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Somatic mutational analysis of FAK in breast cancer: A novel gain-of-function mutation due to deletion of exon 33

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ABSTRACT

Focal adhesion kinase (FAK) regulates cell adhesion, migration, proliferation, and survival. We identified a novel splicing mutant, FAK-Del33 (exon 33 deletion, KF437463), in both breast and thyroid cancers through colony sequencing. Considering the low proportion of mutant transcripts in samples, this mutation was detected by TaqMan-MGB probes based qPCR. In total, three in 21 paired breast tissues were identified with the FAK-Del33 mutation, and no mutations were found in the corresponding normal tissues. When introduced into a breast cell line through lentivirus infection, FAK-Del33 regulated cell motility and migration based on a wound healing assay. We demonstrated that the expression of Tyr397 (main auto-phosphorylation of FAK) was strongly increased in FAK-Del33 overexpressed breast tumor cells compared to wild-type following FAK/Src RTK signaling activation. These results suggest a novel and unique role of the FAK-Del33 mutation in FAK/Src signaling in breast cancer with significant implications for metastatic potential.

1. Introduction

Breast cancer is currently the top cancer reported in women worldwide, both in the developed and in the developing world. It is estimated that there were 458,000 breast cancer-related deaths in 2008 worldwide [1]. Metastasis is the major reason for breast cancer-related deaths. Primary tumors in patients most often metastasize to the axillary lymph nodes or to distant organs after surgery or the administration of chemotherapy [2,3]. To improve breast cancer therapy, new potential therapeutic targets are required.

The potential role of RTKs, including FAK and its activation in breast cancer, is of particular interest. Considering that FAK is involved in tumor formation and progression makes FAK a potentially important new therapeutic target [4]. The small molecule inhibitors targets FAK have been developed for use as potential cancer therapies [5]. Many previous studies have shown that elevated FAK expression correlates with breast tumor malignancy and poor prognosis [6–8]. In animal models, the stable knockdown of FAK expression in breast carcinoma cells inhibits breast carcinoma metastasis to lungs, and FAK deletion could block cancer stem/progenitor self-renewal and invasion [9]. In cell models, FAK-deficient breast cancer cells display enhanced assembly and dynamics of invadopodia, which could be rescued by the expression of intact wild-type FAK [10].

FAK sequence alterations, which result from alternative splicing and/or promoter usage, have been characterized. One transcript from this alternative promoter results in the production of a truncated isoform of FAK, which lacks its N-terminal and catalytic domains, termed FRNK (FAK-related non-kinase). FRNK acts as a dominant negative regulator of FAK, which inhibits the proliferation and migration of vascular smooth muscle cells, induces anoikis in neonatal rat ventricular myocytes and inhibits growth-factor mediated signals to MAP kinase in most FAK cells [11,12]. Various alternative splice variants, which predict changes in the amino acid sequence of FAK, were found in the brain [13–15]. These variants include the following: Pro-Trp-Arg, which defines the FAK + isoform; boxes 28, 6 and 7, in reference to the number of amino acids encoded by these exons; and FAK isoforms including boxes 13, 14 and 31 et al. Altogether, these results show that multiple FAK transcripts are expressed in the adult human brain; however, these transcripts appear to be expressed at low levels in humans, which suggests that these alternative transcripts may be side products and/or play a regulatory role in transcription.

We performed mutational analysis of the FAK gene in breast cancer tissues. Novel somatic alternative splicing mutant of FAK

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was identified in breast and thyroid cancer. We developed a Taqman MGB probe-based technology for mutation isolation in 21 paired samples of breast tumor/normal tissues. The analysis shows the mutation occurred in 3 of the 21 tumor samples. FAK-Del33 mutation was demonstrated to have a positive phenotype with increased cell motility and migration along with FAK/Src pathway activation.

2. Materials and methods

2.1. Breast tissue specimens

For the mutation analysis, fresh tissues (paired tumor/normal tissues) from breast cancer patients were collected after surgery and subjected to RNA extraction using the RNA Trizol reagent (Invitrogen; California).

2.2. Plasmids

The human FAK cDNA plasmids pCR2.1-FAK-Wt and pCR2.1-FAK-Del33 were gifts from Dr. Xinmin Zheng (Cornell University).

2.3. RQ-PCR assay developed for FAK-Del33 mutant detection

RQ-PCR shared a common pair of primers and F/control probes, but differed in the F/mut probe. The following probes and primers were designed: the forward primer in exon 32 ($P_{FAK\text{-}F}$); the reverse primer in exon 34 ($P_{FAK\text{-}R}$); F/control probe in exon 32; and F/mut probe in exon 33 (Fig. S1). The qPCR mixture reaction contained 2 μl 10 × Taq Buffer, 1 U Taq (Promega; Wisconsin), 10 nM primers, 10 nM probe, 25 nM dNTP and 1 μl cDNA (1/5 diluted) in a total volume of 20 μl. We used an ABI PRISM 7500 Sequence Detection System (Applied Biosystems; California) for sample amplification and analysis. The amplification conditions were as follows: 3 min at 95 °C, 10 s at 58 °C, 10 s at 72 °C, followed by 40 cycles.

2.4. Cell lines

All human cancer cell lines [MDA-MB-468, MDA-MB-453, and MDA-MB-435s (isolated from a breast cancer patient, yet melanoma derived)] and the HEK293T cells were obtained from the American Type Culture Collection (ATCC) and cultured following ATCC protocols.

2.5. Cloning and expression of FAK-Wt and FAK-Del33 in cell lines

FAK was overexpressed using a pGIPZ lentiviral vector-based expression system (Open Biosystems, Australia). The ORF regions of FAK-Wt and FAK-Del33 were generated from pCR2.1-FAK through PCR amplification. To generate the lentivirus, the pGIPZ-FAK construct and packaging plasmids (pCMV-VSVG and pCMV-dR8.2) were cotransfected into HEK293T cells using Lipofectamine 2000 (Invitrogen; California). The conditioned media were collected at 48 and 72 h post-transfection, pooled, and filtered through a 0.45-μm filter. Filtered conditioned media were used to infect target cells in the presence of 8 μg/ml Polybrene. Cells were initially infected for 8 h, allowed to recover for 48 h in complete media, and then selected using puromycin over 24 h. The positive cells were continuously cultured for further analysis.

2.6. Antibody and immunoblotting

Cells were extracted using lysis buffer for Western blot and IP (Beyotime, China); the buffer was supplemented with 1% proteinase inhibitor cocktail (Sigma–Aldrich; Missouri), 25 mM NaF, and 1 mM Na_3VO_4. Membranes were incubated with the following primary antibodies: antiphosphotyrosine FAK (Tyr397, Tyr576/7, Tyr925), phosphorylated Src (pTyr419, (n-p)Tyr530), phosphorylated ERK (p-ERK), FAK, GAPDH, Src, and ERK, which were obtained from Cell Signaling Technology. Anti-HA monoclonal antibodies were obtained from Sigma.

2.7. Wound-healing and migration, invasion assay

In the wound-healing assay, cells that were infected with empty vector (NC), wild-type, or FAK-Del33-containing virus were used. Cells were initially seeded uniformly onto 60-mm culture plates with an artificial "wound" carefully created at 0 h using a P-20 pipette tip. Images of the wounds were recorded at 0 h and 24 h from three independent experiments. For Transwell assays, cells were serum-starved overnight in 0.1% bovine serum. Transwell filters (8 μm pore size; Corning) uncoated or coated with fibronectin (10 μg/ml) were used for migration or invasion assays. Cells (3×10^5) in serum-free medium containing 0.1% BSA were added to the upper chambers in triplicate. Then, 20% FBS DMEM was added to the bottom chamber. After 24 h incubation at 37 °C, non-migrated cells on the top of the filters were removed. Cells that had migrated to the bottom of the filters were fixed and stained using Giemsa.

2.8. Cell proliferation assay

Cell proliferation was determined using WST-8 dye (Beyotime, China) according to the manufacturer's instructions. Briefly, 5×10^3 cells/well were seeded in a 96-well flat-bottomed plate, grown at 37 °C for 24–96 h, and then 10 μl WST-8 dye was add to each well; cells were then incubated at 37 °C for 3 h, and the absorbance was determined at 450 nm using a microplate reader.

2.9. Apoptosis flow cytometry assay

The apoptotic cell population was determined using a flow cytometric assay. Briefly, 5×10^5 cells/well were seeded in a 6-well plate, grown at 37 °C overnight, and then placed in serum-starved conditions for a further 24 h. Subsequently, the cells were treated without or with apoptosis inducers A (Apopisa) and B (Apobid) (1:1000; Beyotime, China) in the presence of 10% FBS for 16 h. Cells were trypsinized, washed and re-suspended in PBS. Then, the cultures were stained with Annexin V-FITC and 7-ADD (BD Biosciences, USA), and apoptosis rates were analyzed using a flow cytometer.

2.10. Statistical analysis

Statistical analysis was performed using the SPSS software version 11.0 (Chicago, USA). Differences between the two groups were evaluated using a one-way analysis of variance. A chi-square test was used to calculate the significance of detection rates between two groups. Values of P that were less than 0.005 were considered significant.

3. Results

3.1. Novel FAK mutations in breast cancer

We used RT-PCR to amplify and sequence the coding region of oligo-dT primed FAK cDNAs from 25 paired tumor/normal tissues (6 breast cancer, 10 thyroid cancer, 5 colon cancer, 4 gastric cancers). Multiple independent cDNA plasmid clones were generated

from each sample and sequenced. One alternatively spliced FAK transcript, which skipped the entire exon 33 was identified in 1 of 6 breast cancer samples and in 1 of 10 thyroid cancer samples (Fig. 1A and Table S1).

Considering the low proportion of mutant transcripts in the samples (2–3/10 positive colonies), we developed a real-time quantitative PCR (RQ-PCR) with TaqMan MGB probes for mutation screening. The RQ-PCR that was designed in the present study shared a common pair of primers and an F/control probe, but differed in the F/mut Probe. Because the F/mut probe was designed in exon 33, the probe should not detect signals in the FAK-Del33 mutation plasmid. When we performed single RQ-PCRs for each of the two probes, there was no amplification plot detected in the FAK-Del33 plasmid that was amplified with the F/mut probe, which indicates that the F/mut probe is specific and appropriate for the mutation screening (Fig. 1B and S2). In human tumor tissues, FAK-Del33 appears to be a type of somatic mutation because this mutation only accounts for a proportion of all FAK transcripts. If the sample was contaminated with the FAK-Del33 mutation, then the ΔC_t (F/mut – F/control) would be increased due to decreased DNA template for the F/mut probe [16]. According to this hypothesis, we also performed progressive dilutions of the FAK-Del33 plasmid allele, from 0.1–80%, in a solution of wild-type plasmid allele similar to the non-homogeneous DNA samples. The standard curves for the detection of the mutant allele burden were as follows: $y = 30.97x - 29.65$, and the high correlation coefficients (>0.99 in all experiments) allowed the accurate assessment of the FAK-Del33 mutation in unknown samples (Fig. 1C).

In total, 21 paired breast tumor/normal tissues were analyzed. FAK-Del33 was identified among 3 of 21 breast tumor tissues, T9, T10 and T16. The mean ΔC_t (F/mut – F/control) values are 2.47, 2.16 and 1.48, respectively, for these three samples. According to the standard curves in Fig. 1C, the proportion of mutant allele is 46.8%, 37.3% and 16.2%, respectively. To confirm this result, these samples were amplified with primers that covered exon 33 and reverse sequenced. The sequencing results showed heterozygosis at the end nucleotide of exon 34, followed by exon 33 and exon 32, simultaneously (Fig. S3). Moreover, we did not see the same mutational changes in the normal counterparts of these specimens.

3.2. Mutation of FAK-Del33 regulates cell migration and invasion in breast cancer cells

To investigate the role of FAK-Del33 mutation in tumorigenesis compared with the normal functions of FAK (such as cell migration and invasion), we transfected FAK wild-type or FAK-Del33 into the MDA-MB-468 cell line through lentivirus infection. Our results showed that the FAK transfected cell line was successfully created without any expression difference between FAK-Wt and FAK-Del33 on both mRNA (Fig. 2A and B) and protein levels (Fig. 2C); thus, these cells are reliable for physiological function analysis. The entire molecular weight of FAK-Wt is nearly 125 kDa, whereas the FAK-Del33 is shortened by 2.915 kDa. We used an 8% mini SDS–PAGE (Millipore, USA) because it is practically impossible to examine this slight Mw difference by gel shift. To compensate for this drawback, we showed that the length difference through cDNA amplification covered exon 33. This result demonstrates that a short fragment can be observed in FAK-Del33-expressing cells using an agarose gel (indicated by arrows in Fig. 2B).

We studied the biological functions (motility and migration) of the MDA-MB-468 derived cells using wound healing assay. After 24 h incubation in serum-free media, MDA-MB-468 cells with low metastatic potential slightly migrated. The overexpression of wild-type FAK is significantly different compared with vector alone. However, there was significantly more motility and migrational movements with the FAK-Del33 mutation compared with the wild-type (Fig. 2D). This result indicates that FAK-Del33 is superior to wild-type FAK in regulating cell motility and migration in MDA-MB-468 breast cancer cells. Similar observations were acquired with uncoated Transwell array, FAK-Del33 mutation caused increased migration in response to a FBS stimulus compare to its wild-type (Fig. 2E, upper panel). Similar results were also obtained

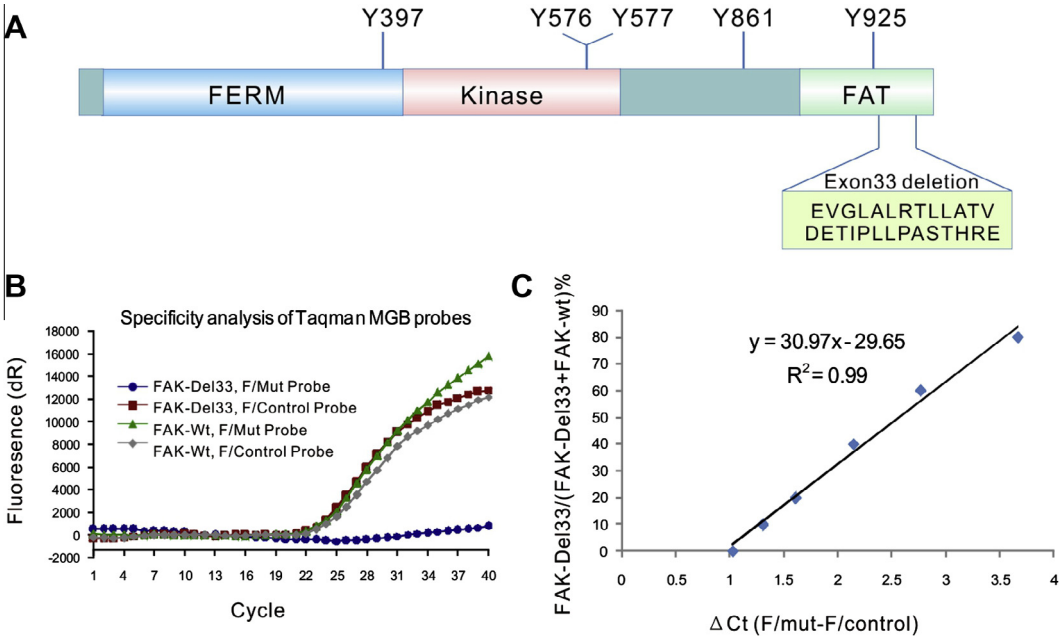

Fig. 1. Novel alteration of FAK in breast cancer. (A) Schematic diagram of the FAK-Del33 mutation that was identified in breast cancer. FAK contains an N-terminal FERM domain, a central tyrosine kinase domain (KD), and a C-terminal focal adhesion targeting (FAT) domain. (B) Specificity analysis of TaqMan MGB probes with wild-type and mutant plasmids. Plasmids were diluted to 0.2 ng/μl and then amplified using F/control and F/mut probes. No amplification plots were detected in the FAK-Del33 mutation plasmid using the F/mut probe. (C) A reference curve of serial dilutions of mutant plasmid mixtures in wild-type plasmid DNA. (D) Representative amplification plot of 3 candidate mutant samples that were tested in duplicate (left); its corresponding DNA sequence is displayed on the right. The arrow shows heterozygosis due to exon 33 deletion.

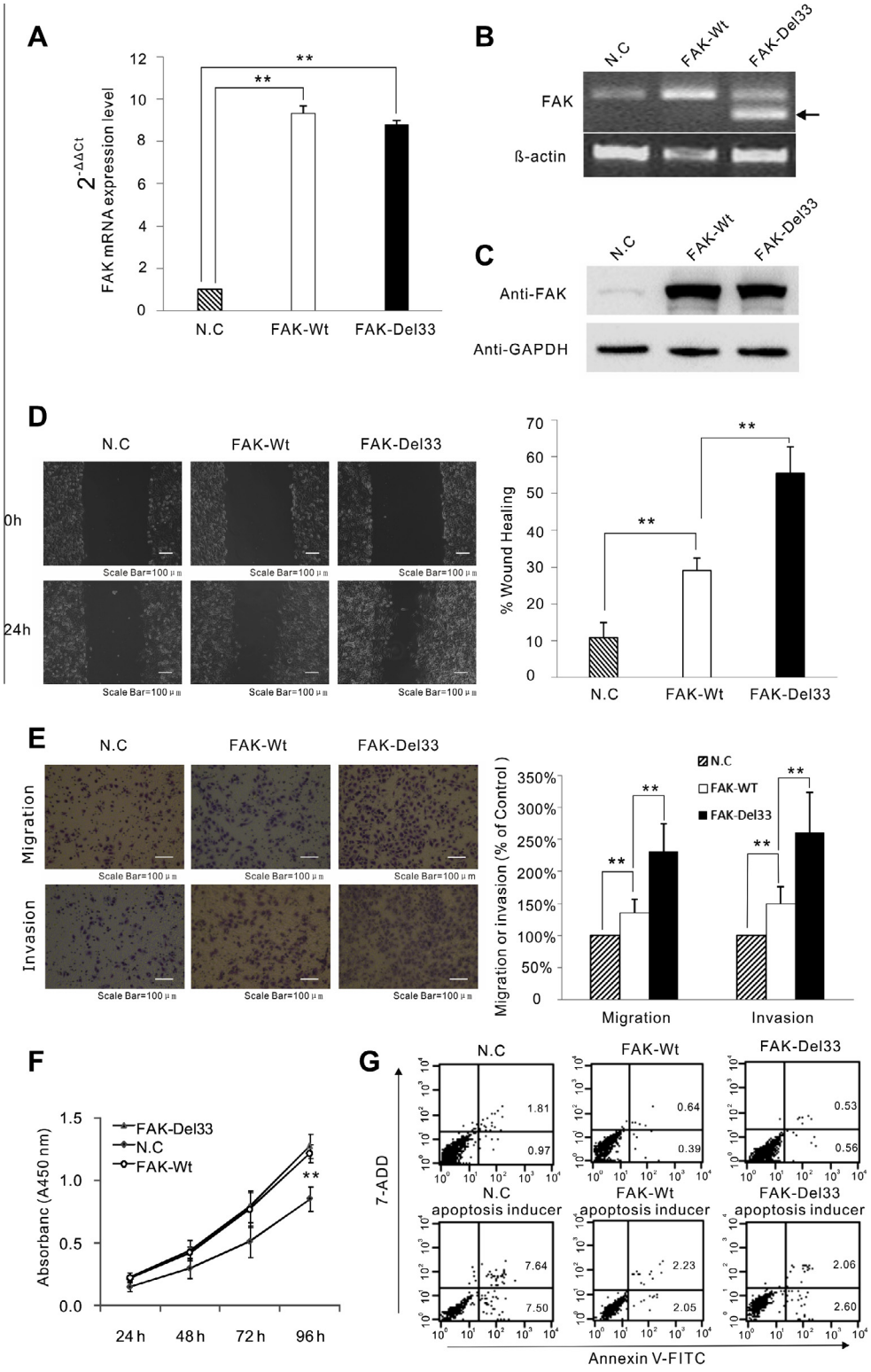

Fig. 2. Mutation of FAK-Del33 regulates cell migration and invasion in breast cancer cells. Quality assessment of MDA-MB-468 derived cells, which were infected with lentivirus that contained empty vector (NC), FAK wild-type, or FAK-Del33. Total RNA was extracted from cells using the Trizol reagent for the detection of FAK by qRT-PCR (A) or relative RT-PCR (B). Arrow indicates a short band (with 81 bp deletion) that was amplified from exogenous-expressed FAK-Del33. (C) In total, 50 μg of whole cell lysates was immunoblotted with antibodies for FAK-Total and GAPDH. (D) Motility and migration analysis by wound healing assay. MDA-MB-468 derived cells were used in the wound healing assay. Digital pictures were then taken at 0 h and at 24 h. (E) Migration and invasion result was shown through Transwells. Relative migration/invasion (*y* axis) = ratio of the number of migrated/invaded cells in test versus control. (F) MDA-MB-468 derived cells were used, and at the indicated time points, the numbers of cells per well were measured based on the absorbance (450 nm) of WST-8. (G) MDA-MB-468 derived cells treated without or with apoptosis inducers, and apoptosis was detected using the flow cytometry.

when MDA-MB-468 derived cells were tested for their ability to invade through Fibronectin-coated filters (Fig. 2E, lower panel). We repeated the same experiment in breast cell lines MDA-MB-435s and acquired similar results (data not shown). These results confirm a direct involvement of FAK-Del33 mutation in regulation of a migratory phenotype.

To study the physiological role of the FAK-Del33 mutation in cell growth, cell proliferation was determined using a WST-8 proliferation assay, and apoptosis was measured by Annexin V/7-ADD. Fig. 2F shows that overexpression of both FAK-Wt and FAK-Del33 promoted cell proliferation in MDA-MB-468 cells to a similar extent. The apoptosis assay (Fig. 2G) shows that overexpression of FAK-Wt or FAK-Del33 significantly reduced cell apoptosis compared with the vector alone. Moreover, these results hold even after treatment with apoptosis inducer. There is no significant difference between FAK-Wt and FAK-Del33 in the proliferation and anti-apoptosis abilities. These data suggest that FAK-Del33 mutation does not change FAK's physiological function in cell growth.

3.3. Role of FAK-Del33 mutation in FAK phosphorylation and downstream target Src

To investigate why FAK-Del33 displays improved cell migration and invasion, we examined the FAK Tyr397 phosphorylation in MDA-MB-468, MDA-MB-453, and MDA-MB-435s. The results

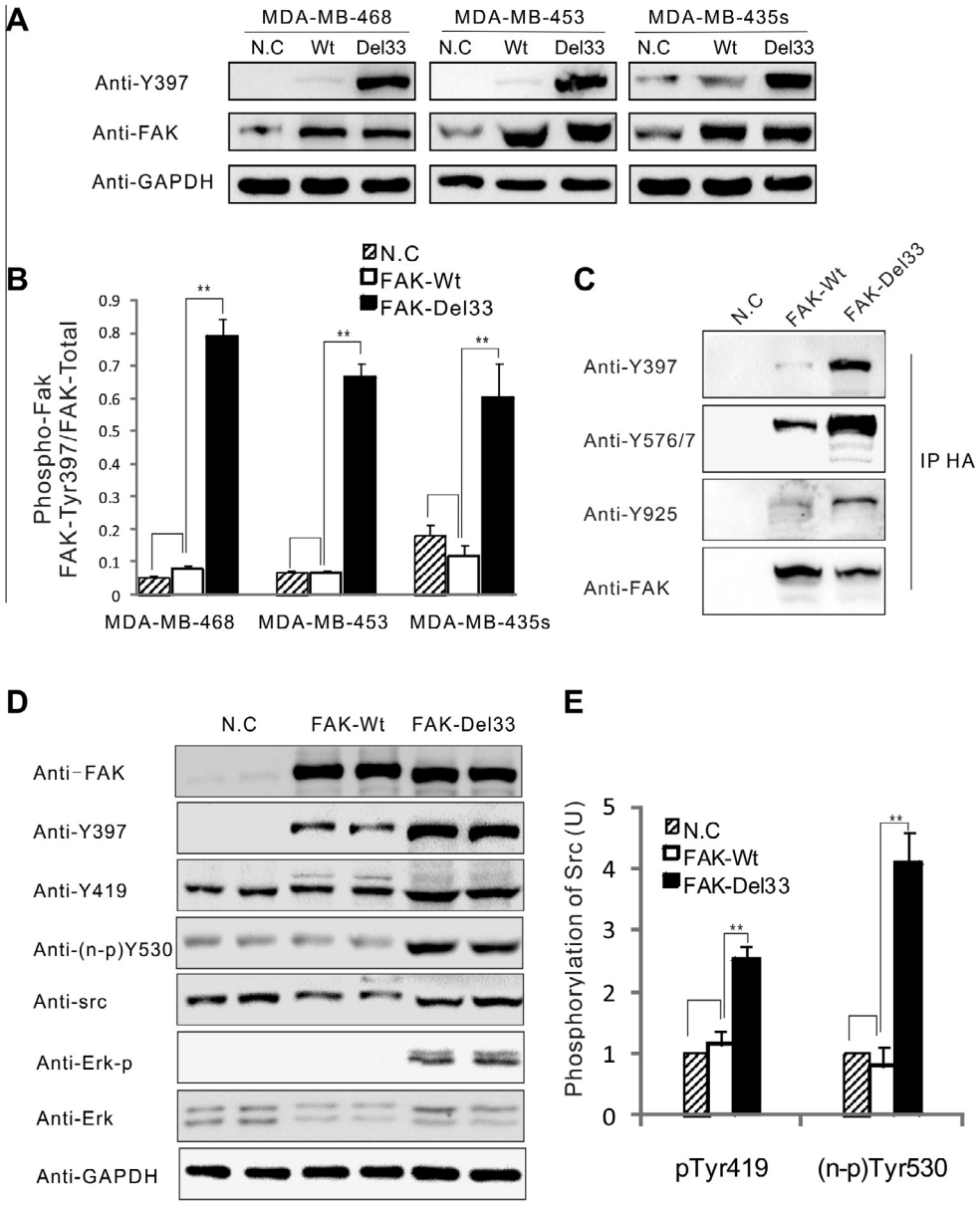

Fig. 3. Role of the FAK-Del33 mutation in FAK phosphorylation and in the downstream target, Src. (A) FAK-Del33 induces Tyr397 hyperphosphorylation. Breast cancer cells MDA-MB-468, -453, and -435s were infected with lentivirus that contained empty vector (NC), FAK wild-type, or FAK-Del33. Equal amounts of cell lysates from these cells were subjected to Western blotting by using the indicated antibodies. (B) The phosphotyrosine immunoblot, as shown in A was analyzed quantitatively using the NIH Image Analysis software program. The relative signal intensity ratio was expressed as an arbitrary unit (U) after the normalization $^{**}P \leqslant 0.005$. (C) FAK-Del33 induces significant phosphorylation of FAK Tyr576/7 and Tyr925. (D) FAK-Del33 induces Src pTyr419 phosphorylation, pTyr530 dephosphorylation and downstream ERK phosphorylation. Each immunoblot that was tested in duplicate was shown. (E) The phosphotyrosine immunoblot, as shown in D (Panel 3–4), was analyzed quantitatively using the NIH Image Analysis software program. The relative signal intensity ratio was expressed as an arbitrary unit (U) after the normalization using the vector-transfected cells (N.C) as the normal standard (1.0 U) $^{**}P \leqslant 0.005$.

demonstrated that FAK-Del33 exhibited preferentially increased tyrosine phosphorylation in Tyr397 compared with its corresponding FAK-Wt (Fig. 3A). After adjusting for the expression levels of the transduced FAK and normalizing for protein loading using the GAPDH immunoblot signal, the relative levels of Tyr397 were 5–10-fold higher in the FAK-Del33-expressing cells than the wild-type cells (Fig. 3B). To investigate the effects of the FAK-Del33 mutation on FAK phosphorylation activity, we examined the other tyrosine phosphorylation of FAK using a phosphospecific antibody. FAK was tagged with the HA protein at the N-terminal, and we used an HA-IP assay to reveal the other phosphorylation sites. As shown in Fig. 3C, FAK-Del33 also significantly induced FAK Tyr576/7 and Tyr925 phosphorylation.

The activation of Src by FAK auto-phosphorylation is central for triggering downstream cellular events. To further study the effects of the FAK-Del33 mutation on FAK/Src signaling, we examined the tyrosine phosphorylation of Src, as well as at its main downstream target p-ERK. We performed overexpression studies in both MDA-MB-468 and 435 s cells, similar results were required and results on MDA-MB-468 are shown. The results demonstrated that the level of pTyr419 on Src was preferentially induced in FAK-Del33-expressing cells (2.21-fold), with the vector-transfected cells normalized to arbitrary unit 1.0 U (Fig. 3D, Panel 3rd). In addition, the signal for the (n-p)Tyr530 dephosphorylation level was (5.17-fold) higher in FAK-Del33-expressing cells (Fig. 3D, Panel 4th). When we detected its downstream p-ERK, the phosphorylation of this protein obviously increased as well (Fig. 3D, Panel 6th). In contrast, the overexpression of wild-type FAK slightly changed the phosphorylation of Src in the confluent culture. These results indicate that FAK-Del33 regulates FAK/Src signaling activation in normal cell cultures.

4. Discussion

A unique splicing mutation with a deletion of exon33 has been detected in the present study and is reported for the first time in human tumors. Aberrant FAK signaling has been described in a variety of human cancers and is always correlated with overexpression but not sequence alteration. There are several alternative transcripts of FAK that have been identified in the human brain [15]; the majority of these transcripts appear to be expressed at low levels in humans, which suggest that these alternative transcripts may play a regulatory role in transcription. Unlike these alternative variants, FAK-Del33 has a relative high incidence in tumor tissues (3/21 breast cancer) with 20–50% of mutant alleles in these samples. We suspect that this mutation may be of clinical significance and may have therapeutic implications.

The FAK-Del33 mutation occurs in the FAT domain of FAK. FAT, which is a C-terminal domain (residues 919–1039 in human FAK), contains multiple protein–protein interactions sites that have been shown to be key regulators of FAK localization to focal adhesion (FA) complexes [17]. FAT is the binding site for the adhesion-associated proteins paxillin and talin. The crucial first step in integrin-mediated FAK activation is the recruitment of FAK to FAs by interactions between its FAT domain and the integrin-associated proteins paxillin [18] and talin [19], which results in auto-phosphorylation at Tyr397 of FAK. A minimal paxillin-binding region of FAK was determined by deletion mutagenesis to encompass residues 919–1042 [18], whereas the talin binding region is localized at residues 965–1012 [19]. According to these data, FAK-Del33 (deleted residues 956–982) may not have the ability to bind paxillin or talin. Subcellular localization demonstrates that FAK-Del33 failed to be recruited to FAs or to induce paxillin phosphorylation on Tyr118 (data not shown), which indicates that the FAK-Del33 mutation may destroy the localization ability of FAT. Interestingly, the FAK-Del33 mutation in the present study shows a gain-of-function alteration of FAK signaling with enhanced tyrosine phosphorylation, as well as enhanced cell motility and migration. The FAK-Del33 mutation even has positive effects on these biochemical and biological functions that are significantly greater than its wild-type counterpart. If FAK-Del33 lost the ability to cluster into FAs, then it may fail to receive integrin signals. Therefore, what types of signals make it auto-phosphorylated? According to the present FAK auto-inhibition activation model, the FERM domain contributes to the regulation of FAK activity. Therefore, the FAT domain that regulates FAK auto-phosphorylation and, subsequently, its biological function is clearly unknown. This possibility would be an interesting point that requires further study. In addition, it would be useful to research the role of FAK outside FAs.

We present evidence that FAK-Del33 promotes cell migration and invasion, which is paralleled with Tyr397 phosphorylation and FAK-Src signaling enhancement. As potential mechanistic explanations, several studies reported that Tyr397 phosphorylation may enhance the invasiveness of cancer cell lines [20,21]. Elevated FAK expression is observed in a large proportion of human breast cancers and correlates with an aggressive phenotype and poor prognosis [5,8,9]. The auto-phosphorylation site of FAK is required for the regulation of cell migration, and two effectors, PI3-kinase and Src kinase, have been shown to function in the FAK-dependent regulation of cell migration [10,22]. In our work, we found that Src was activated with pTyr419 phosphorylation and pTyr530 dephosphorylation, which suggests that FAK-Del33 may promote cell motility and migration through the FAK-Del33/Src signal pathway. For FAK wild-type, although FAK also promotes cell migration and invasion compared to vector transfected cells, it did not exhibit an obvious Src activation.

In the present study, we developed a novel RQ-PCR assay for mutation screening that could be used in high throughput screening and would be sufficiently sensitive for detecting a low FAK-Del33 mutant allele burden. In the present samples, the mutation frequency is 14.2% (3/21) in breast cancer. All of the mutant samples are invasive ductal carcinoma III, and T10 diagnostic with microinvasion. We suspected that FAK-Del33 may have clinically significant relevance, and more samples need to be analyzed to demonstrate this relevance. The altered migration and invasion of the FAK-Del33 mutant would have a significant impact on tumor cell invasion and metastatic potential. An in vivo model to examine the significance of this pathway and the regulation of invasion and metastasis by FAK-Del33 mutation further is warranted. These results strongly imply a unique role for the FAK-Del33 mutation in modulating biological functions and FAK/Src signaling.

5. Conflict of interest statement

None declared.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.11.134.

References

[1] I. Soerjomataram, J. Lortet-Tieulent, D.M. Parkin, et al., Global burden of cancer in 2008: a systematic analysis of disability-adjusted life-years in 12 world regions, Lancet 380 (2012) 1840–1850.

[2] P.D. Bos, X.H. Zhang, C. Nadal, et al., Genes that mediate breast cancer metastasis to the brain, Nature 459 (2009) 1005–1009.
[3] H. Kennecke, R. Yerushalmi, R. Woods, et al., Metastatic behavior of breast cancer subtypes, J. Clin. Oncol. 28 (2010) 3271–3277.
[4] G.W. McLean, N.O. Carragher, E. Avizienyte, et al., The role of focal-adhesion kinase in cancer-a new therapeutic opportunity, Nat. Rev. Cancer 5 (2005) 505–515.
[5] M.J. van Nimwegen, B. van de Water, Focal adhesion kinase: a potential target in cancer therapy, Biochem. Pharmacol. 73 (2007) 597–609.
[6] L.V. Owens, L. Xu, R.J. Craven, et al., Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors, Cancer Res. 55 (1995) 2752–2755.
[7] W.G. Cance, J.E. Harris, M.V. Iacocca, et al., Immunohistochemical analyses of focal adhesion kinase expression in benign and malignant human breast and colon tissues: correlation with preinvasive and invasive phenotypes, Clin. Cancer Res. 6 (2000) 2417–2423.
[8] A.L. Lark, C.A. Livasy, L. Dressler, et al., High focal adhesion kinase expression in invasive breast carcinomas is associated with an aggressive phenotype, Mod. Pathol. 18 (2005) 1289–1294.
[9] M. Luo, H. Fan, T. Nagy, et al., Mammary epithelial-specific ablation of the focal adhesion kinase suppresses mammary tumorigenesis by affecting mammary cancer stem/progenitor cells, Cancer Res. 69 (2009) 466–474.
[10] Z. Xie, K. Sanada, B.A. Samuels, et al., Serine 732 phosphorylation of FAK by Cdk5 is important for microtubule organization, nuclear movement, and neuronal migration, Cell 114 (2003) 469–482.
[11] J.M. Taylor, C.P. Mack, K. Nolan, et al., Selective expression of an endogenous inhibitor of FAK regulates proliferation and migration of vascular smooth muscle cells, Mol. Cell. Biol. 21 (2001) 1565–1572.
[12] M.C. Heidkamp, A.L. Bayer, J.A. Kalina, et al., GFP-FRNK disrupts focal adhesions and induces anoikis in neonatal rat ventricular myocytes, Circ. Res. 90 (2002) 1282–1289.
[13] X. Zhang, C.V. Wright, S.K. Hanks, Cloning of a *Xenopus laevis* cDNA encoding focal adhesion kinase (FAK) and expression during early development, Gene 160 (1995) 219–222.
[14] J.M. Corsi, E. Rouer, J.A. Girault, et al., Organization and post-transcriptional processing of focal adhesion kinase gene, BMC Genomics 7 (2006) 198.
[15] F. Burgaya, J.A. Girault, Cloning of focal adhesion kinase, pp125FAK, from rat brain reveals multiple transcripts with different patterns of expression, Brain Res. Mol. Brain Res. 37 (1996) 63–73.
[16] G.R. Ruan, B. Jiang, L.D. Li, et al., MPL W515L/K mutations in 343 Chinese adults with JAK2V617F mutation-negative chronic myeloproliferative disorders detected by a newly developed RQ-PCR based on TaqMan MGB probes, Hematol. Oncol. 28 (2010) 33–39.
[17] Y. Shen, M.D. Schaller, Focal adhesion targeting: the critical determinant of FAK regulation and substrate phosphorylation, Mol. Biol. Cell 10 (1999) 2507–2518.
[18] I. Hayashi, K. Vuori, R.C. Liddington, The focal adhesion targeting (FAT) region of focal adhesion kinase is a four-helix bundle that binds paxillin, Nat. Struct. Biol. 9 (2002) 101–106.
[19] H.C. Chen, P.A. Appeddu, J.T. Parsons, et al., Interaction of focal adhesion kinase with cytoskeletal protein talin, J. Biol. Chem. 270 (1995) 16995–16999.
[20] S.M. Hyder, C. Chiappetta, G.M. Stancel, Pharmacological and endogenous progestins induce vascular endothelial growth factor expression in human breast cancer cells, Int. J. Cancer 92 (2001) 469–473.
[21] S. Kato, M. Pinto, A. Carvajal, et al., Progesterone increases tissue factor gene expression, procoagulant activity, and invasion in the breast cancer cell line ZR-75-1, J. Clin. Endocrinol. Metab. 90 (2005) 1181–1188.
[22] J.T. Parsons, Focal adhesion kinase: the first ten years, J. Cell Sci. 116 (2003) 1409–1416.