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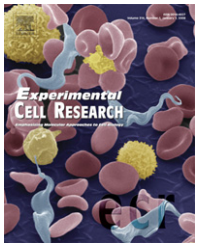

Research Article

TGFβ-induced EMT requires focal adhesion kinase (FAK) signaling ☆

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ABSTRACT

The epithelial-to-mesenchymal transition (EMT) is a crucial process, occurring both during development and tumor progression, by which an epithelial cell undergoes a conversion to a mesenchymal phenotype, dissociates from initial contacts and migrates to secondary sites. We recently reported that in hepatocytes the multifunctional cytokine TGFβ induces a full EMT characterized by (i) Snail induction, (ii) E-cadherin delocalization and down-regulation, (iii) down-regulation of the hepatocyte transcriptional factor HNF4α and (iv) up-regulation of mesenchymal and invasiveness markers. In particular, we showed that Snail directly causes the transcriptional down-regulation of E-cadherin and HNF4, while it is not sufficient for the up-regulation of mesenchymal and invasiveness EMT markers. In this paper, we show that in hepatocytes TGFβ induces a Src-dependent activation of the focal adhesion protein FAK. More relevantly, we gathered results indicating that FAK signaling is required for (i) transcriptional up-regulation of mesenchymal and invasiveness markers and (ii) delocalization of membrane-bound E-cadherin. Our results provide the first evidence of FAK functional role in TGFβ-mediated EMT in hepatocytes.

Introduction

The epithelial-to-mesenchymal transition (EMT) is a crucial step of tumorigenesis, when non-invasive and non-metastatic tumor cells lose their epithelial phenotype, acquire invasive properties, infiltrate surrounding tissues and metastasize to secondary sites. The most compelling evidence that EMT regulates invasiveness and tumor aggressiveness comes from the observation that markers of EMT are present at the tumor–host interface but not in the bulk tumor [1–4]. Turning an epithelial cell into a mesenchymal cell requires loss of epithelial polarity, alteration in cellular architecture and acquisition of migration capacity. Hallmarks for EMT include dissolution of cell–cell contacts, production of transcription factors able to inhibit

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Epithelial cadherin (E-cadherin) expression (e.g. Snail), increased expression of mesenchymal markers (e.g. fibronectin), induction of focal adhesion turnover and secretion of proteolytic enzymes involved in matrix degradation, such as the matrix metalloproteinases (MMPs) [5].

The multifunctional cytokine Transforming Growth Factor β (TGFβ), which normally functions to prevent the proliferation, regulates differentiation and provokes migration of epithelial cells [6–8]. TGFβ has also been characterized as an important inducer of EMT [9] via several downstream pathways, including RhoA [10], Ras/MAPK [11,12] and Jagged1/Notch [13]. Furthermore, TGFβ is a regulator of cellular microenvironment and extracellular matrix (ECM) remodeling [14–16].

The non-receptor focal adhesion kinase (FAK) is a major protein of the focal adhesion complex that integrates signals from growth factors and integrins to control cell adhesion, migration and invasion [17]. FAK is up-regulated in various epithelial cancers [18]. Moreover, FAK expression correlates with tumor invasiveness [19] and increased FAK activity and/or expression frequently associate with metastatic tumors [20].

Evidence of FAK implication in the TGFβ-induced EMT is limited to the observation that FAK undergoes to specific phosphorylations during this process [21]; however, direct evidence of its functional role is yet to be unveiled.

We previously demonstrated that in MMH (Murine Met Hepatocytes) cells TGFβ induces a full EMT characterized by (i) disorganization of epithelial cell membrane and down-regulation of cytoskeleton markers, (ii) decrease of hepatocyte transcriptional factors HNF1 and HNF4 and (iii) up-regulation of mesenchymal and invasiveness markers. We also showed that while Snail over-expression causes down-regulation of epithelial markers, it is not sufficient for the up-regulation of mesenchymal and invasiveness markers [22]. The latter observation suggested that other effectors are necessary for the full accomplishment of the TGFβ-induced EMT of the hepatocyte.

Here, we demonstrate that FAK plays a role in TGFβ-induced up-regulation of EMT mesenchymal and invasiveness markers as well as in the delocalization of membrane-bound E-cadherin.

Materials and methods

Cell lines and culture conditions

MMHs are a number of differentiated, polarized and non-tumorigenic hepatocytic cell lines initially described in Amicone et al. [23]. In this work, we used, as MMH parental cell, the epithelial clone described as E14 MMH cell line in Spagnoli and colleagues [24]. MMH-FRNK and MMH-S1034 cells were generated by transfection of parental MMH cells by Lipofectamine 2000 (Invitrogen, San Diego, CA) with pCDNA3.1 FRNK and pCDNA3.1 FRNK-S1034 constructs, respectively. Since pooled populations of transfected hepatocytes were heterogeneous for FRNK and FRNK-S1034 expression (data not shown), several clones were selected and expanded. Two clones that expressed comparable levels of FRNK (clones 1 and 11), and one FRNK-S1034 clone (clone 7) as control, were chosen to examine morphological and biochemical features of the TGFβ-induced EMT.

Cells were grown in RPMI 1640, supplemented with 10% FCS, 50 ng/ml EGF, 30 ng/ml IGF II (PeproTech Inc, Rocky Hill, NJ), 10 μg/ml insulin (Roche, Mannheim, Germany) and antibiotics, using collagen I (Transduction Laboratories, Lexington, UK) coated dishes (Falcon-BD, Franklin Lakes, NJ). For TGFβ treatments, normal medium was supplemented with 2 ng/ml of this cytokine (Gibco BRL, Gaithersburg, MD) for the indicated times. For treatments with pyrazolopyrimidine PP-2 (PP2) (Calbiochem, Merck Chemicals Ltd., Beeston, Nottingham, UK), normal medium was supplemented with 10 μM of this Src inhibitor for the indicated times. PP2 was added 30 min before TGFβ addition.

Immunofluorescence

For indirect immunofluorescence analyses, cells were grown on collagen I-coated 35 mm Ibidi dishes (Ibidi, GmbH Integrated BioDiagnostics, München, Germany) in the absence or presence of TGFβ and/or PP2, fixed and treated as previously described [25]. The antibodies were used as follows: mouse monoclonal anti-E-cadherin antibody (Transduction Laboratories, Lexington, UK) 2.5 μg/ml; rabbit polyclonal anti HNF4α antibody (Santa Cruz Biotechnology, Santa Cruz, CA) 4 μg/ml; mouse monoclonal anti-paxillin antibody (BD Pharmingen Biosciences, San Diego, CA) 5 μg/ml; rabbit polyclonal anti-FAK P-Y^{861} phospho-specific antibody (Biosource International, Camarillo, CA) diluted 1/50; rabbit polyclonal anti-FAK P-Y^{925} phospho-specific antibody (Santa Cruz Biotechnology, Santa Cruz, CA) 4 μg/ml. Secondary antibodies (Alexa Fluor 488- and 594-labeled, 2 μg/ml and 4 μg/ml respectively) were from Molecular Probes (Invitrogen, San Diego, CA). As control, cells were also treated according to the same protocols only with secondary antibodies (data not shown). Fluorescence imaging was performed using sequential acquisition of different channels on a Leica TCS2 confocal microscope.

Immunoprecipitations and Western blotting

Cells were lysed on ice in lysis buffer containing 50 mM Tris HCl pH 8.0, 150 mM NaCl, 5 mM EGTA pH 8.0, 50 mM NaF pH 8.0, 10% glycerol, 1.5 mM $MgCl_2$, 1% Triton X-100 containing freshly added protease and phosphatase inhibitors cocktail tablets (Complete protease inhibitor cocktail tablets, PhosStop phosphatase inhibitor cocktail tablets, Roche, Monza, IT). Lysates were clarified by centrifugation at 4 °C and protein concentrations were determined by Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA) and confirmed by Coomassie staining.

For immunoprecipitation analyses, aliquots of cellular lysates were incubated with 1 μg polyclonal anti-FAK antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1.5 h at 4 °C. Immunocomplexes were collected on protein A–sepharose beads. The beads were washed three times with lysis buffer supplemented with 1% NP-40 then boiled for 3 min in sodium dodecyl sulfate (SDS) sample buffer. For Western blot analyses, equal amount of proteins from cell lysates were resolved on 10% polyacrylamide gel electrophoresis (SDS–PAGE) and

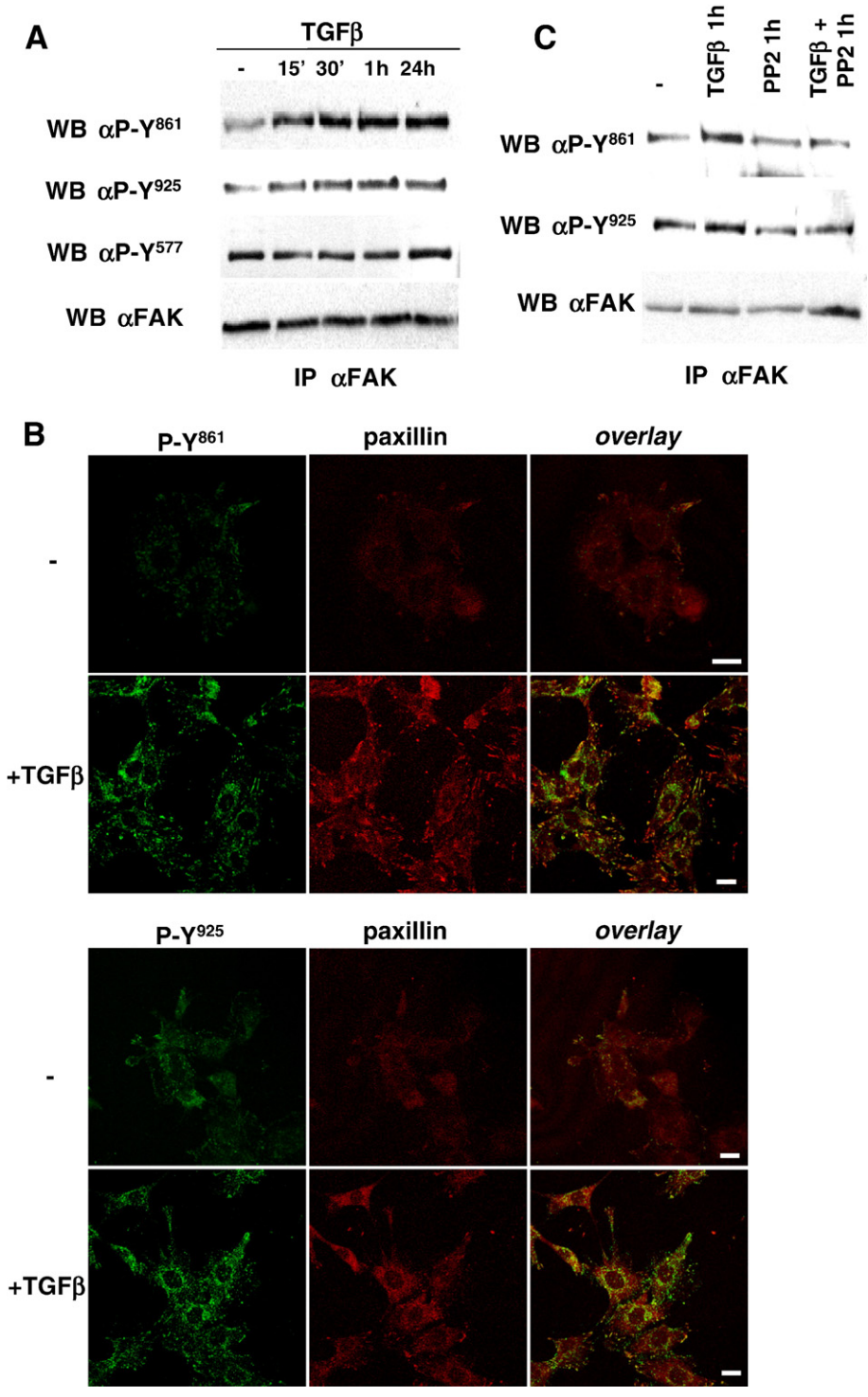
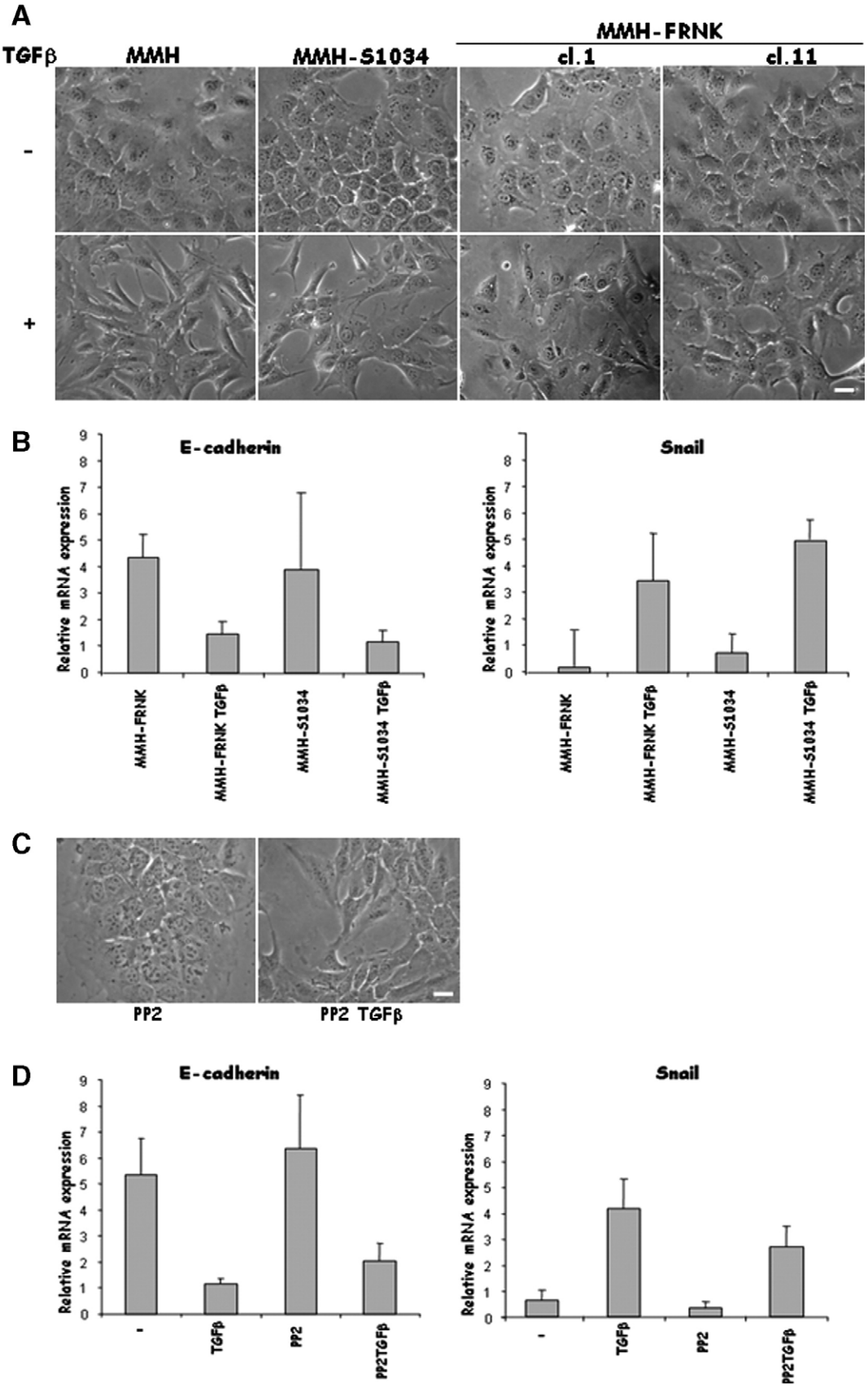


Fig. 1 – FAK is activated and localizes in focal adhesions during the TGFβ-induced EMT. (A) Immunoblotting analyses showing TGFβ-induced FAK phosphorylations. MMH cells were grown in 2% fetal bovine serum for 24 h and treated with TGFβ (2 ng/ml) for the indicated time. Untreated cells (–) served as control. Total proteins were immunoprecipitated with anti-FAK antibody and analyzed for phospho-specific FAK Tyr-861 (Y^{861}), Tyr-925 (Y^{925}), Tyr-577 (Y^{577}) and total FAK. (B) Immunofluorescence analyses showing co-localization of FAK and Paxillin. MMH cells were grown with or without TGFβ for 24 h, fixed and stained for phospho-specific FAK Tyr-861 (Y^{861}), Tyr-925 (Y^{925}) and paxillin. Fluorescence imaging was performed using sequential acquisition of different channels on a Leica confocal microscope. Coincident green and red signals are shown in yellow (each indicated calibration bar in the right panels is 20 μm). (C) Immunoblotting analyses showing PP2 interference on TGFβ-induced FAK phosphorylations. MMH cells grown as in (A) were treated or not with TGFβ and/or PP2 (10 μM) for 1 h. Total protein extracts were immunoprecipitated with anti-FAK antibody and analyzed as in (A).

transferred to PVDF membranes (Millipore, Billerica, MA). Blots were blocked in Tris buffered saline plus 0.05% Tween-20 (TBS-T) containing 5% non-fat dried milk and probed with mouse monoclonal antibody anti-E-cadherin (Transduction Laboratories, Lexington, UK), 0.25μg/ml; mouse monoclonal anti-α tubulin antibody (Santa Cruz Biotechnology, Santa Cruz, CA),



0.2 μg/ml (as control, data not shown); rabbit polyclonal anti-FAK P-Y^{577} phospho-specific antibody (Biosource International, Camarillo, CA) diluted 1/1000; rabbit polyclonal anti-FAK P-Y^{861} phospho-specific antibody (Biosource International, Camarillo, CA) diluted 1/1000; rabbit polyclonal anti-FAK P-Y^{925} phospho-specific antibody (Santa Cruz Biotechnology, Santa Cruz, CA), 0.2 μg/ml; mouse monoclonal anti-FAK antibody (Transduction Laboratories, Lexington, UK), 0.5 μg/ml. Immune complexes were detected with horseradish peroxidase-conjugated species-specific secondary antiserum (Bio-Rad Laboratories) followed by enhanced chemiluminescence reaction (Pierce Chemical, Rockford, IL).

RNA extraction, reverse transcription PCR and real time quantitative PCR (qRT-PCR)

Total RNA was extracted from cultured cells using an RNA extraction kit (NucleoSpin® RNA II, Machery-Nagel, Germany) according to the manufacturer's instructions. Single-stranded cDNA was obtained by reverse transcription of 1 μg of total RNA using MMLV-reverse-transcriptase (Promega, MI, Italia). cDNA was amplified by PCR using GoTaq enzyme (Promega, MI, Italia). qRT-PCRs were performed using BioRad-iQ-iCycler with SYBR green fluorophore; the reactions were carried out using iQ™ SYBR® Green Supermix (BioRad Laboratories, Hercules, CA) in 20 μl volume in a 96-well plate; 40 ng of cDNA was used as template and cycling parameters were 95 °C for 3 min followed by 45 cycles of 95 °C for 30 s, 60 °C for 1 min, 72 °C for 30 s, 60 °C+0.5 °C for 10 min. Specificity of each amplicon has been determined by melting curve method. Fluorescence intensities were analyzed using the manufacturer's software and relative amounts with respect to the housekeeping gene β-actin were obtained using the $2^{-\Delta\Delta Ct}$ method. Primer sequences: E-cadherin sense 5′-gattacgagggcagtggttct-3′, antisense 5′-catgtccgccagcttcttga-3′ (GenBank accession no.NM_009864.2, from 2633 to 2758); MMP9 sense 5′-cccagatgatgggagagaagc-3′, antisense 5′-cacagcgtggtgttcgaatg-3′ (GenBank accession no. NM_013599.2, from 182 to 307); HNF4α1 sense 5′-cggcatggatatggccg-3′, antisense 5′-gggacgtgtcattgccca-3′ (GenBank accession no.NM_008261.2, from 141 to 241); Snail-1 sense 5′-gtctgcacgacctgtggaaag-3′, antisense 5′-gttggagcggtcagcaaaag-3′ (GenBank accession no.NM_011427.2, from 604 to 729); fibronectin sense 5′-tgaagtcgcaaggaaacaagc-3′, antisense 5′-tgaacgggaggacacaggg-3′ (GenBank accession no.NM_010233.1, from 1157 to 1282); β-actin sense 5′-accacaccttctacaatgag-3′, antisense 5′-aggtctcaaacatgatctgg-3′ (GenBank accession no. NM_0073932, from 339 to 458).

Results

FAK is activated during the TGFβ-induced EMT

In the attempt to clarify the role of FAK signaling in TGFβ-mediated EMT of hepatocytes, we first investigated the kinetics of activation of this kinase in response to TGFβ.

The FAK phosphorylation levels on Tyr-861 (in the C-terminal domain), Tyr-577 (in the activation loop of the catalytic domain) and Tyr-925 (in the Grb2 SH2-binding site) were analyzed by using anti-phospho-specific antibodies. In fact, while FAK has been described to have multiple tyrosine phosphorylation sites, Tyr-861, Tyr-577 and Tyr-925 have been previously correlated to EMT, migration and tumorigenesis [21,26]. As shown in Fig. 1A, we found that 15 min of TGFβ treatment was sufficient to increase FAK basal phosphorylation at Tyr-861 and Tyr-925 residues. The activation persisted for 24 h, when the cells underwent a complete EMT (Fig. 2A; [22]). On the contrary the level of Tyr-577 phosphorylation did not significantly change in comparison to that of untreated cells (Fig. 1A). Furthermore, immunostaining analyses showed that FAK translocated to newly formed TGFβ-induced focal adhesions, co-localizing with the focal adhesion marker Paxillin (Fig. 1B).

Since phosphorylation of both Tyr-861 and Tyr-925 residues has been described to be mediated by the Src family kinases [27], we treated MMH cells with the Src inhibitor PP2 demonstrating that the TGFβ-mediated FAK activation required Src activity (Fig. 1C).

These results demonstrate that (i) FAK is activated by TGFβ in an Src-dependent manner and (ii) FAK localizes in TGFβ-induced focal adhesions in hepatocytes undergoing EMT.

Interfering with FAK signaling does not block TGFβ-induced phenotypic transition and E-cadherin transcriptional down-regulation

In order to determine the role of FAK signaling in TGFβ-induced EMT, we stably expressed in MMHs the dominant negative inhibitor of FAK function named FRNK ([28], MMH-FRNK). As a control, we used both parental cells and MMH over-expressing a mutated FRNK protein FRNK-S1034 that is not able to localize to the focal adhesions and therefore does not function as a dominant negative (MMH-S1034). Several clones were obtained and analysis was carried out on MMH cells, two MMH-FRNK and one MMH-S1034 clones.

Fig. 2 – FAK signaling is not required for TGFβ-induced phenotypic transition and E-cadherin transcriptional down-regulation. (A) TGFβ induces a phenotypic transition in MMH-FRNK, MMH-S1034 and parental cells. Phase-contrast micrographs of MMH cells, MMH-FRNK and MMH-S1034 treated or not with TGFβ for 24 h. Two MMH-FRNK clones (cl.1 and 11) that expressed comparable levels of FRNK, and one MMH-S1034 clone as control, were analyzed (calibration bar is 20 μm and applies to all images). (B) Interfering with FAK signaling does not influence Snail and E-cadherin transcriptional regulation. Analyses by qRT-PCR of Snail and E-cadherin expression in MMH-S1034 and in MMH-FRNK cells treated or not with TGFβ for 24 h. Data are the mean ± SEM for experiments in triplicate. (C) TGFβ induces a phenotypic transition in PP2-treated MMH cells. Phase-contrast micrographs of MMH cells treated with the Src inhibitor PP2 ± TGFβ for 24 h (calibration bar is 20 μm and applies to all images). (D) Interfering with Src signaling does not influence Snail and E-cadherin transcriptional regulation. qRT-PCR performed as in (B) on MMH (–) and PP2 treated MMH cells (PP2) after 24 h of TGFβ treatment (TGFβ and PP2 TGFβ, respectively). Data are the mean ± SEM for experiments in triplicate.

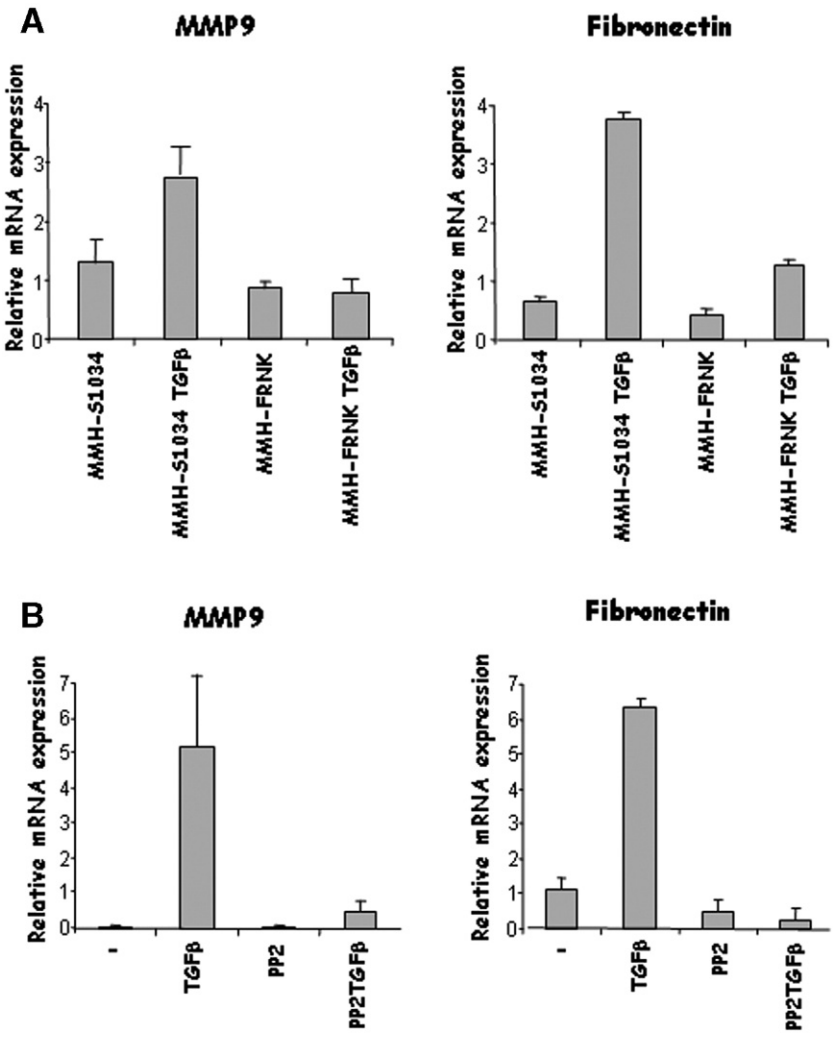
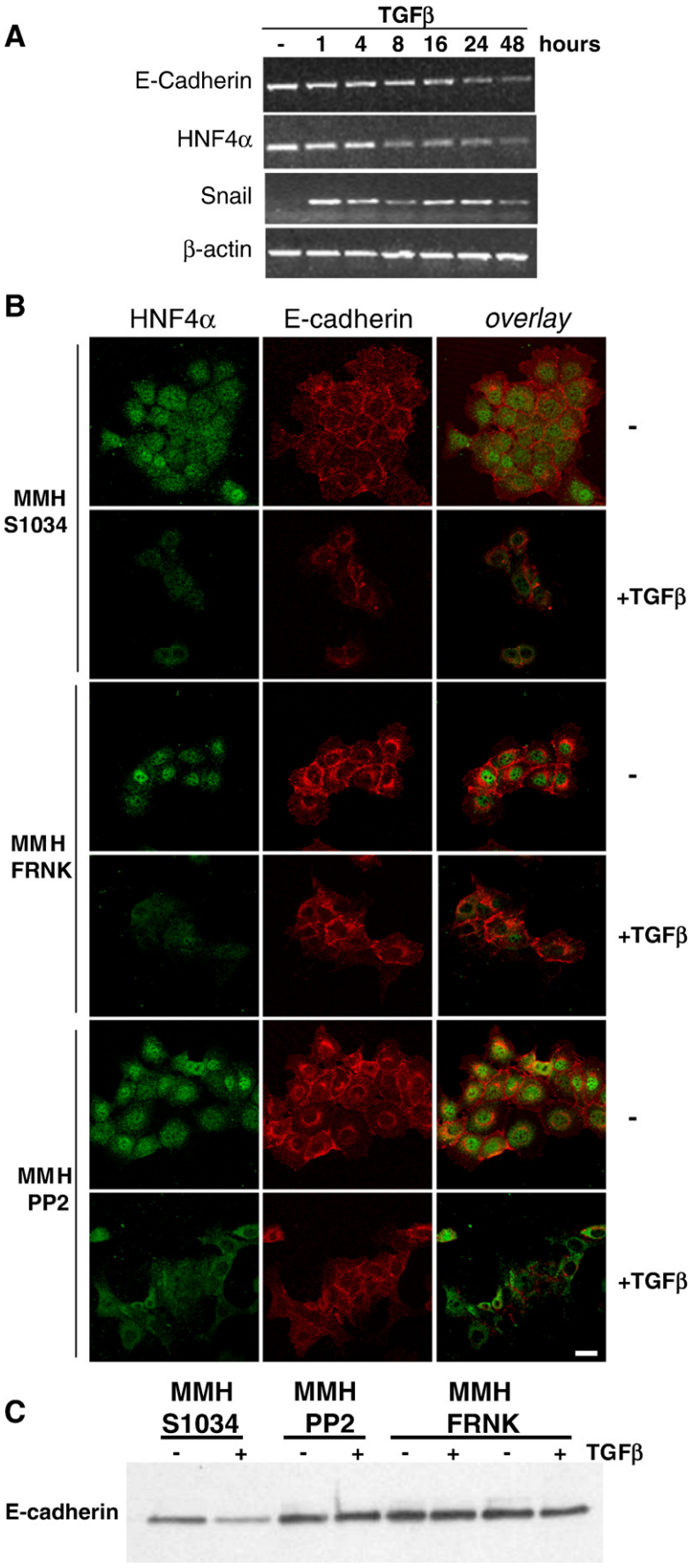


Fig. 3 – TGFβ-induced up-regulation of EMT mesenchymal and invasiveness markers requires FAK signaling. Analysis by qRT-PCR, performed as in Fig. 2B, of MMP9 and Fibronectin gene expression in MMH-FRNK (A) and in PP2-treated MMH (B, PP2), compared to MMH-S1034 and untreated MMH cells (–), after 24 h of TGFβ treatment (MMH-FRNK TGFβ and PP2 TGFβ, respectively). Data are the mean ± SEM for experiments in triplicate.

First, we analyzed whether FRNK interfered with TGFβ-induced EMT. As shown in Fig. 2A, MMH cells and MMH-S1034 cells acquired a fibroblastic phenotype after 24 h of TGFβ treatment. After the same time of TGFβ treatment MMH-FRNK cells, while appeared less dispersed than MMH cells and MMH-S1034, still showed a elongated phenotype. The latter observation indicated that TGFβ was able to induce a phenotypic transition resembling an EMT also in hepatocytes with impaired FAK function. Furthermore, quantitative qRT-PCR analyses showed that in MMH-FRNK cells TGFβ was able to transcriptionally up-regulate Snail; this, in turn, caused a clear drop in the transcript levels of the Snail major target gene E-cadherin (Fig. 2B). When MMH hepatocytes were treated only with Src inhibitor PP2 cell–cell contacts appeared tighter, as previously reported for other cell systems (Fig. 2C, [16]). Notably, cells treated with both TGFβ and PP2 became elongated (Fig. 2C), up-regulated Snail mRNA and, conversely, down-regulated E-cadherin mRNA (Fig. 2D).

Fig. 4 – TGFβ-induced delocalization from membrane and degradation of E-cadherin during EMT require FAK signaling. (A) TGFβ-induced modulation of EMT markers expression. Semiquantitative RT-PCR analyses were performed on mRNAs from untreated (–) or TGFβ-treated MMH cells for the indicated time. β-Actin amplification was used for template normalization. (B) E-cadherin and HNF4α immunofluorescence analyses in TGFβ-treated MMH-S1034, MMH-FRNK and MMH-PP2 cells. MMH-S1034, MMH-FRNK and PP2-treated MMH (MMH-PP2) cells were grown in the absence or presence of TGFβ (for 12 h), fixed and co-stained for E-cadherin and HNF4α. Fluorescence imaging was performed as in Fig. 1B (calibration bar in the last panel is 20 μm and applies to all images). (C) Western blot analyses of E-cadherin expression in MMH-S1034, MMH-FRNK and PP2-treated MMH cells with or without TGFβ. MMH-S1034 and MMH-FRNK cells were grown in the absence or presence of TGFβ for 12 h. PP2-treated MMH cells (MMH PP2) were treated for 12 h with PP2 with or without TGFβ.


A
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Snail
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B
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MMH PP2
-
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C
MMH S1034
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MMH FRNK
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E-cadherin

These results suggest that Src/FAK signaling is not required during TGFβ-induced EMT of the hepatocytes (i) to regulate the expression of the EMT master gene Snail and (ii) to modulate the expression of the Snail target gene E-cadherin.

FAK signaling is required for TGFβ-induced up-regulation of mesenchymal markers during MMH cells EMT

We next asked whether Src-FAK signaling could play a role in TGFβ-induced up-regulation of mesenchymal and invasiveness markers. We analyzed the modulation of expression of the mesenchymal marker fibronectin and of MMP-9 in MMH-FRNK and MMH-S1034 cell clones, treated or not with TGFβ. MMP-9 and fibronectin are both defined as hallmarks of EMT and are modulated by TGFβ, but not by Snail, in MMH cells [22]. qRT-PCR analysis showed that, in the presence of TGFβ, the expression of fibronectin and MMP9 was not significantly up-regulated in MMH-FRNK clones compared to MMH-S1034 and MMH cells (Fig. 3A). Moreover, Src inhibitor PP2 abolished TGFβ-induced up-regulation of MMP-9 and fibronectin expression in MMH cells (Fig. 3B). This evidence suggests a significant role for mesenchymal phenotype acquisition in hepatocytes through Src/FAK signaling.

FAK signaling is required in hepatocytes for TGFβ-induced delocalization and degradation of E-cadherin

The observation that Src signaling activation may trigger internalization and degradation of E-cadherin [29] leads us to hypothesize that Src/FAK could also have a similar role in TGFβ-mediated EMT. To address this point we compared membrane-bound E-cadherin in MMH-FRNK, in MMH-S1034 and in PP2-treated MMH cells undergoing an EMT. In order to rule out the possibility that the decrease of membrane-bound E-cadherin could be the result of a transcriptional down-regulation, the analyses were carried out at 12 h. In fact, in our experimental cell system, a time course of TGFβ treatment showed that E-cadherin transcriptional repression was detectable only after 24 h, while the onset of Snail expression was observed as early as 1 h and HNF4α started to decline after 4 h (Fig. 4A).

As shown in Fig. 4B, 12 h of TGFβ treatment caused the expected morphological change as well as nuclear HNF4α down-regulation in all the three cell lines, thus providing evidence for a TGFβ-induced transition. Notably, in the meantime the E-cadherin staining continued to decorate the cellular membrane both in MMH-FRNK and in PP2-treated MMHs (Fig. 4B). Furthermore, Western blot analyses showed that, after 12 h of TGFβ treatment, E-cadherin levels were decreased in MMH-S1034 cells but were unmodified in MMH-FRNK and PP2-treated cells (Fig. 4C). These data indicated that in hepatocytes Src/FAK signaling is required during TGFβ-induced EMT for E-cadherin membrane delocalization and degradation.

Discussion

Our main finding is that TGFβ-induced EMT requires an Src-mediated FAK activation for (i) transcriptional up-regulation of mesenchymal and invasiveness markers (i.e. MMP9 and fibronectin) and (ii) delocalization of membrane-bound E-cadherin. To our knowledge, our results provide the first evidence of a specific FAK functional role in TGFβ-mediated EMT of the hepatocyte. Here, we show that after TGFβ treatment FAK (i) is activated by Src-dependent phosphorylation on specific 861 and 925 tyrosines and (ii) is localized to focal adhesions of hepatocytes undergoing TGFβ-induced EMT. The impairment of Src/FAK function does not affect TGFβ-mediated Snail activation and E-cadherin transcriptional down-regulation but, notably, it impedes the transcriptional up-regulation of mesenchymal and invasiveness markers. Our results imply that Src/FAK signaling is necessary for the full accomplishment of the TGFβ-induced EMT in hepatocytes.

It is well established that Src signaling induces a switch in adhesion type predominance from cadherin- to integrin-based adhesions, promoting a more motile phenotype. In fact, the Src signaling pathway is an important component of adhesion changes associated with the EMT in cancer [30]. Since c-Src is activated by TGFβ in various cell types [31,32] it has been hypothesized that Src pathway is likely a driving force also in TGFβ-mediated EMT, however, previous investigations yielded contradictory results. While Galliher and Schiemannn found that Src-mediated TGFβ receptor type II phosphorylation enhances the EMT [15], Maeda and colleagues concluded that Src activation is not necessary for this process [16]. These discrepancies may be ascribed to different EMT readout used by the authors.

Concerning FAK, its Src-dependent activation has been proposed to cause negative regulation of E-cadherin mediated cell–cell contacts [33], but its role in regulating intercellular adhesion appears more complex. In fact it has been shown that suppression of FAK specific signaling determines a phenotype similar to that of cells over-expressing constitutively activated Src [34]. This discrepancy may be based on differential phosphorylation of FAK, as proposed by Nakamura and co-workers [21] that speculated that different modes and qualities of FAK tyrosine phosphorylation are likely to evoke different downstream signaling cascades. Interestingly, specific FAK phosphorylation on Y925 facilitates the angiogenic switch in tumor progression [26]. Finally, the diversity of responses to Src/FAK- or FAK-induced effects on cell–cell adhesion has been also ascribed to different cell types [30].

We also observe that E-cadherin transcriptional down-regulation is a late event of TGFβ-mediated EMT of hepatocytes, while in early phases the Src/FAK signaling mediates the membrane-bound E-cadherin delocalization and degradation (Figs. 4B and C). This is in accord with a recent report [35] that highlights the relevance of both transcriptional and post-translational TGFβ-dependent mechanisms of E-cadherin regulation during tumor progression. Future experiments are required to clarify whether FAK is also involved in the reported Src-mediated E-cadherin delivery to lysosomes [29] and/or ubiquitination/degradation [36].

Our results provide new insights into the molecular mechanisms underlying TGFβ-induced EMT, unraveling a key role of specific FAK activation in controlling both acquisition of mesenchymal and invasiveness markers as well as destabilization of cell–cell contacts by E-cadherin delocalization.

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