Transglutaminase-2 in cell adhesion
All roads lead to paxillin?

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Role of paxillin in cell adhesion

Paxillin, a 68 kDa conserved cytoskeletal adhesome protein, is one of the earliest proteins to be detected in the nascent adhesions and is therefore clearly important in the assembly and disassembly of focal adhesions in eukaryotic cells. As a multi-domain adaptor protein crucial in the coordinated recruitment of a network of other adhesome proteins, paxillin is vital for cell-extracellular matrix adhesion, signal transduction, as well as cell migration. These biological processes are regulated by the subcellular localization of paxillin, as well as post-translational modification of paxillin. Mechanistically, these two events may involve protein–protein interactions.

Paxillin has multiple tyrosine, serine, and threonine phosphorylation sites, which are indispensable for normal cell adhesion, and is tightly regulated. These potential phosphorylation sites are targeted by various kinases activated by adhesion signaling and growth factors. The role of phosphorylation in adhesion complex signaling is relatively less understood compared with other conventional signaling paradigms and kinase cascades. Paxillin comprises of several structural domains. The N-terminal of paxillin contains five leucine and aspartate-rich regions known as LD motifs (LD1–5). These 13 amino acids-long LD motifs with the consensus sequence of LDXLLXXL are highly conserved among species. At the C-terminal, there are four zinc-finger motifs known as LIM (Lin-11, Isl-1, Mec-3) domains (LIM1–4). Phosphorylation of different domains of paxillin has distinct functional effects, and has been associated with the formation of focal adhesions and actin stress fibers. In particular, the LD motifs...
control most of its signaling activities and the phosphorylation of these LD motifs is related to recruitment of downstream signaling molecules. For example, it has been reported that phosphorylation of paxillin LD4 domain at serine\(^{273}\) can regulate Rho GTPase signaling.\(^{11}\) On the other hand, phosphorylation on serine and threonine residues of LIM2 and 3 domains are essential for the localization of paxillin to the focal adhesions.\(^{8}\) Such molecular processes regulate maturation and turnover rate of focal adhesion complexes.\(^{8}\)

What are the consequences of paxillin phosphorylation? Phosphorylation of paxillin leads to recruitment of signaling molecules, regulation of focal adhesion, and thus, cell migration. Although tyrosine phosphorylation of paxillin is more commonly studied,\(^{1}\) its serine residues are also heavily phosphorylated during cell adhesion and, thus, may be important. One key serine residue (Ser\(^{178}\)) is located between the LD2 and LD3 motifs of paxillin, and is therefore not surprisingly important for signaling. It was previously demonstrated that mutation of Ser\(^{178}\) of paxillin to Ala\(^{178}\) (an amino-acid that cannot be phosphorylated) disrupted normal cytoskeletal remodeling, cell migration, and adhesion.\(^{12}\)

**Tranglutaminase-2 as a Regulator of Adhesion and Paxillin Phosphorylation**

Paxillin can be phosphorylated by a variety of kinases.\(^{8}\) However, the upstream regulators involved in paxillin phosphorylation are not well characterized.
One such candidate molecule may be the transglutaminase (TG)-2, a ubiquitous protein known to be important for skin fibroblast adhesion and migration. TG-2 is a member of the transglutaminase family that is best known for catalyzing post-translational modification of proteins via calcium-dependent cross-linking/transamidating activity. This catalytic action of TG-2 results in the formation of isopeptide bond that is resistant to mechanical and proteolytic degradation. TG-2 is a multifunctional protein (Fig. 1). Besides its cross-linking activity, TG-2 acts as a G-protein in signaling, a cell surface adhesion molecule via non-covalent binding of matrix molecules, and even as a protein kinase.

The effect of TG-2 on cell adhesion may be mediated by a few mechanisms. Cell adhesion to surrounding substrate is greatly influenced by the rigidity of the matrix. In some cases, the enzymatic cross-linking function of TG-2 has been documented to contribute to the stability of extracellular matrix by cross-linking matrix molecules. In some reports, the adhesion signaling may not require the enzymatic transamidase function of TG-2. TG-2 may interact non-covalently with an array of matrix molecules such as integrin, growth factor receptors, and other cell surface or ECM proteins, such as fibronectin, to trigger adhesion signaling. Heparan sulfate proteoglycan (HSPG) syndecan-4, a transmembrane glycosylated protein, has also been reported as an important binding partner of TG-2. Syndecan-4 increases the concentration of the TG-2/fibronectin molecules in the matrix and, thereby, promotes the cross-linking of fibronectin. The increase in extracellular cross-linking will eventually raise the stiffness of the extracellular matrix. Since the fibronectin can also interact with the cell surface integrins, this process not only increases the supportive properties of the matrix, but may also result in integrin clustering through outside-in signaling.

When TG-2 was reduced by short hairpin RNA in cultured human corneal epithelial (HCE-T) cells, the cytoskeleton of the cells, as evidenced by f-actin imaging, was disrupted. Using time-lapse imaging, these cells also showed a slower velocity of directional cell movement. This highlights the vital role of TG-2 in cellular adhesion and migration respectively. Previously extracellular TG-2 has been known to mediate integrin clustering leading to outside-in signaling, resulting in activation of downstream cell motility effectors, the rho proteins. The exact mechanism of how TG-2 achieves this remains to be defined.

Recently, our group has shown that phosphorylation of Ser78 paxillin is dependent on TG-2 status in cultured HCE-T cells using immunoblots with phosphoserine-specific antibody. This is a robust finding because the effect of TG-2 on paxillin phosphorylation does not require a specific extracellular ligand. The same results were obtained when the cells were grown on extracellular matrix proteins (fibronectin, laminin) coated surfaces. In other reports, the TG-2-dependent adhesion signaling required specific interaction between syndecan-4/TG-2 and fibronectin in the matrix.

The relationship of TG-2 with paxillin phosphorylation was also demonstrated in vivo. Superficial mechanical wounds were constructed in the central cornea of homozygous TG-2-knockout and control mice, and observed at intervals until the epithelium was fully healed. In TG-2-knockout mice, reduced phospho-Ser78 paxillin was found at the leading edge of the migrating epithelial cells. This was associated with lack of strong adhesion between the leading edge of the migrating epithelium prior to closure and delayed wound closure. It is currently unknown whether the targeting of paxillin or phosphorylated form of paxillin to the leading edge of migrating cells is dependent on TG-2.

**Transglutaminase-2 and Phosphorylation of Other Adhesomes**

Apart from paxillin, examples of other important adhesomes include vinculin, talin, zyxin, and focal adhesion kinase (FAK). TG-2 was associated with phosphorylation of vinculin at Tyr822, and FAK at Tyr397 but not at Tyr176 (another residue known to be associated with adhesion signaling). In addition, TG-2 was linked to the activities of both the Rac1 and Cdc42 RhoGTPase.
proteins. Interestingly, it also participates in the phosphorylation of the cytoplasmic tail of β-3 integrin, previously known to be important in the signaling to Rac1.25

Once Ser<sup>178</sup> is phosphorylated, a chain of events can take place, which facilitates paxillin to coordinate the recruitment of other adhesome members. Phosphorylation of paxillin at Ser<sup>178</sup> by activated c-Jun N-terminal kinase (JNK) facilitates the binding of FAK<sup>26,27</sup> to paxillin. The bound FAK then further facilitates phosphorylation of paxillin at Tyr 31 and 118, which, in turn, promotes binding of vinculin to paxillin.<sup>8,28</sup> These changes suggest that phosphorylation of Ser<sup>178</sup> paxillin may be upstream to phosphorylation of FAK and vinculin (Fig. 2). If TG-2 only affects phosphorylation of one adhesome initially, interaction with the most upstream Ser<sup>178</sup> paxillin may be sufficient to interfere with post-translational changes of downstream FAK and vinculin.

**Transglutaminase-2 Affects Adhesion in Diverse Cell Types**

TG-2’s role in cell adhesion has already been demonstrated in more than one cell lineage.<sup>14,29</sup> The effect of TG-2 on cell adhesion has been reported in NIH mouse 3T3 fibroblasts,<sup>29</sup> human umbilical endothelial cells,<sup>28</sup> human corneal epithelial cells,<sup>24</sup> astrocytes,<sup>30</sup> and a myelogenous leukemia cell line.<sup>31</sup>

Despite the possible multiplicity of extracellular and intrinsic factors that exist in different tissues, some aspects of the TG-2-paxillin relationship<sup>24</sup> may be evolutionarily conserved. If so, this commentary addresses a fundamental issue, which will likely impact adhesion and cellular behavior in biology.

**Hypothesis**

Based on the initial assumption that TG-2 interacts most directly with paxillin (see above), three hypotheses (Fig. 3) can be proposed to explain the relationship between paxillin and TG-2 in this recent work.<sup>24</sup> Since differential biochemical roles of TG-2 (cross-linking and non-cross-linking) have been implicated in fibroblast-mediated wound healing,<sup>32</sup> each of these current hypotheses involves one distinct biochemical function of TG-2. These hypotheses are not necessarily mutually exclusive.

The first hypothesis is that TG-2 acts as a scaffolding or adaptor molecule, which targets paxillin non-covalently to the focal adhesion. Via further binding to other adhesome proteins, TG-2 facilitates recruitment of these adhesome members, which may or may not be kinases. Due to the close proximity between the newly recruited molecules and paxillin, kinases present in the complex may have easier access to phosphorylate paxillin at Ser<sup>178</sup>. If this hypothesis is true, TG-2 fulfills the definition of an adhesome and should be regarded as a bona fide adhesome member.<sup>3</sup>

There have been a few studies which evaluate TG-2 as an integrin co-receptor.<sup>22,33-35</sup> These suggest that TG-2 may serve as a “scaffolding protein” in the extracellular compartment. Although there is no direct evidence that TG-2 can act as an intracellular adaptor protein, there were at least three findings that support this possibility.

First, TG-2 mediates the activation of protein kinase Cα, leading to its binding with β1 integrins.<sup>22</sup> Second, when TG-2 transcripts are targeted with antisense oligonucleotides, there is reduced detection of PKCα in the membrane.
fraction. 32 Lastly, there is evidence that paxillin can bind PKC. 36 Taken together, there is a possibility that TG-2 can interact with paxillin through direct or indirect translocation of kinases to the membrane. A second hypothesis is that TG-2’s cross-linking function may be involved in paxillin phosphorylation. This can occur through upstream processes, such as crosslinking of matrix or cell membrane proteins like syndecan-4 as described above. Alternatively, TG-2 may be able to cross-link an intracellular kinase such as c-Jun N-terminal kinase (JNK). In experiments with human corneal epithelial cells, the JNK can be co-immunoprecipitated with paxillin in the absence of the JNK inhibitor (SP600125) but in the presence of this inhibitor, the JNK no longer interacts with paxillin. 32

By serving as a transamidase, TG-2 oligomerises and activates dual leucine zipper kinase or Dlk (MAP3K12), which then activated JNK. The activated JNK (pJNK) is therefore the immediate kinase phosphorylating paxillin. This mechanism of TG-2 on JNK has been reported in the context of cell death signaling. 37,38 Consistent with this observation, silencing of TG-2 has been associated with reduced pJNK in malignancies. 37,39,40 Moreover, it has been reported that JNK can localize to paxillin-containing focal adhesions in corneal epithelial cells. 6,12,41 Various studies have shown that pJNK can be a kinase for Ser78 of paxillin. 74 Our in vitro kinase screening assay (data unpublished) with a panel of 229 kinases shows that JNK is a relatively powerful kinase for recombinant human paxillin substrate.

Lastly, TG-2 itself may be the kinase that phosphorylates paxillin. Although the kinase function of TG-2 is not well described, there are reports that described TG-2 acts as a serine/threonine kinase. 35-37 One study has found TG-2 serving as a kinase that phosphorylates insulin-like growth factor binding protein (IGFBP)-3. TG-2 purified from guinea pig liver as well as recombinant human TG-2 were able to phosphorylate IGFBP-3. In contrast to the transamidase function, the intrinsic kinase activity of TG-2 could be inhibited by calcium in a concentration-dependent way. 17 Another study found TG-2 to phosphorylate the transcription factor p53. Since the phosphorylation occurs at Ser66 and Ser69 of p53, which are important for interactions with its main inhibitor Mdm2, this kinase activity is important functionally. 35 It is also interesting that the TG-2 can phosphorylate histone proteins H1 and H3 in chromatin preparations. For example, TG-2 phosphorylates the histone H1 at Ser10 of H3, and since this enhances acetylation, it may have further effects on the epigenetics and control of gene expression. 35

**Other Intricacies in the Paxillin Regulation of Cell Adhesion**

Apart from the full-length paxillin called paxillinα protein mentioned above, other members of the paxillin family also exist, e.g., Hic-5, a natural paxillin antagonist. Hic-5’s LD-domains compete with paxillin for its binding partners. Recently, a shorter form of paxillin called paxillinδ, which lacks the LDI domain, has been reported. It strongly localizes to focal adhesions, suppresses the tyrosine phosphorylation of full-length paxillin, and competitively inhibits integrin signaling. Tyrosine signaling downstream of the LD1 domain of paxillinα is inhibited by paxillinδ. Since the original Ser78 residue of paxillinα is intact in paxillinδ, it remains to be shown if the phospho-Ser78-dependent adhesion function is suppressed when both paxillin isoforms are present. 13

**Conclusion**

Further research should focus on whether TG-2 binds to paxillin or another adhesion protein. If TG-2 binds paxillin directly, it is relevant to determine the specific part of TG-2 that binds paxillin as well as the specific domain of paxillin that is involved in this interaction. In addition, it may be important to investigate if conformational changes of paxillin occur due to this interaction.

It is possible that TG-2-paxillin relationships may vary in the presence of different cell substrates or tissue origins. This requires clarification in further studies.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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