shown by Western blot and zymography analyses (Fig. 1A). The functional implication of Slug was demonstrated by using Slug siRNA. In a modified Boyden chamber invasion assay, cell invasion induced by Fhit silencing was reduced by 66\% (\( P = 0.001 \)) and 40\% (\( P < 0.01 \)) when cells were cotransfected with Slug siRNA 1 and 2, respectively (Fig. 1B and Supplementary Figs. S1A and S2A and S2B). The inhibition of cell invasion by Slug siRNA 1 and 2 was correlated with a lower increase of vimentin and MMP-9 expression. The induction of vimentin by Fhit siRNA was reduced by 62\% (\( P < 0.001 \)) and 48\% (\( P < 0.05 \)) in the presence of Slug siRNA 1 and 2 and that of MMP-9 by 61\% (\( P < 0.001 \)) and 51\% (\( P < 0.01 \)), respectively (Fig. 1C and Supplementary Figs. S1B and S3A and S3B).

**Cell invasion induced by Fhit inhibition requires ERK1/2 signaling pathway**

Next, aiming at identifying the signaling cascade acting upstream the transcription factor Slug, we performed a large screening of different pharmacologic inhibitors of known intracellular signaling pathways in a modified Boyden chamber invasion assay (Supplementary Fig. S4). We found that Fhit siRNA-induced cell invasion was ERK dependent. Indeed, the invasive capacities of Fhit-silenced cells were decreased by 64\% in the presence of the PD98059 inhibitor (\( P < 0.05 \); Fig. 2A and Supplementary Fig. S2C). Another inhibitor of the same pathway, U0126, was also able to reduce by 74\% cell invasion induced by Fhit siRNA transfection (\( P < 0.05 \); data not shown). Moreover, the increase of ERK1/2 phosphorylation by Fhit silencing was demonstrated by Western blot analysis (Fig. 2B). The effect of PD98059 was also observed on the expression level of Fhit targets. Indeed, a 65\% reduction of vimentin level (\( P < 0.01 \)) was observed in Fhit-silenced cells treated with PD98059 and a 54\% for MMP-9 (\( P < 0.05 \)). The level of Slug was also affected by the PD98059 treatment (reduction by 41\%, \( P < 0.05 \)) supporting a cascade of regulation in which ERK acts upstream to Slug (Fig. 2B and Supplementary Fig. S3C).

**Cell invasion induced by Fhit inhibition depends on Src**

In addition, we observed that PP1, a Src kinase inhibitor, was as potent as PD98059 to decrease cell invasion in Fhit-silenced cells (by 66\%, \( P < 0.01 \); Fig. 3A and Supplementary Fig. S2D) in comparison with other pharmacologic inhibitors (Supplementary Fig. S4). Src activation by Fhit silencing was as potent as PD98059 to decrease cell invasion in Fhit-silenced cells treated with DMSO or PD98059.

was confirmed by the increase of Src phosphorylation as demonstrated by Western blot analysis (Fig. 3B). PP1 inhibitor effect on cell invasion was correlated with a down-regulation of Fhit targets. The induction of vimentin, MMP-9, and Slug expression by Fhit inhibition was reduced by 77\% (\( P < 0.05 \)), 63\% (\( P < 0.01 \)), and 65\% (\( P < 0.05 \)), respectively (Fig. 3B and Supplementary Fig. S3D). Moreover, the fact that the phosphorylation state of ERK1/2 is affected by PP1 suggests that Src acts upstream ERK1/2 in the signaling cascade (Fig. 3B). Combined treatment with both inhibitors, PD98059 and PP1, had no significant additional effect on cell invasion thus supporting this hypothesis (data not shown). Because PP1 and phospho-Src antibody target several Src family tyrosine kinase members, the specificity of Src involvement was confirmed by an in vitro kinase assay using enolase as Src substrate on c-Src immunoprecipitated from lysates of scrambled and Fhit siRNA-transfected cells (Fig. 3C).
Cell invasion induced by Fhit inhibition involves EGFR

Finally, we investigated which cell surface receptor is implicated in activation of this Src/ERK/Slug cascade. We found that the increase of invasive capacities induced by Fhit silencing in bronchial cells HBE4-E6/E7 is 59% reduced by gefitinib, thus suggesting the implication of receptor tyrosine kinases (RTK; \( P < 0.05 \); Fig. 4A). Indeed, the selective inhibitor of EGFR tyrosine kinase, gefitinib, decreases by 51% invasion of Fhit-silenced cells \( (P < 0.01) \) whereas other RTK inhibitors, FGF and PDGF inhibitors, have no significant impact (Fig. 4A and Supplementary Fig. S2E). Moreover, the increase of EGFR phosphorylation by Fhit silencing was demonstrated by Western blot analysis (Fig. 4B). Upregulation of vimentin, MMP-9, and Slug after Fhit siRNA transfection is respectively 35% \( (P < 0.05) \), 48% \( (P < 0.001) \), and 50% \( (P < 0.01) \) lower in the presence of gefitinib (Fig. 4B and Supplementary Fig. S3E). We also observed that gefitinib influences the phosphorylation state of Src and ERK kinases, supporting a link with the Src/ERK cascade depicted above (Fig. 4B). In addition, we found that the effect of EGFR inhibition by gefitinib on vimentin and MMP-9 expression is counteracted by Src overexpression in Fhit siRNA-transfected cells (Supplementary Fig. S5). Results obtained with gefitinib on cell invasion and Fhit targets were reproduced with an EGFR neutralizing antibody, thus precisely involving EGFR in the signaling pathway (Fig. 4A and C and Supplementary Figs. S2F and S3F). Furthermore, the ectopic expression of Fhit in Fhit-deficient lung cancer cells NCI-H1299 previously described to decrease their invasive capacities (19), induces a downregulation of the EGFR/Src/ERK/Slug signaling pathway concomitantly with a decrease of MMP-9 production and vimentin promoter activity (Fig. 5). Finally, the in vivo relevance of these findings was assessed by demonstrating by Western blot analysis an inverse correlation between Fhit and phospho-EGFR levels in protein extracts from a series of squamous cell lung carcinoma samples \( (r = -0.4478, P < 0.05) \), especially those of TNM stage III \( (r = -0.8000, P < 0.05) \); Fig. 6).

Discussion

In this study, we demonstrated that one of the pathways engaged by Fhit to control lung tumor invasion is the regulation of 2 of its main target genes, MMP-9 and vimentin, by an EGFR/Src/ERK/Slug signaling cascade (Fig. 7). First, we found that Fhit silencing induces MMP-9 and vimentin overexpression along with cell invasion in a Slug-dependent way. Slug is a member of the Snail family of transcription factors known to be key mediators of EMT during both physiologic processes such as embryonic development and pathologic processes such as metastatic progression (22, 23). Slug has been particularly well described in the context of lung cancer progression. Indeed, Slug overexpression is an indicator of poor overall survival in patients with lung cancer and, in agreement with our observations, Slug promotes invasion of lung cancer cells in vitro and in mouse models (13). Moreover, it was shown that Slug facilitates invasion of both pancreatic and oral cancer cells through upregulation of MMP-9 (24, 25). In addition, Vuoriluoto and colleagues previously demonstrated that Slug induces vimentin expression and migration in mammary cells (26). Vimentin, cell invasion, and migration were also found regulated by exogenous modulation of Slug in a leiomyosarcoma cell line (27).

Also, we demonstrated that ERK signaling acts upstream Slug in this cascade of regulation. Accordingly, Slug expression is controlled by Erk5 in keratinocytes during wound healing (28). A direct link between ERK activation and Slug induction was also shown to play a role in E-cadherin

![Figure 3](image-url)
repression in colon adenocarcinoma cells (29). Chen and colleagues showed that ERK pathway regulates breast cancer cell migration by maintaining Slug expression (30). A ERK/Slug pathway is involved in a mesenchymal-to-epithelial transition induced by clusterin silencing in lung adenocarcinoma cells (31). Finally, an association between ERK1/2

![Figure 4. Cell invasion induced by Fhit inhibition involves EGFR. A, comparison of the invasive properties of HBE4-E6/E7 cells transfected with scrambled siRNA or Fhit siRNA in the presence of DMSO or either genistein (10 μmol/L), a PDGFR inhibitor (0.1 μmol/L), a FGFR inhibitor (2 μmol/L) or gefitinib (0.8 μmol/L), or in the presence of IgG1; or a neutralizing EGFR antibody (2 μg/mL) using a modified Boyden chamber assay. B, Western blot analysis of Fhit, phospho-EGFR, total EGFR, phospho-Src, total Src, phospho-ERK1/2, total ERK1/2, Slug, vimentin levels, and zymography analysis of MMP-9 levels in HBE4-E6/E7-transfected cells treated with DMSO or gefitinib. C, Western blot analysis of Fhit, phospho-EGFR, total EGFR, phospho-Src, total Src, phospho-ERK1/2, total ERK1/2, Slug, vimentin levels, and zymography analysis of MMP-9 levels in HBE4-E6/E7-transfected cells treated with IgG1, or a neutralizing EGFR antibody.]
signaling and MMP-9 regulation by Slug was demonstrated by Joseph and colleagues (25).

Furthermore, we showed a link between Src and ERK signaling in the regulation of MMP-9, vimentin, and cell invasion. This finding was supported by previous studies demonstrating a c-Src–mediated migration/invasion of breast cancer cells and hepatocellular carcinoma cells by downstream activation of the ERK pathway (32, 33). Src and MEK inhibitors have a combined effect on melanoma invasion (34). Src is overexpressed or hyperactivated in cancers of which lung carcinomas and is tightly associated with neoplastic invasion and EMT (35). Indeed, Src is known to promote the expression of MMPs such as MMP-9 by activating ERK and PI3K signaling (36). Src modulation also impacts vimentin expression in rat squamous bladder carcinoma cells and Wei and colleagues described a cross-linking between vimentin and c-Src in mediating prostate cancer invasion and metastasis (37, 38).

Finally, we identified EGFR as the receptor orchestrating the Src/ERK/Slug pathway modulated by Fhit silencing and/or Fhit ectopic expression. Altogether, these results thus show that Fhit regulates the EGFR signaling pathway, thereby inhibiting tumor cell invasion through the modulation of MMP-9 and vimentin levels. This is in line with our in vivo data showing an inverse correlation between Fhit and phospho-EGFR level in squamous cell lung carcinomas and the previous work of Arnoux and colleagues demonstrating the control of cell motility in keratinocytes by a EGFR/Erk5/Slug cascade (28). EGFR is considered as a major inducer of EMT (39). In that way, it has been shown that EGF acts in synergy with TGF-β1 to increase migration of human renal cells by upregulating MMP-9 through ERK signaling (40). In addition, EGFR activation promotes migration and

![Figure 5](image-url)  
**Figure 5.** Ectopic expression of Fhit in NCI-H1299 cells induces a downregulation of the EGFR/Src/ERK/Slug signaling pathway. A, Western blot analysis of Fhit, phospho-EGFR, total EGFR, phospho-Src, total Src, phospho-ERK1/2, total ERK1/2, Slug, and GAPDH levels in NCI-H1299-mock and NCI-H1299-Fhit stable transfectants. B, zymography analysis of MMP-9 levels in NCI-H1299-mock and NCI-H1299-Fhit stable transfectants. C, vimentin promoter reporter assay. NCI-H1299-mock and NCI-H1299-Fhit transfectants were subjected to a transient transfection with a 1.5-kb vimentin promoter firefly luciferase reporter vector followed by a luciferase activity assay. The firefly luciferase activity was normalized to the activity of the Renilla luciferase used as internal control. The data are expressed as fold induction relative to the empty vector–transfected NCI-H1299-mock cells. *, P < 0.05.

![Figure 6](image-url)  
**Figure 6.** Inverse correlation between Fhit and pEGFR levels in squamous cell lung carcinomas. A, Fhit and phospho-EGFR levels were analyzed by Western blotting in protein extracts from a series of 22 SCLC human samples (6 TNM stage I, 7 stage II, and 9 stage III). B, the correlation coefficient r was calculated using a Spearman’s rank-correlation analysis in the entire tumor series and also in the TNM stage III tumors.
invasion of head and neck squamous cell carcinoma cells by inducing vimentin and MMP-9 in a ERK1/2-dependent way (41). The mechanism by which Fhit regulates EGFR signaling remains unknown. Fhit and Src have been shown to reciprocally coimmunoprecipitate (42). One hypothesis may therefore be that Fhit sequestrates Src, thereby impeding it to associate with and mediating the phosphorylation and hyperactivation of EGFR. Accordingly, Src, known to be a key downstream of activated EGFR family members, is also able to form a complex with EGFR to potentiates its oncogenic activity (43). Interestingly, Fhit has also been described as a physiologic target of Src upon stimulation of EGFR leading to its proteasome degradation (42, 44). Therefore, Fhit is a substrate of EGFR/Src and its downregulation by various mechanisms such as promoter methylation or loss of heterozygosity may also influence the EGFR/Src signaling thus creating a regulatory loop between Fhit, EGFR and Src to sustain invasion of carcinoma cells.

In conclusion, this study provides new insights into the role of Fhit in the control of invasive phenotype of lung tumor cells. Interestingly, our results show the involvement of EGFR in the Fhit-driven process of EMT regulation. A better understanding of the mechanisms of regulation of EGFR signaling may be particularly helpful in the context of lung cancer and EMT-associated resistance to targeted therapy.

References

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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