

P055 Targeted deletion of *tgm-2* uncovers a role for extracellular transglutaminase-2 in RhoA downregulation during fibroblast adhesion to fibronectin

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Lack of transglutaminase-2 (TG2) in primary embryonic fibroblasts from *tgm-2* -null mice (TG2^{-/-}MEF) results in hyperactivation of RhoA, which is accompanied by decreased cell migration and accelerated cell spreading leading to anomalous focal adhesions, functions that are regulated by inhibition of RhoA during initial cell adhesion to fibronectin (FN). Decreased auto-phosphorylation of focal adhesion kinase at Tyr-397 (FAK-P₃₉₇) and inhibition of the GTPase activating protein RhoGAP during early cell adhesion, account for the defect in adhesion-dependent RhoA regulation found in TG2^{-/-}MEF. Reconstitution of TG2^{-/-}MEF by adhesion to FN with extracellular TG2 (Verderio *et al* J. Biol. Chem. 2003) increases FAK-P₃₉₇, restores normal RhoA activity and compensates the defect in focal adhesions and cell migration. This finding is consistent with TG2 externalisation and binding to extracellular matrix FN in primary fibroblasts. TG2 contribution to focal adhesions on FN is dependent on signalling through heparan sulphate proteoglycans (HSPG), since restoration of normal focal adhesions in TG2^{-/-}MEF reconstituted with matrix TG2 is RGD-independent and requires cell-surface heparan sulphate chains. Moreover, dermal fibroblast isolated from syndecan-4-null mice are defective in TG2-mediated RGD-independent FAK-P₃₉₇ and cell spreading compared to wild type fibroblasts. Our results establish a novel link between TG2 function and downregulation of RhoA leading to focal adhesions via a HSPG-dependent pathway, and provide new insights into the mechanism of action of TG2 in fibroblast adhesion.