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Mini review

Post-translational modifications of integrin ligands as pathogenic mechanisms in disease

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ABSTRACT

Protein post-translational modifications like glycation, carbamylation and citrullination increase the functional diversity of the proteome but in disease situations might do more harm than good. Post-translational modifications of ECM proteins are thus appearing as mechanisms, which contribute to tissue dysfunction in chronic kidney disease, in diabetes and in various inflammatory diseases. In chronic renal failure, carbamylation could lead to kidney fibrosis. In diabetes, high glucose levels lead to non-enzymatic glycation and cross-linking of collagens, which contribute to tissue stiffening with consequences for cardiovascular and renal functions. In inflammatory diseases, citrullination deiminates arginine residues with possible consequences for integrin-mediated cell adhesion to RGD- and GFOGER sequences in ECM proteins. Citrullination of fibronectin was in one study suggested to affect cell adhesion by modifying the heparin-binding site and not the RGD site. In a recent publication citrullination of GFOGER sequences in collagen II was demonstrated to selectively affect α10β1 and α11β1 integrin-mediated cell adhesion to collagen II, with consequences for synovial fibroblast and stem cell adhesion and migration. The implications of citrullination affecting integrin binding in disease open up a new area of study and might have implications for the pathogenesis of inflammatory diseases like rheumatoid arthritis and periodontitis.

Contents

1. Introduction

Glycation, citrullination and carbamylation are three post-translational modifications that can change the interacting properties of proteins containing lysine and arginine in their interactive domains (Fig. 1).

Thinking about these post-translational modifications from a matrix perspective, ECM proteins like fibronectin and collagens represent targets where post-translational changes can potentially affect cell adhesion, physicochemical properties and antigenicity.

A major group of cell surface receptors for the ECM are integrins (Barczyk et al., 2010). Whereas substantial data has been collected on how integrins interact with other signaling receptors to integrate extracellular information (Legate et al., 2009), less is known about how integrins actually interact with their ligands *in vivo*.

Questions that need to be considered include details of how ligand organization, availability and post-translational modifications change the ability of integrins to bind ligand. A recent study suggests that citrullination of collagen II affects integrin binding and implies integrins

Abbreviations: AGE, advanced glycation end-product; ECM, extracellular matrix; CKD, chronic kidney disease; MMP, matrix metalloproteinase; PAD, peptidyl-arginine deiminase; PPAD, *P. gingivalis* peptidyl-arginine deiminase; RA, rheumatoid arthritis.

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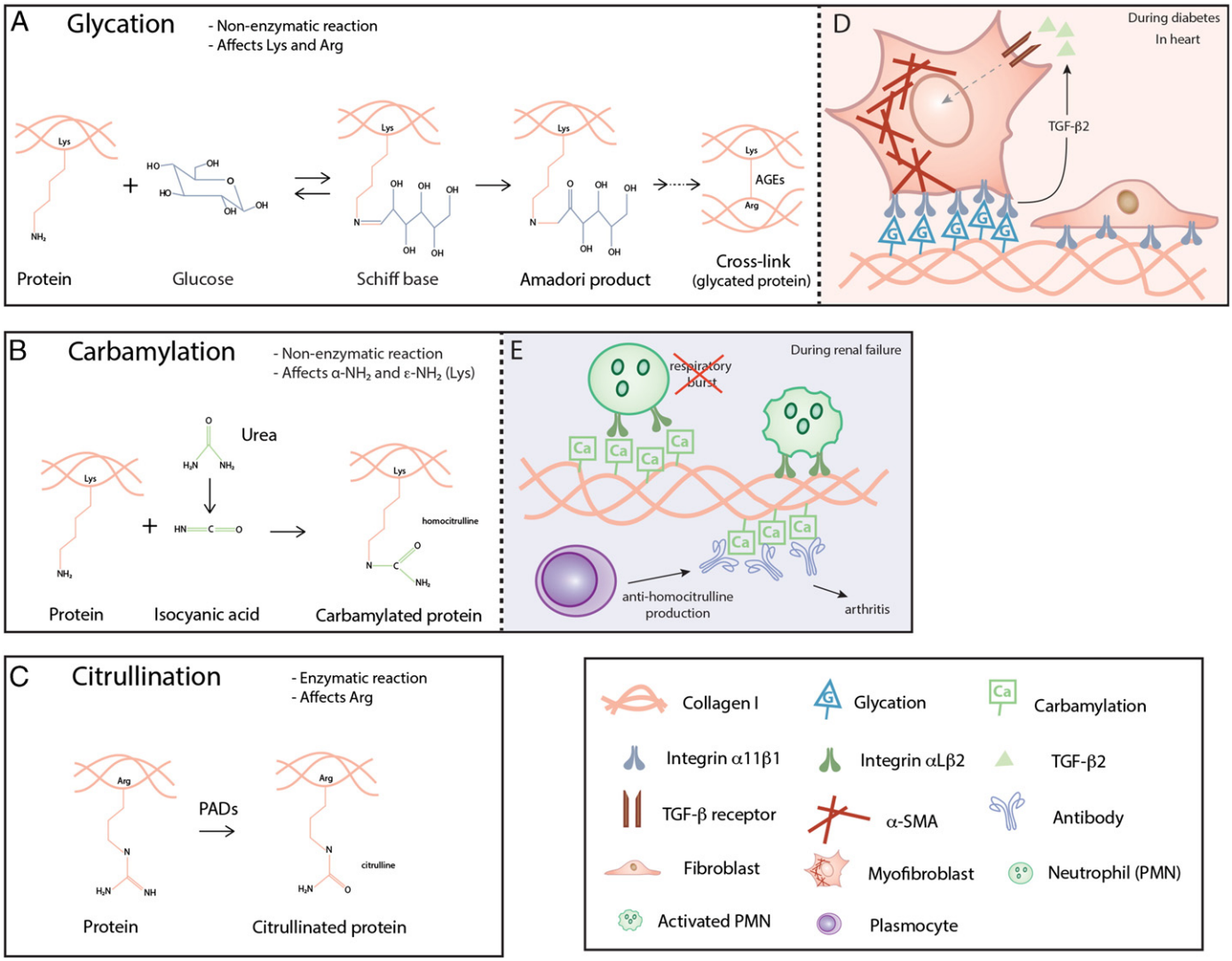

Fig. 1. ECM post-translational modifications and their involvement in disease. Biochemical reactions of glycation (A), carbamylation (B) and citrullination (C) are depicted. PADs: peptidyl-arginine deiminases. D. With an increase of glucose concentration, glycation of collagen I induces differentiation of cardiac fibroblasts into myofibroblasts, via autocrine secretion of TGF-β2 (Talior-Volodarsky et al., 2012). E. Carbamylation of collagen I impaired inflammatory cell functions like respiratory burst (Jaisson et al., 2007). Carbamylation can also promote autoimmune response leading to arthritis (Shi et al., 2014).

to be involved in the pathogenesis of rheumatoid arthritis (Sipila et al., 2014). In relation to this publication we provide a short summary of citrullination with a brief overview on glycation and carbamylation since these are post-translational modifications that affect the same amino acids, and make the link to diseases other than arthritis.

1.1. Mechanisms of extracellular post-translational modifications and their consequences for cell–matrix interactions

Glycation is the non-enzymatically covalent reaction whereby reducing sugars like glucose form bonds to amino groups in amino acids like lysine and arginine (Bucala and Cerami, 1992). Glycated collagen can then form non-enzymatic crosslinks known as advanced glycation end-products (AGEs) (Gautieri et al., 2014). The involvement of integrins in biological effects of glycation and AGEs needs further study, but studies so far indicate that glycation interferes with cell adhesion to collagen I (Avery and Bailey, 2006; McCarthy et al., 2004; Morita et al., 2005).

Carbamylation leads to the non-enzymatic addition of urea-derived isocyanic acid to α-NH_2 (protein N-termini) and ε-NH_2 (lysine residues), and has been shown to affect collagen I triple helix stability and sensitivity to MMP cleavage (Jaisson et al., 2007). Available data suggest that carbamylation does not primarily affect cell adhesion since lysine residues are not present in cell adhesive sequences used by integrins. However, carbamylation could induce the structure modification of ECM proteins that may result in the lack of recognition by integrins (Jaisson et al., 2006).

On the contrary, citrullination is an enzymatic process that thus differs from glycation and carbamylation by physiological roles. Citrullination contributes to distinct functions such as skin protection and gene regulation by affecting keratin and histones, respectively. Hypercitrullination of histones is also involved in immune responses, essential in the formation of highly decondensed chromatin structures (Baka et al., 2012). Citrullination requires enzymes called peptidyl-arginine deiminases (PADs), which act to replace the primary ketimine group (=NH) by a ketone group (=O). PADs are intracellular enzymes and belong to a family of 5 members in humans, PAD 1–4 and PAD-6 (Yoshida et al., 2006). Under some conditions like inflammation, the enzymes can be released into the extracellular space. PAD-4 expressed by neutrophils is considered to be a major enzyme involved in extracellular citrullination in inflammation. Citrullination, leads to deimination of arginine and has the potential to modify cell adhesive properties of proteins containing the RGD sequence (fibronectin, fibrinogen) and GFOGER-like sequences (collagens). Citrullination has been observed

to occur in fibronectin and to affect its cell-adhesive properties (Shelef et al., 2012), although, somewhat surprisingly, no direct link to the RGD site was demonstrated in that study, but instead suggesting that arginines in the heparin-binding domain are the targets of citrullination in fibronectin. The molecular mechanism of cell adhesion to collagen has long been a subject of controversy (Zeltz et al., 2014). *In vitro* experiments have convincingly established that cells using β1 integrin receptors bind directly to GFOGER sequences in monomeric triple helical collagens (Carafoli et al., 2013; Emsley et al., 2000; Farndale et al., 2008; Knight et al., 2000; Zhang et al., 2003) and indirectly to RGD-sites in COLINBRIs using β1- and αv-integrins (Reyhani et al., 2014; Zeltz et al., 2014).

1.2. Post-translational modifications of ECM in disease

Collagens are non-enzymatically glycated not only during diabetes with increased levels of glucose but also during aging, leading to increased tissue stiffness (Gautieri et al., 2014), which is suggested to be a pathophysiological factor in renal, cardiac, and blood vessel dysfunction (Willemsen et al., 2012). Interestingly, in diabetic cardiomyopathy, myofibroblast differentiation is induced by glycated collagen I, in a mechanism that seems to involve integrin α11β1-mediated autocrine secretion of TGF-β2 (Talior-Volodarsky et al., 2012) (Fig. 1D).

In vitro experiments and clinical studies have suggested the potential involvement of carbamylated proteins in chronic kidney disease (CKD) complications like atherosclerosis. A recent study in a mouse model demonstrates that the chronic increase of urea, as seen in CKD, increases the carbamylation rate of plasma and tissue proteins, including collagens (Pietrement et al., 2013). Lysine carbamylation could induce autoimmune response via production of anti-homocitrulline antibodies, leading to arthritis (Jaisson et al., 2011) (Fig. 1E).

Citrullination occurs in rheumatoid arthritis (RA) and antibodies to citrullinated proteins are found in several inflammatory diseases (Makrygiannakis et al., 2006). An interesting link exists between citrullination, rheumatoid arthritis and periodontitis (PD). Subjects with PD are at an increased risk to develop RA, and vice versa. Current findings support the idea that PD could be a factor in the initiation of autoimmune inflammatory responses that occur in RA. The citrullination in periodontitis may lead to the breaking of immunotolerance to citrullinated proteins, contributing to the development of RA (Koziel et al., 2014). The leading pathogen in chronic periodontitis, *Porphyromonas gingivalis*, produces a bacterial enzyme, *P. gingivalis* peptidyl-arginine deiminase (PPAD). In support of a role of this enzyme in the development of arthritis, inoculation of animals with a PPAD knockout *P. gingivalis* strain prior to immunization with collagen II had no influence on clinical development or progression of the experimental arthritis (Maresz et al., 2013). This is in contrast to mice that had been inoculated with a wildtype PPAD producing *P. gingivalis* strain and where collagen II-induced arthritis was exacerbated by infection with the periodontal pathogen. In addition, in a model for experimental autoimmune arthritis antibodies against citrullinated proteins have been found to be pathogenic (Kuhn et al., 2006).

2. An unexpected role casting in the synovium- or what a difference an imine group makes

An interesting study by Sipila et al. asks the question if citrullination of collagen II affects cell adhesion by modifying the GFOGER sequence (Sipila et al., 2014). The answer is yes. However, a systematic comparison of the binding of the four β1 integrin collagen receptors, α1β1, α2β1, α10β1 and α11β1, somewhat surprisingly reveals that it is mainly the binding of α10β1 and α11β1 to collagen II that is affected by citrullination. A modest decrease in cell migration mediated by these receptors on citrullinated collagen II is also observed. The authors suggest that the reduced cell adhesion might interfere with the migration of synovial fibroblasts and mesenchymal stem cells *in vivo*.

Why is it surprising that the chondrocyte α10β1 and fibroblasts α11β1 are identified in these assays?

1. α1β1 and α2β1 have been studied in the context of cartilage and bone in a limited number of studies, but were still prior to this study the main candidate collagen receptors to be affected by collagen citrullination. Since both α1β1 and α2β1 are expressed on inflammatory cells and mesenchymal stem cells, one could envision that reduced cell adhesion due to citrullination would affect inflammatory cell adhesion in the inflamed synovia. However, this does not appear to be a likely scenario. Instead the results from Sipila et al. suggest that α10β1 and α11β1 play leading roles on non-immune cells in the synovia.
2. .It is maybe not so surprising that citrullination affects GFOGER-dependent cell adhesion to collagen II, but the more surprising is the selective effect for two of the four collagen-binding integrins, although all four collagen receptors bind the GFOGER sequence. The reason for this can be understood in structural terms. A comparison of the atomic structure of the four I domains interacting with a trimeric GFOGER peptide reveals that α10 - and α11 I-domains interact with the charged arginine in GFOGER in uncitrullinated collagen. The arginine is sandwiched between two negative residues in the α10- and α11 I-domains. For integrin binding to the citrullinated collagen, there is no charge neutralization for α10β1- and α11β1-mediated binding, resulting in a weaker interaction.

The study is important since it explains the effect of citrullination of collagen II at the molecular level, however a number of questions remain unanswered.

- α11β1 is expressed on cultured mesenchymal stem cells, but it will be important to determine if this expression also extends to the mesenchymal stem cells in joints *in vivo*.
- α10β1 and α11β1 are expressed on chondrocytes and synovial fibroblasts, respectively and it will be important to determine to what extent cells in synovia are in contact with citrullinated collagen II.
- Since collagens form fibrillar structures, it is unclear to what extent the integrin-binding GFOGER sequences are available to cells in mature cartilage and synovia matrices *in vivo*. It is possible that the availability of GFOGER for extracellular citrullination is largely restricted to dynamic situations where ECM is turned over and GFOGER motifs are exposed (Zeltz et al., 2014).
- α11β1 is normally found in cells residing in matrices rich in collagen I, so the question arises if citrullination of collagen I also occurs in the synovial membrane and has the same effect as that observed when collagen II is citrullinated.
- Since α11β1 is also expressed in human periodontal ligament fibroblasts (Barczyk et al., 2009, 2013) it would be interesting to establish if α11β1 has a function in periodontal disease in the context of citrullination. Maybe a reduced adhesion of periodontal ligament fibroblasts to the periodontal collagen I will contribute to a disorganized matrix more susceptible to inflammatory cell destruction.
- To test if the findings have an effect as suggested by the authors it would be important to show proof of principle in animal models.

Some of the issues raised above are schematically summarized in Fig. 2.

3. Conclusion

In summary, the recent findings of post-translational modifications of cell adhesion sites in ECM proteins open up a new field of study. Citrullination appears to be an important mechanism in inflammatory disease and with a possible link to integrin-mediated adhesion we will surely hear more about this subject in the future.

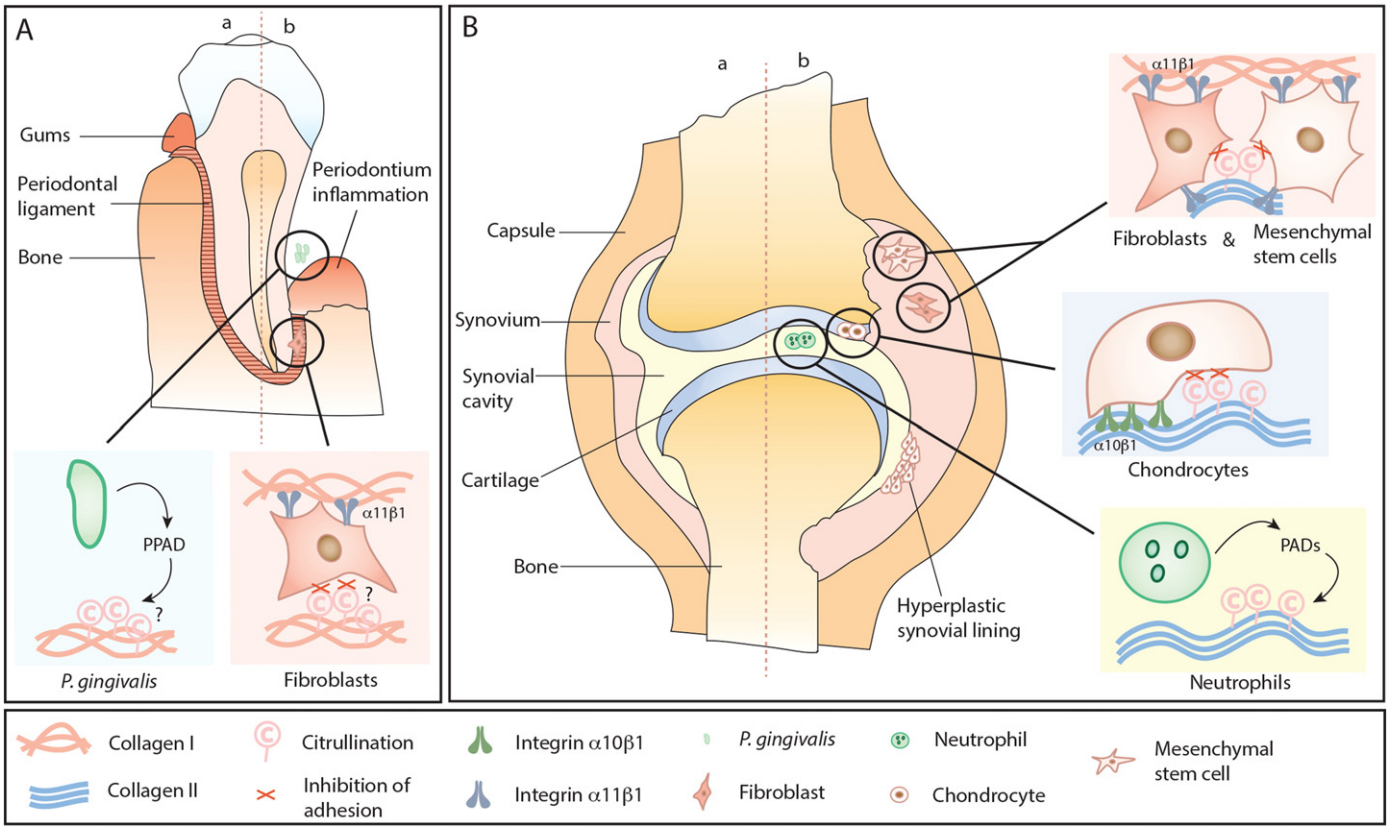

Fig. 2. Schematic view of the potential role of citrullinated collagen in periodontitis and in the arthritic synovium. A. Schematic representation of the potential role of citrullination in periodontitis. Human periodontal ligament in normal uninflamed state (a) and inflamed state (b) is shown. Bacterial *P. gingivalis* peptidyl-arginine deiminase (PPAD) is suggested to citrullinate collagen I to affect integrin α11β1-mediated interactions with collagen I. B. Schematic representation of normal synovium (a) and arthritic synovium (b) is shown. For simplicity only cells expected to express integrin α10β1 (chondrocytes) and integrin α11β1 (synovial mesenchymal stem cells and synovial fibroblasts) are depicted. Neutrophil-released peptidyl-arginine deiminases (PADs) are suggested to citrullinate collagen II in cartilage.
Modified from Smolen and Steiner (2003).

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