

Significance of the syndecan-4-transglutaminase-2 interaction

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Transglutaminase type 2 (TG2) (EC 2.3.2.13) is a multifunctional ubiquitous protein which has been implicated in the pathogenesis of many apparently unrelated diseases such as tissue fibrosis^{1, 2, 3}, celiac disease⁴, cancers^{5, 6}, neurodegenerative disorders⁷ and type II diabetes⁸. Although TG2 is capable of different biological activities, overall its expression and activation is believed to occur as a response to tissue injury and/or cell stress. Protein cross-linking is the enzymatic reaction for which TG2 is better known and depends on Ca²⁺ binding and GTP dissociation from TG2, conditions which are favoured in the extracellular environment or following cell injury and loss of Ca²⁺ homeostasis. Protein cross-linking leads to the formation of intra- or inter-molecular Nε(γ-glutamyl)lysine bonds between the γ-carboxamide group of a peptide-bound Gln residue and either the ε-amino group of a peptide-bound Lys residue or a primary amino group of polyamines, resulting into protein polymerisation/modification⁹. TG2 causes additional post-translational modifications among which protein deamidation of Gln residues contributes to the development of disorders caused by gluten sensitivity (e.g. celiac disease)⁴.

TG2-mediated protein crosslinking has been convincingly linked to normal and abnormal wound repair processes. General consensus exist that the externalisation of TG2 and activation of its cross-linking activity are responsible for extracellular matrix (ECM) stabilisation and resistance to matrix metalloproteinase degradation¹⁰, and that uncontrolled cross-linking as a consequence of chronic cell insult and increased secretion of TG2 is implicated in the pathogenesis of tissue fibrosis^{1, 2, 3}. Not all the externalised TG2 is believed to be enzymatically active, despite the favourable conditions of low Ca²⁺/GTP in the extracellular environment¹¹. Most TG2 is thought to remain latent and be activated on demand e.g. for cell repair/proliferation in response to tissue injury/stress^{12, 13, 14} or for modulation of the ECM^{15, 16, 17} e.g. by transforming growth factor beta^{18, 19} in response to oxidative stress^{20, 21} and by the hypoxia inducible factor-1 in solid tumours²². TG2 cross-linking may be controlled by the tight binding of TG2 to large substrates like fibronectin (FN)^{23, 24}, which can further complex with collagen²⁵, and by TG2 internalisation and subsequent lysosomal degradation²⁶. In recent years it has become clear that the influence of TG2 on cell-matrix interactions also depends on cross-linking

independent mechanisms. TG2 has been ascribed an adhesive role by acting as either an intracellular GTPase²⁷, an integrin- β_1 co-receptor²⁸, or a structural protein supporting adhesion-mediated cell signalling independently from the Arg-Gly-Asp (RGD) cell binding domain of FN²⁹. Despite the importance of TG2-externalisation for most of the cross-linking dependent and independent roles, the mechanism of TG2 secretion is elusive. TG2 lacks a leader peptide and it is not post-translationally modified for classic externalisation through the endoplasmic reticulum/Golgi route⁹. Among the influencing factors are the requirements for TG2-active-state conformation^{30, 31} and an intact N-terminal FN binding site³².

Recent studies by Scarpellini et al³³ and Telci et al³⁴ published in the Journal of Biological Chemistry have highlighted a novel interaction of TG2 with the heparan sulphate proteoglycan (HSPG) syndecan-4 (S4), and suggested that this interaction may be important for controlling the cell-surface trafficking and cross-linking activity of TG2³³, and for mediating the adhesion-dependent signalling role of matrix TG2³⁴.

Early work from Bergamini's group described affinity purification by heparin-sepharose as an effective step for TG2 purification from erythrocytes³⁵. More recently, the involvement of cell-surface HSPGs in TG2-mediated RGD-independent cell adhesion was hypothesised²⁹, since pre-treatment of cells with HS-degrading enzyme led to the disruption of this distinct cell adhesion process. This earlier work has prompted further investigations on the binding interaction between TG2 and heparin, a highly sulphated analogue of heparan sulphate (HS) glycosaminoglycan chains, which only exist covalently bound to the core protein of cell-surface proteoglycans e.g. syndecans, glypicans and secreted proteoglycans³⁶. HSPGs bind extracellular ligands through the HS and influence their biological activity by affecting protein stability, activity, conformation, as well as cell-surface localisation, membrane secretion/internalisation and protein interactions^{36, 37}. Among the HSPG-subfamilies, the syndecans act as co-receptors for both ECM components and soluble ligands³⁸, and S4 has overlapping roles with extracellular TG2 in wound healing and fibrosis³⁹, thus suggesting the implication of syndecans in the biological function of TG2.

The hypothesis that S4/HS could regulate TG2 was recently tested using a range of in vitro and cell-based approaches³³. Initially, TG2-heparin interaction was studied at equilibrium by heparin/HS solid-phase assays and in real-time by surface plasmon resonance. The TG2-heparin/HS interaction was saturable and the apparent dissociation constant was in the low nM range ($K_D \sim 20$ nM by solid phase and $K_D \sim 90$ nM by SPR), suggesting high affinity of TG2 for heparin/HS, comparable to that for the classic binding partner FN⁴⁰. Next, in cell systems it was established that the co-association of cell membrane TG2 with S4 is not mediated by FN (which binds both TG2 and HS), but largely depends on TG2 association with the HS chains of S4³³. The significance of the TG2-S4 interaction was then studied in primary dermal fibroblasts using a combination of S4 knock-out/knock-in experiments. It was shown that lack of S4/HS leads to a lower level of cell-surface TG2 antigen and cross-linking activity, and a parallel accumulation of cytosolic TG2 in primary fibroblasts but no changes in the total level of TG2 expression. Since heparin did not have a direct regulatory effect on TG2 enzymatic activity³³, this finding led to the suggestion that the HS chains of S4 may direct the cell-surface trafficking and localization of TG2 at cell-matrix adhesions. Indeed, complexes of TG2 and S4 were detected at cell-matrix adhesions by immunofluorescence³³. Hence, cell-surface HS may affect the externalization of TG2 in a similar way to that recently reported for fibroblast growth factor-2⁴¹. Functional inhibition of HS with surfen, a small antagonist of HS⁴², or heparitinase digestion led to even higher alterations in cell-surface TG2 activity, suggesting that more cell-surface HSPGs are likely to be implicated³³. Since the endocytic receptor LRP1 responsible for TG2 internalisation has been shown to act in concert with HSPGs^{26, 37}, the possibility of a dual function of HSPGs in TG2 externalisation and internalisation is intriguing. However, published data so far support a role for HSPGs in the trafficking of TG2 to the cell-surface since S4-null fibroblasts display an accumulation of intracellular TG2³³. Given the variety of roles of HS, the high affinity binding of TG2 for HS is likely to influence the function of TG2 widely in the extracellular environment. Consistent with this idea, Telci et al recently showed that once TG2 is externalised and bound to FN, S4 may act as a cell-surface receptor mediating the RGD-independent outside-in signalling role of matrix TG2 in partnership with integrin-

β_1 ³⁴. The interaction of matrix TG2 with S4 has been shown to mediate activation of protein kinase C α and its further interaction with integrin- β_1 , thus leading to activation of focal adhesion kinase and survival ERK1/2 mitogen activated protein kinases. This pathway, which is RGD-independent, relies on increased deposition of TG2 in the ECM e.g. by cell secretion following cell lysis or erythrocytes rupture at wound sites. Hence, TG2 mediated RGD-independent cell adhesion is likely to be prevalent in restricted conditions of matrix breakdown, accumulation of RGD peptides of FN and competitive block of integrin-mediated RGD-dependent pathway. It is envisaged that in these conditions, TG2 degradation would be limited by binding to both FN and HS^{25, 10, 43}, hence TG2 would be able to rescue cell adhesion and facilitate wound repair.

In conclusion, characterisation of the high affinity binding of TG2 to HS has opened the way to new hypotheses on how TG2 may be externalised and its function regulated in the ECM. Studies so far have led to the identification of S4 as a new component of the cell-surface trafficking of TG2 and a novel cell-surface receptor for matrix TG2. It is anticipated that the flexibility and length of the HS chains would allow for this dual interaction of S4 with TG2 to take place. TG2 and S4 are molecules that are both increased in conditions of tissue fibrosis in vivo^{44, 3, 45}. If their interaction is confirmed in vivo, alteration of S4-TG2 association could become an attractive target for the control of tissue fibrosis.

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