

# Regulation of tensin-promoted cell migration by its focal adhesion binding and Src homology domain 2

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Tensin1 is an actin- and phosphotyrosine-binding protein that localizes to focal adhesions. Recently, we have shown that both tensin1 and a new family member, tensin2, promote cell migration [Chen, Duncan, Bozorgchami and Lo (2002) *Proc. Natl. Acad. Sci. U.S.A.* 99, 733–738]. Since localization of proteins to particular intracellular compartments often regulates their functions, and Src homology domain 2 may mediate signals related to cell migration, we hypothesize that tensin-mediated cell migration is regulated by the focal adhesion localization and the Src homology domain 2 of tensin. To test this hypothesis, we have analysed the effects of a series of tensin1 mutants on cell migration. Our results have shown that (1) tensin1 contains two

focal adhesion-binding sites, (2) the wild-type tensin1 significantly promotes cell migration, (3) mutants with one focal adhesion-binding site do not promote cell migration, (4) the non-focal adhesion localized mutant suppresses cell migration and (5) the mutant that is not able to bind to phosphotyrosine-containing proteins has no effect on cell migration. These results have indicated that focal adhesion localization of tensin1 and the phosphotyrosine-binding activity are two critical factors in regulating tensin-mediated cell migration.

**Key words:** actin binding, NPXY sequence, phosphotyrosine binding domain, wound healing.

## INTRODUCTION

Cell migration is a fundamental aspect in numerous normal and pathological processes, including embryonic development, wound healing, inflammation and metastasis of tumour cells [1,2]. Various factors such as growth factors and extracellular matrix (ECM) proteins in the cellular microenvironment participate in the regulation of cell migration. ECM proteins interact with cells via integrin transmembrane proteins [3]. The cytoplasmic tails of integrins bind to cytoskeletal proteins providing a physical link between the actin cytoskeleton and the ECM [4,5]. Ligation of integrins by the ECM initiates a cascade of intracellular signalling events involving the activation of tyrosine kinases, the phosphorylation of cytoskeletal substrates and the assembly of focal adhesions. These biochemical modifications eventually bring about diverse biological responses, including cell migration [6–9]. Studies have demonstrated the involvement of focal adhesion molecules in cell migration. For example, overexpression of Fak or Cas promotes cell migration [10,11], whereas expression of vinculin or Ena/VASP (vasodilator-stimulated phosphoprotein) suppresses motility [12,13]. These findings indicate that various mechanisms are involved in regulation of cell migration at focal adhesion sites.

Tensin1 is an actin-binding protein localized to focal adhesions [14]. The N-terminal of tensin1 binds to F-actin [14], whereas the centre region retards the G-actin polymerization [14]. The C-terminus contains the Src homology domain 2 (SH2 domain) [15] and the phosphotyrosine-binding (PTB) domain. In addition, tensin1 is a substrate of protein tyrosine kinases. The tyrosine phosphorylation of tensin1 is enhanced by ECM, growth factors or oncogenes [16–18]. These findings suggest that tensin1 plays important roles in organizing the actin cytoskeleton and mediating signal transduction [14]. Furthermore, analysis of tensin1 knockout mice has demonstrated a critical role of tensin1 in renal function and wound healing [19,20]. To examine the role of

tensin in cell migration, we have analysed previously cells isolated from tensin1 knockout mice and cells expressing recombinant tensin. The results showed that tensin1-null cells migrated significantly slower than their normal counterparts, and ectopic expression of tensin1 had an opposite effect [21]. Furthermore, we have identified a new tensin family member, tensin2, which contains 1410-amino-acid residues and shares extensive homology with tensin1 at its N- and C-termini, whereas the middle regions are divergent. Ectopic expression of tensin2 also enhanced cell migration, suggesting that tensin represents a gene family that positively regulates cell motility [21].

In the present study, we have examined the mechanism of tensin-promoted cell migration. Since targeting of signalling protein complexes to focal adhesions is critical for the control of various cellular functions, we began by mapping the focal adhesion-binding (FAB) sites on tensin1 using deletion mutagenesis and transfection analysis. We then generated mutants that specifically inactivated the FAB sites without disrupting the binding to actin and phosphotyrosine (pTyr)-containing proteins. These mutants were introduced into cells to define the structural requirements for subcellular localization of the protein and to determine how this affects cell migration. Our results have demonstrated that the localization of tensin1 to the focal adhesion site is a critical step in the regulatory process of cell migration. In addition, since SH2 domains may mediate signals related to cell migration, we have inactivated the SH2 domain and shown that this mutant abolished tensin-promoted cell migration, indicating that a functional SH2 domain is also required for tensin-mediated cell migration.

## MATERIALS AND METHODS

### DNA constructs and transfection

To construct chicken tensin1 expression vectors, cDNA fragments were generated using convenient restriction enzyme sites

Abbreviations used: ECM, extracellular matrix; FAB, focal-adhesion binding; GFP, green fluorescent protein; GST, glutathione S-transferase; PTB, phosphotyrosine binding; pTyr, phosphotyrosine; SH2 domain, Src homology domain 2.

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or PCR amplification, and introduced into the mammalian expression vector, pEGFP (ClonTech, Palo Alto, CA, U.S.A.), for transfection assays, or the bacterial expression vector, pGEX, for expressing recombinant proteins. The pEGFP constructs were transfected into indicated cell lines using SuperFect reagents (Qiagen, Chatsworth, CA, U.S.A.) according to the manufacturer's instructions. Stable transfectants were generated using G418 selection (600  $\mu\text{g}/\text{ml}$ ) for 10 days, and the surviving colonies were picked using filters pre-wetted with trypsin, and subsequently transferred to 24-well plates. Positive clones were verified by green fluorescent protein (GFP) expression and Western-blot analysis. NIH 3T3 and HEK-293 cells were purchased from A.T.C.C. and human renal epithelial cells from Clonetics (San Diego, CA, U.S.A.).

#### Actin co-sedimentation assay

Rabbit skeletal-muscle actin was purchased from Cytoskeleton Inc. (Denver, CO, U.S.A.). Protein aggregates were removed from recombinant proteins and G-actin by centrifugation at 150000  $g$  for 30 min in a Beckman TL-100 tabletop ultracentrifuge (Beckman, Fullerton, CA, U.S.A.). Recombinant proteins were then mixed with G-actin in polymerization buffer [5 mM Tris/HCl (pH 8)/50 mM KCl/0.2 mM  $\text{CaCl}_2$ /2 mM  $\text{MgCl}_2$ /1 mM ATP], incubated for 1 h at room temperature (25  $^\circ\text{C}$ ), and ultracentrifuged at 150000  $g$  for 30 min to pellet the actin filaments and their associated proteins. The samples were analysed by SDS/PAGE followed by Coomassie Blue staining.

#### pTyr-protein binding assay

Cell cultures were treated with lysis buffer [50 mM Tris (pH 7.5)/150 mM NaCl/1 mM EDTA/1% Nonidet P40], including protease inhibitors (1 mM PMSF, 10  $\mu\text{g}/\text{ml}$  leupeptin and 10  $\mu\text{g}/\text{ml}$  pepstatin), and centrifuged at 13000 rev./min in a microfuge for 15 min at 4  $^\circ\text{C}$ . Cell lysates prepared from Src-transformed rat fibroblasts were incubated with indicated glutathione S-transferase (GST) fusion proteins for 1 h at 4  $^\circ\text{C}$ . Glutathione-agarose beads (30  $\mu\text{l}$ ) were added to the sample and incubated for 1 h. The associated proteins captured on the beads were washed three times with the lysis buffer, dissociated in SDS/PAGE sample buffer and analysed by immunoblotting with anti-pTyr antibodies (PY100).

#### Boyden chamber assay

Cell migration assays were performed using modified Boyden chambers. The lower surface of the membrane was coated with 10  $\mu\text{g}/\text{ml}$  fibronectin for 2 h at 37  $^\circ\text{C}$ , and the lower chamber was filled with 0.6 ml Dulbecco's modified Eagle's medium with 10% (v/v) foetal bovine serum. Cells were harvested with trypsin/EDTA, washed once with serum-free Dulbecco's modified Eagle's medium, containing 20  $\mu\text{g}/\text{ml}$  trypsin inhibitor, and resuspended to  $1 \times 10^6$  cells/ml. The suspension (100  $\mu\text{l}$ ) was added to the upper chamber and the cells were allowed to migrate at 37  $^\circ\text{C}$  (5%  $\text{CO}_2$ ) for 15 h. The upper surface of the membrane was wiped with a cotton tip to remove non-migratory cells mechanically, whereas migratory cells attached to the lower surface were fixed at room temperature for 30 min in 10% formalin, and stained for 20 min (1% crystal violet and 2% ethanol in 100 mM borate buffer, pH 9.0). The stained cells were photographed with a CCD camera attached to the inverted phase-contrast microscope. Four fields per chamber were photographed and cell numbers were counted. Cell migration rates were determined by the total cell numbers per field. The background migration rate

was evaluated on BSA-coated membranes and subtracted from all data, and each cell line in duplicate wells was analysed in three independent experiments. Data are presented as means  $\pm$  S.D. Mann-Whitney  $U$  test was used for statistical analysis.  $P < 0.01$  was considered to be statistically significant.

## RESULTS

### Tensin1 contains two FAB sites

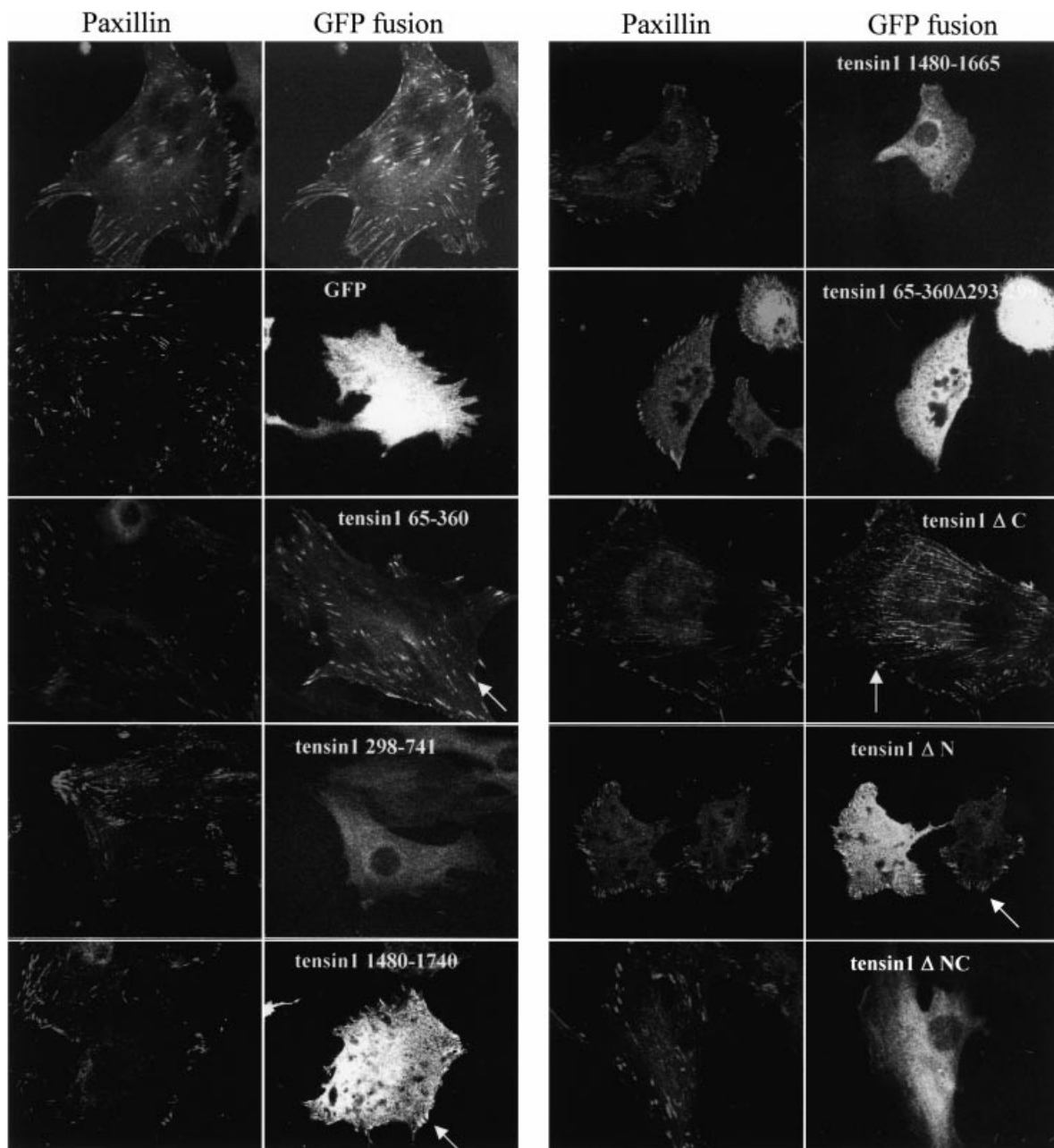
To explore the association between tensin and focal adhesions, and the role of tensin's focal adhesion localization in cell migration, we characterized the FAB sites in the protein. To track the localization of tensin1, we transfected the GFP-tensin1 construct into NIH 3T3 cells. As shown in Figure 1, tensin1 fused with GFP targeted normally to focal adhesions and was co-localized with other focal adhesion molecules such as paxillin (Figure 1) and vinculin (results not shown). In contrast, GFP alone was found in the cytoplasm.

Since tensin1 and tensin2 sequences are highly conserved at the N- and C-terminal portions, and both proteins are localized to focal adhesions, the FAB activity resided quite probably in these conserved regions. GFP-tensin1 1-741 and GFP-tensin1 741-1744 were generated to test this possibility. Focal adhesion targeting was observed with both chimaeras (results not shown), indicating that there are at least two independent FAB sites within tensin1.

To dissect FAB activities further, we engineered a series of GFP-fusion chimaeras and tested their localization in NIH 3T3 cells. A summary of GFP-fusion constructs is shown in Figure 2(A). Among the mutants generated to characterize the FAB site in the N-terminal half (FAB-N), we found that tensin1 residues 1-428, 65-428 (results not shown) and 65-360 (Figure 1) retained their FAB properties, whereas residues 298-741 (Figure 1), 1-292, 154-428 and 100-360 did not (results not shown). The shortest polypeptide containing the FAB-N localizes to residues 65-360.

Among the mutants generated to analyse the FAB site in the C-terminal half (FAB-C), those containing tensin1 residues 889-1744, 1239-1744, 1418-1744 (results not shown) and 1480-1740 (Figure 1) fusion proteins were detected at focal adhesions. In contrast, GFP fused to residues 889-1744 $\Delta$ 1418-1583, 1239-1593 (results not shown) and 1480-1663 (Figure 1) were found in the cytoplasm. These results indicate that the shortest fragment containing the FAB-C is from residues 1480 to 1740. It appeared that the FAB-N site of tensin1 targeted more efficiently to focal adhesions than the FAB-C site, since fragments containing the FAB-N site localized to both central and peripheral focal adhesions. In contrast, constructs with the FAB-C site showed an intense cytoplasmic distribution and smaller focal adhesions at the cell periphery. To examine whether expression of tensin1 mutants affects the structure of focal adhesions, cells were also labelled with paxillin (Figure 1) or vinculin (results not shown). We found that all transfectants contained paxillin-positive focal adhesion plaques, demonstrating that the absence of focal adhesion localization of tensin mutants could not be attributed to a lack of plaque formation. However, expression of some mutants appeared to affect complex formation. Cells transfected with the FAB-C site (such as residues 1480-1740) formed smaller and fewer adhesion plaques, whereas non-focal adhesion-localizing constructs (such as residues 298-741) and fragments with FAB-N site showed no effect on complex formation.

The expression of each GFP-fusion construct was analysed by protein immunoblotting using anti-GFP antibodies, confirming that the expressed sizes of the fusion proteins were as predicted (Figure 2B). These constructs were also transfected into HEK-293



**Figure 1** Localization of tensin1 GFP-fusion proteins in NIH 3T3 cells

Cells grown on coverslips were transiently transfected with the indicated GFP constructs. After 24 h, cells were fixed with methanol for 10 min at  $-20^{\circ}\text{C}$ . After labelling with anti-paxillin antibodies, cells were visualized with a Zeiss LSM 510 laser scanning microscope. GFP-tensin1 wild-type, 65–360, 1480–1740, tensin1 $\Delta\text{C}$  and tensin1 $\Delta\text{N}$  are localized to focal adhesions (arrows).

cells and the same localization results were observed (results not shown), indicating that the mechanism regulating focal adhesion localization of tensin1 is preserved in cells of different germinal origins.

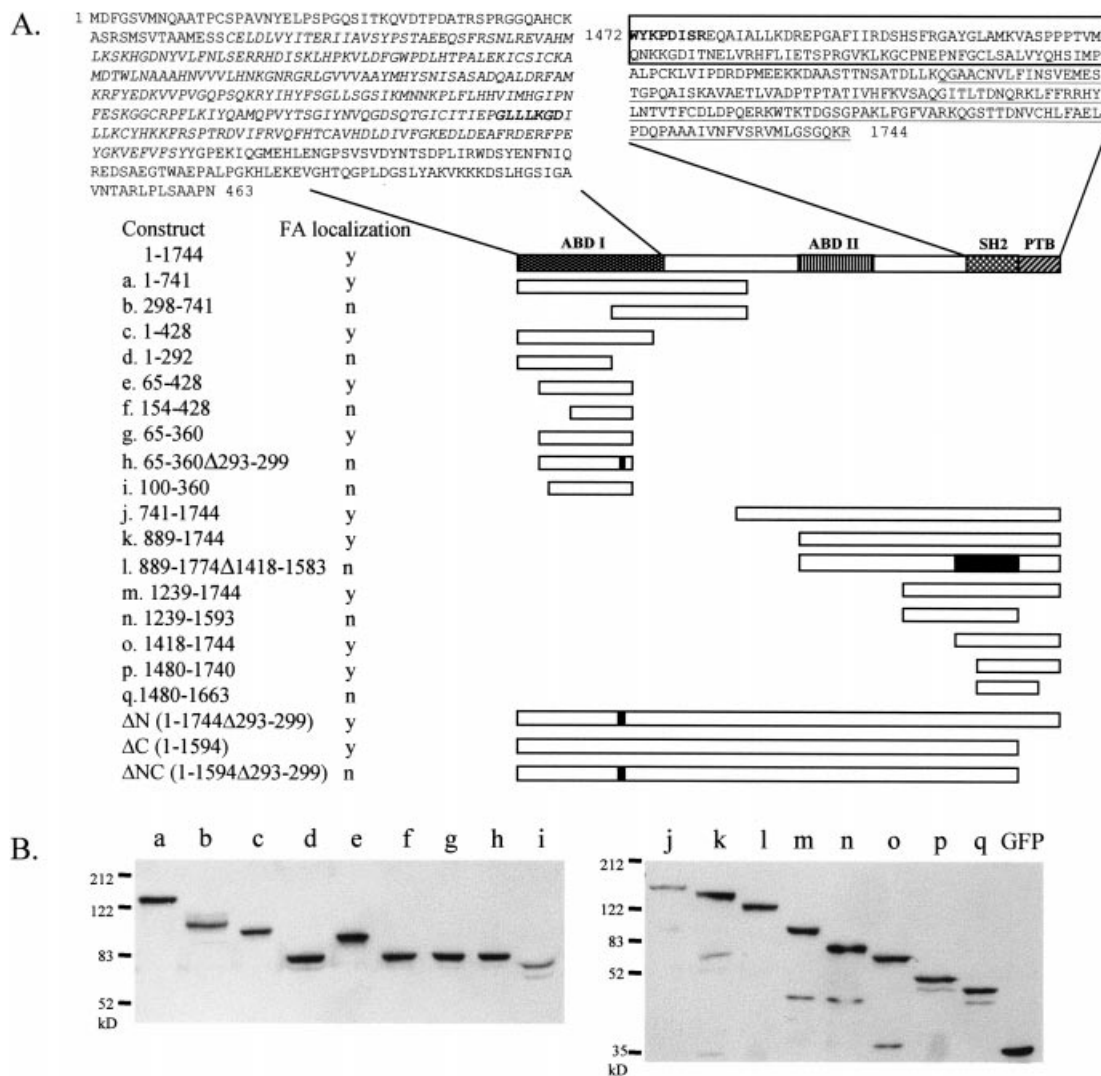
#### Deletion of amino acids 293–298 in FAB-N abolishes the focal adhesion targeting but not the actin-binding activity

Since the FAB-N overlaps within the actin-binding region (residues 1–463), we examined whether FAB-N targeting property could be separated from the actin-binding activity. The deletion of seven residues (293–299) in tensin1 65–360 resulted in

the loss of focal adhesion localization (Figure 1). We then expressed tensin1 65–360 and 65–360 $\Delta\text{293–299}$  as GST-fusion proteins for the actin-binding assay, and found both GST 65–360 and GST 65–360 $\Delta\text{293–299}$  co-sedimented with actin filaments (Figure 3). This finding demonstrates that the actin-binding activity does not require a functional FAB site.

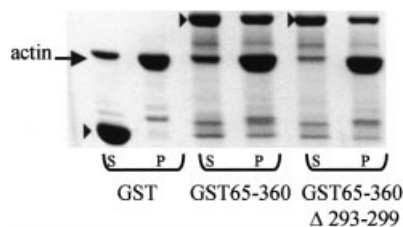
#### Molecular dissection of pTyr and FAB activities in the C-terminus of tensin1

Transfection analysis of GFP-tensin1 1417–1744 and GFP-tensin1 1480–1740 revealed that both fragments localized to



**Figure 2 Summary of the focal adhesion localization of tensin1 GFP-fusion proteins**

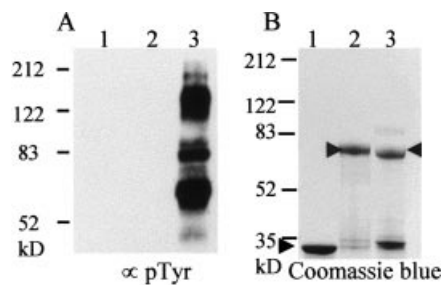
(A) Various tensin1 fragments were expressed as GFP-fusion proteins. Their relative positions within tensin1 are shown. The ability of the fusion proteins to localize to focal adhesions (FA) is summarized. y, yes; n, no. Solid bars indicate the deletion regions. Amino acid sequences of actin-binding domain (ABD) I, SH2 (box) and PTB (underline) domains are shown. Deleted residues are in bold. FAB-N (65–360) sequences are in italic. (B) Cell lysate prepared from each transfectant was immunoblotted with anti-GFP antibodies to examine GFP-fusion protein expressions in these cells. Lower bands detected in some samples were probably degradation products.



**Figure 3 Binding of tensin1 fragments to actin filaments**

GST or GST-fusion proteins (arrowheads) were used for actin co-sedimentation assay. After ultracentrifugation, proteins in supernatant (S) and pellet (P) were recovered and separated by SDS/PAGE, and detected by Coomassie Blue staining. The presence of GST-tensin1 65–360 and GST-tensin1 65–360 $\Delta$ 293–299, but not GST in the actin pellet fractions suggests that both GST-fusion proteins are capable of interacting with actin filaments. Arrow shows actin.

focal adhesions. Because the first construct contained the SH2 (residues 1472–1570) and PTB (residues 1601–1744) domains, whereas the second construct had eight residues of the SH2 domain (residues 1472–1479) deleted but the PTB remained intact, it was likely that the second construct had lost the pTyr-binding activity of the SH2 domain. To examine this possibility and to test whether PTB domain of tensin1 interacts with pTyr proteins, cell lysates prepared from Src-transformed rat (Src-Rat) fibroblasts were incubated with GST, GST-tensin1 1417–1744 or GST-tensin1 1417–1744 $\Delta$ 1472–1479. After extensive washes, pTyr proteins associated with GST or GST-fusion proteins were analysed by immunoblotting. Although GST-tensin1 1417–1744 interacted with several pTyr proteins, neither GST nor GST-tensin1 1417–1744 $\Delta$ 1472–1479 could bind these proteins under the same condition (Figure 4). These results indicate that the FAB-C targeting activity does not require a functional SH2 domain, and that the PTB domain of tensin1



**Figure 4** Deletion of eight residues (1472–1479) from the SH2 domain of tensin1 eliminates its pTyr-protein-binding activity

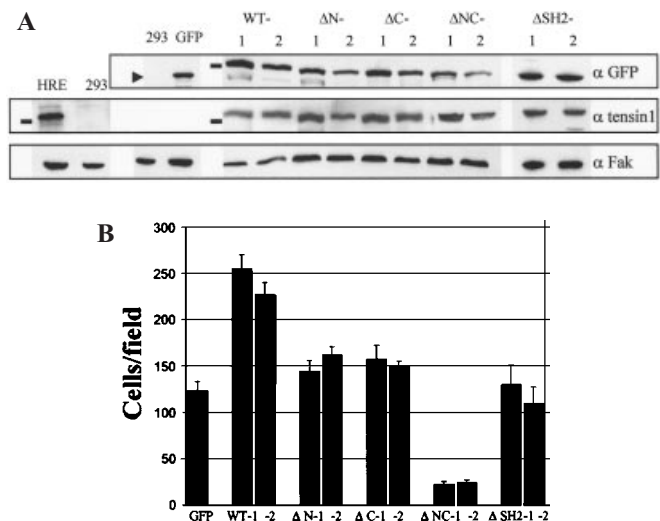
(A) Cell lysate from Src-rat fibroblasts was first incubated with GST (lane 1), GST-tensin1 1417–1744Δ1472–1479 (lane 2) or GST-tensin1 1417–1744 (lane 3) fusion proteins; then glutathione-agarose beads were added to the samples. The associated proteins captured on the beads were analysed by protein immunoblotting with anti-pTyr antibodies. Note that only GST-tensin1 1417–1744 is able to bind to pTyr proteins. (B) Loading of the GST and GST-fusion proteins (arrowheads) in (A) was assessed by Coomassie Blue staining. Lower bands detected in lane 3 were degradation products.

does not interact with pTyr proteins found in Src-transformed cells but is required for FAB-C targeting. However, the PTB domain alone is not sufficient, because GFP-tensin1 889–1744Δ1418–1583, which contains the PTB domain, cannot target to focal adhesions (results not shown; Figure 2).

#### Both FAB sites are involved in regulation of tensin1-mediated cell migration

To examine whether tensin1 subcellular localization plays a role in cell migration, we generated GFP chimaeras fused with tensin1 mutants which specifically inactivated FAB-N (ΔN, deletion of residues 293–299), FAB-C (ΔC, deletion of residues 1594–1744) or both FAB sites (ΔNC). As shown in Figure 1, both GFP-tensin1ΔN and GFP-tensin1ΔC were detected at focal adhesions, whereas GFP-tensin1ΔNC was diffusely distributed in the cytoplasm. These results further confirm that there are two independent FAB sites in tensin1.

We used these constructs to establish HEK-293 cell lines stably expressing GFP, GFP-tensin1, GFP-tensin1ΔN, GFP-tensin1ΔC or GFP-tensin1ΔNC. We chose HEK-293 cells because tensin1-null mice developed significant kidney defects, and because no detectable tensin1 expression was found in these cells, in contrast with normal kidney cells (Figure 5A). In addition, HEK-293 cells migrate much more slowly than NIH 3T3 cells, making it easier to detect subtle effects of mutant expression on promoting cell migration. Stable transfectants, which expressed levels of tensin1 mutant protein similar to those of endogenous protein in normal kidney cells, were selected (Figure 5A) and were analysed for cell migration using the Boyden chamber assay (Figure 5B). Our results showed that expression of wild-type tensin1 significantly promoted cell migration on fibronectin, both the GFP-tensin1ΔN and GFP-tensin1ΔC transfectants migrated at a similar rate to the HEK-293 GFP control cells, and the GFP-tensin1ΔNC cells migrated significantly slower than control cells. These results demonstrate that (1) re-expression of tensin1 alone is capable of promoting cell migration, (2) localization of tensin1 to focal adhesions by a single FAB site has very weak, if any, effect in promoting cell migration in HEK-293 cells and (3) non-focal adhesion-localized tensin1 mutant (GFP-tensin1ΔNC) suppresses cell motility.



**Figure 5** Expression of GFP-tensin1 promoted cell migration

(A) Total cell lysates (30 μg) prepared from normal human renal epithelial cells (HRE) or HEK-293 cells were immunoblotted with anti-tensin1 antibodies to examine its expression levels in these cells. Note the lack of tensin1 expression in HEK-293 cells. Cell lysates (30 μg) isolated from HEK-293 cells, GFP control cells, GFP-tensin1 WT, GFP-tensin1ΔN, GFP-tensin1ΔC, GFP-tensin1ΔNC and GFP-tensin1ΔSH2 transfectants (two stable clones from each GFP-tensin1 chimaera) were immunoblotted with anti-GFP (upper panel) or anti-tensin1 (middle) antibodies to show the expression levels of recombinant proteins. Smaller bands detected in some tensin1 transfectants are degradation products. The blots in the upper panel were stripped and probed with anti-Fak antibodies to show the equal loading (bottom panel). Bars show 200 kDa markers. Arrow indicates a 30 kDa marker. (B) Migration of these stable clones was analysed using the Boyden chamber. GFP-tensin1 WT cells migrated significantly faster than the GFP-expressing cells, whereas GFP-tensin1ΔNC moved much slower than the control cells. GFP-tensin1ΔN, GFP-tensin1ΔC and GFP-tensin1ΔSH2 mutants had no effect on cell migration. Error bars are S.D.  $P < 0.01$  was considered to be statistically significant.

#### The SH2 domain is critical for tensin1-mediated cell migration

Since the SH2 domain is important in transducing signals by binding to pTyr proteins, it is probable that the SH2 domain of tensin1 mediates signals in regulating cell migration. To test this, we have deleted residues 1472–1479 (tensin1ΔSH2), which abolished the pTyr-binding activity as shown earlier, and transfected into HEK-293 cells for migration assays. As shown in Figure 5(B), HEK 293-cells stably expressing GFP-tensin1ΔSH2 migrated significantly slower than GFP-tensin1 cells but similar to GFP control cells, indicating that the pTyr binding of the SH2 domain is critical for tensin1-promoted cell migration.

#### DISCUSSION

In the present study, we have investigated the role of focal adhesion localization of tensin1 and the role of the SH2 domain of tensin1 in cell migration. In addition to its characterized actin-binding and FAB function, the FAB-N sequence shares homology with auxilin, cyclin G-associated kinase and PTEN (phosphatase and tensin homologue deleted from chromosome 10)/MMAC 1 (mutated in multiple advanced cancers). However, the significance of this similarity is not clear, since none of these molecules binds to F-actin, and only cyclin G-associated kinase has been found at focal adhesions [22]. Our analysis of various tensin1 mutants reveals that the actin-binding activity and focal adhesion localization could be independent. A similar scenario exists at the C-terminal 326 residues, where we have determined

that the interaction of tensin1 with focal adhesions via FAB-C does not require the presence of a functional SH2 domain. In contrast, the PTB domain within the FAB-C is necessary but not sufficient for the focal adhesion localization. We speculate that a competition between pTyr-protein interactions and focal adhesion localization may occur at the C-terminal region of tensin1, and a similar competition may exist between actin filaments and focal adhesions at the N-terminus. The dynamic nature of tensin1 phosphorylation may also affect the interactions with their associated proteins, providing a potential regulatory mechanism by competitive binding. It will require the identification of molecules binding to FAB-N and FAB-C to address this possibility.

PTB domains are initially identified as domains which bind in a pTyr-dependent fashion to peptides that form a  $\beta$  turn. In contrast with SH2 domains, the PTB domain-binding specificity is determined by residues N-terminal to the pTyr [23]. For example, the peptide ligand for the Shc PTB domain has been found to be the sequence NPXpY [23]. In a previous study [24], the PTB domains have also been found to participate in pTyr-independent interactions. They bind to NPXY without being tyrosine-phosphorylated. It is surprising that the NPXY sequence is a general internalization signal for proteins [25] and many integrin  $\beta$  subunits contain this motif. Their similarities to the PTB domain-binding sequence may not be coincidental. Although the NPXY sequence is probably not involved in the integrin internalization [26], it has been demonstrated that the NPXY motifs are required for integrin activation and binding to the PTB-like domain in talin in a pTyr-independent fashion [27]. It is possible that the NPXY sequence might also serve as a binding site for the PTB domain in tensin1. Our studies showed that the PTB domain in tensin1 did not interact with pTyr proteins. However, it was critical for FAB-C targeting activity. Whether this PTB domain interacts with integrin  $\beta$  tail remains to be investigated.

The presence of two apparent FAB sites in a molecule is not surprising, since talin, vinculin and vinxin also contain at least two independent targeting sites [28–30]. We hypothesize that the following advantages may exist for having two functional FAB sites versus a single one: (1) two functional independent FAB sites may allow the recruitment of tensin1 to focal adhesions by two different molecules, or two separated sites in a molecule, which may potentially be activated/regulated by distinct signals; (2) tensin1 can anchor to focal adhesions with only one FAB site, allowing the other to interact with other focal adhesion constituents or regulatory molecules such as short actin filaments or pTyr proteins; (3) simultaneous interactions of both FAB sites with their targets may increase the binding affinity of tensin1 to focal adhesion sites; (4) by interaction of focal adhesions with one or both FAB sites, tensin1 may regulate its function in a sequential way. Indeed, our results have indicated a role for multiple FAB sites in the regulation of focal adhesion structure and cell migration. It appears that FAB-N is responsible for localizing tensin1 to classical focal adhesions without altering their structure, whereas FAB-C plays a role in focal adhesion formation through its ability to redirect paxillin and vinculin to smaller focal adhesion plaques. However, these contrasting characteristics do not seem to affect the migration phenotypes in our assays, since both GFP-tensin1 $\Delta$ N and GFP-tensin1 $\Delta$ C mutants migrate at a similar rate, and GFP-tensin1 $\Delta$ NC transfectants form classical focal adhesions but their migration rate is the slowest. Our results do reveal a correlation between cell motility and the availability of FAB sites, indicating that the subcellular localization of tensin1 is a way of regulating cell motility, presumably by shuttling between the cytoplasm and the

focal adhesions. In fact, many focal adhesion molecules, including tensin1, vinculin, talin and Fak are present in at least two pools in the cells (Triton X-100-soluble and -insoluble) [31–34].

Based on our findings, we propose a working model to describe the role of tensin1 in cell migration. Tensin1 is present in at least two different pools in a cell, either in the cytoplasm or the focal adhesion complex. Cytoplasmic tensin1 can suppress cell migration by (presumably) sequestering/inactivating downstream regulators, whereas tensin1 is able to promote cell motility when it is associated with focal adhesions via both FAB sites. However, focal adhesion localization of tensin1 is not sufficient for promoting motility. It also requires a functional SH2 domain that is able to interact with tyrosine-phosphorylated regulators. Three factors are involved in tensin1-mediated cell migration: subcellular localization, binding affinity to focal adhesion constituents and downstream regulators of cell motility. Nonetheless, how do cells maintain two pools of endogenous tensin1 *in vivo*? What molecules interact with FAB sites and the SH2 domain? Are there regulators that associate with tensin1 other than FAB and SH2 domains? These are several aspects of this model that remain to be tested.

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