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INVITED REVIEW

# Transglutaminase 2 as a biomarker of osteoarthritis: an update

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**Abstract** Osteoarthritis is a progressive joint disease characterized by cartilage degradation and bone remodelling. Under physiologic conditions, articular cartilage displays a stable chondrocyte phenotype, whereas in osteoarthritis a chondrocyte hypertrophy develops near the sites of cartilage surface damage and associates to the pathologic expression of type X collagen. Transglutaminases (TGs) include a family of $Ca^{2+}$-dependent enzymes that catalyze the formation of $\gamma$-glutamyl cross-links. Their substrates include a variety of intracellular and extracellular macromolecular components. TGs are ubiquitously and abundantly expressed and implicated in a variety of physiopathological processes. TGs activity is modulated by inflammatory cytokines. TG2 (also known as tissue transglutaminase) mediates the hypertrophic differentiation of joint chondrocytes and interleukin-1-induced calcification. Histomorphometrical and biomolecular investigations document increased TG2 expression in human and experimental osteoarthritis. Consequently, the level of TG2 expression may represent an adjuvant additional marker to monitor tissue remodelling occurring in osteoarthritic joint tissue. Experimental induction of osteoarthritis in TG2 knockout mice is followed from reduced cartilage destruction and increased osteophyte formation compared to wild-type mice, suggesting a different influence on joint bone and cartilage remodelling. The capacity of transamidation by TG2 to regulate activation of latent TGF-$\beta$ seems to have a potential impact on the regulation of inflammatory response in osteoarthritic tissues. Additional studies are needed to define TG2-regulated pathways that are differently modulated in osteoblasts and chondrocytes during osteoarthritis.

**Keywords** Osteoarthritis · Transglutaminase 2 · Joint tissue · Transforming growth factor-beta

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## Introduction

Osteoarthritis (OA) is a slowly progressing chronic joint disease that affects not only most people over the age of 65 (Petersson and Jacobsson 2002) but also non-human mammals, including horse and dog (Poole 1996). The aetiology of OA is complex and involves many cellular and biochemical processes. Degradation of articular cartilage and osteophyte formations are the major features of OA (Petersson and Jacobsson 2002). Synovitis, another important characteristic observed in OA, occurs as a secondary inflammatory symptom following cartilage biochemical and mechanical stress (Goldenberg and Cohen 1978).

Articular cartilage is predominantly composed of collagen, proteoglycans and glycoproteins (Eyre 2004; Todhunter 1996). Adult cartilage does not contain vascular supply, so chondrocytes metabolism is largely anaerobic and nutrition is achieved by diffusion through the synovial fluid (Garvican et al. 2010). Chondrocytes in normal articular cartilage remain largely in a resting state and do

not undergo terminal differentiation (Goldring 2000). In OA, chondrocyte hypertrophy develops, typically near sites of cartilage surface damage (Poole et al. 1989). Chondrocyte hypertrophy is likely to promote successive calcification and co-localizes with deposits of calcium pyrophosphate crystals (Ishikawa et al. 1989). In normal condition, cartilage extracellular matrix is a dynamic equilibrium. In particular, the balance between anabolic and catabolic activities of chondrocytes maintains the structural and functional integrity of articular cartilage (Goldring and Goldring 2010). In OA, a deregulated balance between proteinases degrading the extracellular matrix and their inhibitors likely occurs in favor of proteolysis (Soder et al. 2005). Transglutaminases (TGs) catalyze a calcium-dependent transamidation reaction that produces covalent cross-linking of available substrate glutamine residues to a primary amino group and modify the extracellular matrix through effects including protein cross-linking and stabilization (Chen and Mehta 1999). TG transamidation catalytic activity is increases in OA joint cartilage (Rosenthal et al. 1997). TG2 is also expressed from hypertrophic chondrocytes (Aeschlimann and Thomazy 2000; De Laurenzi and Melino 2001; Nurminskaya et al. 2003). In osteoarthritic process, chondrocytes undergo hypertrophy and an accelerated turn-over and, successively, calcification of the pericellular matrix becomes prevalent in sclerotic subchondral bone (Ryan and McCarty 1997). Transglutaminase 2 (TG2) is an essential mediator of interleukin-1beta (IL-$\beta$1)-induced calcification, as well as hypertrophic differentiation and calcification in articular chondrocytes in vivo and in vitro (Aeschlimann et al. 1996; Aeschlimann and Thomazy 2000; Nurminskaya et al. 2003; Johnson and Terkeltaub 2005). In osteoarthritic cartilage, hypertrophic differentiation develops within the population of human chondrocytes (von der Mark et al. 1992). Chondrocyte hypertrophy is also documented in mouse knee cartilage within the first few weeks following joint instability-induced experimental OA and promotes disease progression (Kamekura et al. 2006). In this review, we describe the specific contribution of TG2 to joint tissue remodelling, with particular reference to the interplay with inflammatory mediators of OA.

## The role of transglutaminases

The TGs are a family of $Ca^{2+}$-dependent enzymes that catalyze the formation of $\gamma$-glutamyl cross-links. Their substrates include a variety of intracellular and extracellular macromolecular components, so they can alter several properties of tissues in which TGs are active. The TGs family consists of at least five members, including the plasma (also called coagulation factor XIII) and the tissue/cytosolic form (Greenberg et al. 1991; Fesus et al. 1996). TGs are expressed ubiquitously and abundantly, and implicated in a variety of cellular processes, such as differentiation, cell death, inflammation, cell migration, and wound healing (for a review, see Fesus and Piacentini 2002; Lorand and Graham 2003; Fésüs and Szondy 2005; Ientile et al. 2007; Collighan and Griffin 2009; Sarang et al. 2009).

All TGs seem to be capable to cross-link a broad, similar range of intracellular and extracellular substrates. Intracellular substrates include proteins of the actin cytoskeleton (Zhu et al. 1994) and histones (Ballestar et al. 1996); extracellular substrates comprise several certain collagen types, osteopontin, vitronectin and osteonectin (Mosher and Proctor 1980; Prince et al. 1991; Beninati et al. 1994; Aeschlimann et al. 1996). Many TGs are found in the extracellular matrix of hypertrophic cartilage, and the extent at of cross-linking alters the susceptibility of the matrix to proteolysis, so affecting its removal rate during the development. Fibronectin, for example, is sensitive to cleavage by tissue metalloproteinases only after it has been cross-linked by TGs (Bini et al. 1996). Several functions have been proposed for TGs. For plasma TG, they include the stabilization of the fibrin matrix during blood clotting. In the epidermis, TG mediates the cross-linking of the keratin envelop during epidermal cell differentiation (Thacher 1989).

TGs activity has also been reported to influence cell growth, differentiation (Borge et al. 1996; Katoh et al. 1996) and apoptosis (Borge et al. 1996; Cummings 1996; Fesus et al. 1996). Moreover, tissue TGs are also implicated in other processes, including the determination of cell shape by the cross-linking of cytoskeletal actin with associated proteins (Nemes et al. 1997), the promotion of cell adhesion (Borge et al. 1996; Ueki et al. 1996) and the stabilization of the extracellular matrix (Aeschlimann et al. 1995). TGs are also wound repair enzymes implicated in tissue remodelling following injury, in pulmonary and dermal fibrosis, cardiac hypertrophy and arterial reshaping (Lorand and Graham 2003). IL-1$\beta$, TNF$\alpha$, as well as nitric oxide, peroxynitrite donors, and chemokines induce TG activity in chondrocytes (Johnson et al. 2001, 2003; Heinkel et al. 2004; Johnson and Terkeltaub 2005).

Transglutaminase 2 (TG2), also known as tissue transglutaminase or transglutaminase C catalyzes protein cross-linking via transamidation of glutamine residues to lysine residues in a $Ca^{2+}$-dependent manner (Lorand and Graham 2003; Facchiano and Facchiano 2009). The cross-linking generates proteolytically resistant $\gamma$-glutamyl-lysine bonds. TG2 is monomeric and cytosolic, and it is conditionally expressed in certain cells including hepatocytes and erythrocytes. TG2 is upregulated in cells undergoing apoptosis (Borge et al. 1996; Cummings 1996; Fesus et al.

1996). Besides the classical cross-linking activity, TG2 displays several other biochemical functions. In rat hepatocytes, TG2 can act as a high-molecular weight GTP-binding protein by mediating α1-adrenergic receptor signaling (Nakaoka et al. 1994). TG2 contains protein disulfide isomerase (PDI) (Hasegawa et al. 2003), protein kinase (Mishra and Murphy 2004), and displays DNA nuclease activities (Takeuchi et al. 1998). In addition, TG2 mediates cell-extracellular matrix connections interacting directly with fibronectin, integrins, and syndecan (Telci et al. 2008; Collighan and Griffin 2009). A critical concentration of calcium is the only known requirement for TG2 activation (Fesus et al. 1996). As concerning articular cartilage, exogenous nanomolar TG2 is sufficient to directly induce hypertrophic differentiation in chondrocytes in organ culture (Johnson and Terkeltaub 2005). Yet, it is uncertain how TG2 is secreted, since it lacks a signal peptide (Lorand and Graham 2003).

## Physiological expression of transglutaminase 2 in osteoarticular tissues

In physiologic endochondral growth plate mineralization, chondrocytes undergo a multi-step differentiation process with an ordered progression from a resting to proliferative state, followed by maturation to a terminally differentiated hypertrophic state (Erlebacher et al. 1995). In normal articular cartilage, chondrocytes remain largely in a resting state and do not undergo terminal differentiation or mineralize their matrix (Goldring 2000). Instead, hypertrophic chondrocytes are capable to remodel and mineralize extracellular matrix (Alini et al. 1996), possibly by the upregulation of matrix metalloproteinase-13 that mediates matrix degradation occurring in OA (Krane 2001). In addition, hypertrophic chondrocytes demonstrate a marked increase of the release of mineralization-competent secretory vesicles (Alini et al. 1996) and an increased alkaline phosphatase activity (Gerstenfeld and Shapiro 1996; Alini et al. 1996). The latter mediates both calcification and growth plate organization (Harmey et al. 2004) and promotes chondrocyte hypertrophy (Alini et al. 1996). TG2 is essential to promote cultured chondrocyte maturation and hypertrophy in response to several signals, including retinoic acid, CXCL1 and the calgranulin S100A11 (Johnson et al. 2003, 2008; Merz et al. 2003). TG2 is also implicated in modifications of the extracellular matrix modulating osteoblasts maturation and bone mineral accumulation (Nurminskaya and Kaartinen 2006). Changes in TG2 expression and FXIIIA release have been also identified in the physiological maturation of growth plate chondrocytes (Johnson et al. 2001, 2003; Summey et al. 2002; Heinkel et al. 2004; Johnson and Terkeltaub 2005). Upregulation of TG2-transamidation activity via transfection associates with a potent upregulation of the chondrocyte capacity to calcify surrounding matrix (Johnson et al. 2001). Exogenous nanomolar amounts of TG2 are sufficient to induce chondrocytes hypertrophic differentiation in organ culture of articular cartilage (Johnson and Terkeltaub 2005). In addition, paracrine and juxtacrine effects of TG2 released from chondrocytes modulate osteoblast differentiation through extracellular TG2-induced cAMP-dependent protein kinase signaling (Nurminskaya et al. 2003; Nurminskaya and Kaartinen 2006). In the latter, a metalloproteinase13-dependent process progresses through resting, proliferative, pre-hypertrophic and hypertrophic differentiation and terminal chondrocytes death (Aeschlimann et al. 1993; Borge et al. 1996). Extracellular matrix remodelling is partly due to a shift in cartilage-specific collagens from type II to type X collagen as well as by enhanced metalloproteinase-13, ADAMTS-5 and reduced aggrecan expression (Nurminskaya and Linsenmayer 1996; Nurminskaya et al. 1998).

## Joint expression of transglutaminase 2 during osteoarthritis

TG2 expression, similarly to the total TG catalytic activity and TG-catalyzed isopeptide bond formation, is increased in chondrocytes and extracellular matrix of human knee cartilage during OA (Figs. 1, 2; Johnson et al. 2001; Summey et al. 2002; Rosenthal et al. 1997). In OA, foci of articular chondrocyte hypertrophy develop, typically near sites of altered cartilage surface (Poole et al. 1989; von der Mark et al. 1992). OA-induced chondrocyte hypertrophy promotes matrix remodeling and repairs (Vignon et al. 1983), but also calcification since hypertrophic chondrocytes co-localized with deposits of hydroxyapatite and calcium pyrophosphate dihydrate crystals (Ishikawa et al. 1989). The latter stimulate intra-articular inflammation and sustain cartilage damage contributing to the progression of OA (McCarthy 1999; Terkeltaub 2002). Cartilage contains a variety of potential TG2 substrates including collagen subtypes, fibronectin and the mineralization-regulatory protein osteopontin (Kaartinen et al. 2002). Furthermore, TG2 transamidation catalytic activity has been shown to increase in joint cartilage in parallel with OA severity or ageing (Rosenthal et al. 1997; Johnson et al. 2001). Finally, inflammatory mediators implicated in OA, including IL-1$\beta$ (Attur et al. 1998) stimulate chondrocyte matrix calcification (Johnson et al. 2001). Cell surface TG2 serves as an integrin co-receptor for fibronectin (Hang et al. 2005), whereas extracellular TG2 promotes integrin clustering and RhoA activation (Janiak et al. 2006). Increased FXIIIA expression and extrusion directly stimulate matrix calcification in chondrocytes (Johnson et al. 2001; Nurminskaya

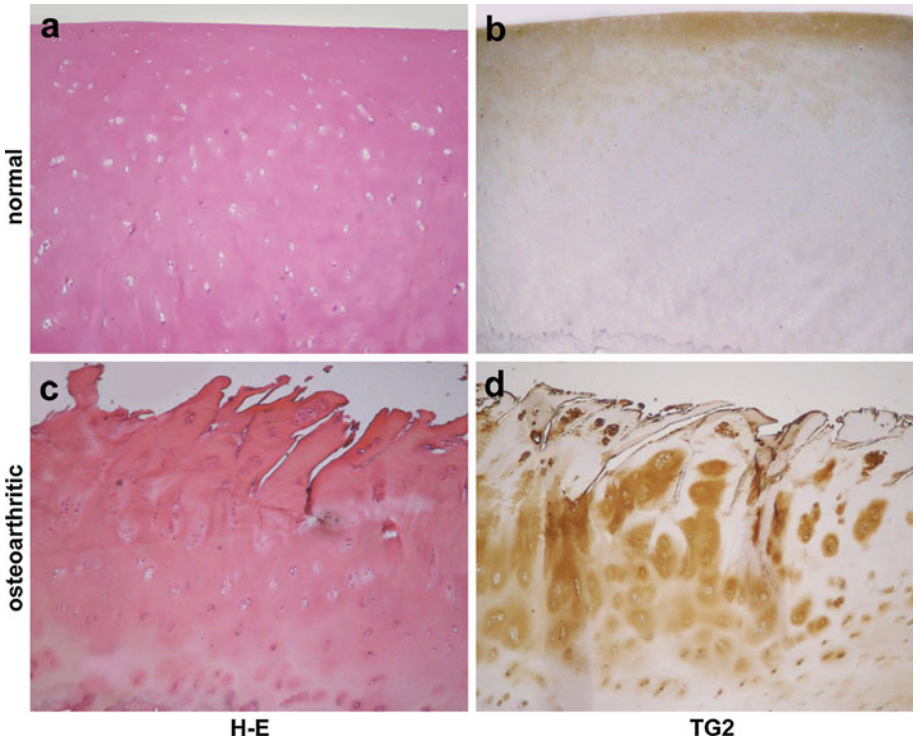


**Fig. 1** Human osteoarthritis and transglutaminase 2 (TG2) expression. In serial sections, representative histology of human head femur articular cartilage showing **a** smooth surface of normal cartilage with **b** low and focal expression of TG2. **c** Marked cartilage destruction in osteoarthritic degeneration of human head femur articular cartilage, where **d** immunodetection shows marked and diffuse TG2 expression in osteoarthritic chondrocytes and extracellular matrix; **a** and **c** Haematoxylin–Eosin (H–E) stain; **b** and **d** diaminobenzidine as chromogen; original magnification ×100

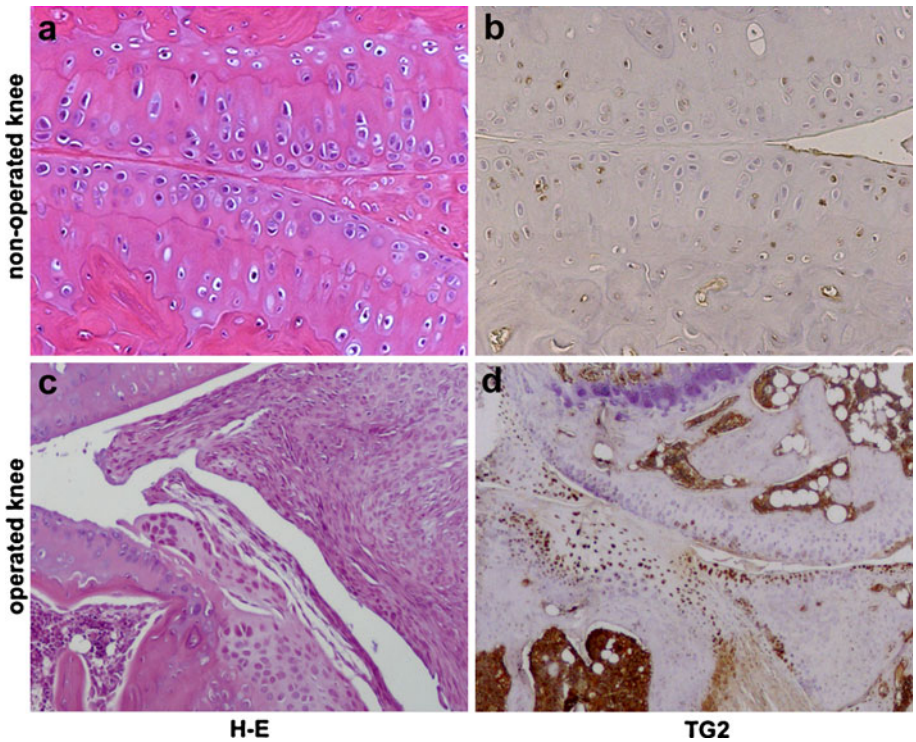


**Fig. 2** Experimental induction of osteoarthritis in mice and transglutaminase 2 (TG2) expression. **a** Normal cartilage in non-operated mouse knee characterized from **b** slight and focal TG2 immunodetection; **c** osteoarthritic cartilage destruction four weeks after surgical induction of knee instability is parallel to **d** the diffuse TG2 expression in wild-type mice; **a**, **c** Haematoxylin–Eosin (H–E); **b** and **d** diaminobenzidine as chromogen; original magnification ×100

et al. 1998, 2003). In the absence of TG2 or lack of the promotion of TG2 externalization, FXIIIA is unable to induce hypertrophy.

TG2 has been shown to cluster cell surface integrins and lead to integrin-dependent signaling and activation (Janiak et al. 2006). In murine joint cartilage after induction of experimental OA, upregulation of $\alpha 1\beta 1$ integrin develops in the superficial and upper mid-zone; in this condition, $\alpha 1\beta 1$ integrin appears to mediate cartilage-matrix remodeling (Zemmyo et al. 2003). Moreover, $\alpha 1\beta 1$-integrin is augmented in hypertrophic chondrocytes of OA articular cartilage (Zemmyo et al. 2003). Antibody-induced cross-

linking of $\alpha1\beta1$ integrin induces TG2 mobilization. Conversely, antibody-mediated blocking of $\alpha1$-integrin subunit suppresses the capacity of FXIIIA to induce both TG2 mobilization and type X collagen expression, but it does not block TG2-induced type X collagen expression (Johnson et al. 2008). Also $\alpha5\beta1$ integrin is involved, being essential for exogenous TG2 to promote chondrocyte hypertrophy (Tanaka et al. 2007). Specifically, FXIIIA is likely to cluster $\alpha1\beta1$ integrin and thereby it primes chondrocytes for TG2-dependent induction of hypertrophy (Tanaka et al. 2007). Multimerization of secreted S100A11 calgranulin in cultured human chondrocytes occurs in response to stimulation with CXCL8 (Johnson et al. 2001). TG2 plays a major role in mediating CXCL8-induced chondrocyte hypertrophic differentiation (Poole et al. 1989). It has been hypothesized that TG2 mediates transamidation of S100A11, so promoting the conversion of the secreted calgranulin into an inflammatory cytokine-like promoter of chondrocyte hypertrophy (Cecil and Terkeltaub 2008).

## Role of transglutaminase 2 in the induction of osteoarthritis

TG2 release is critical for the repair of multiple forms of tissue injury (Lorand and Graham 2003; Verderio et al. 2005; Bakker et al. 2005). The possibility to induce surgically experimental lesions in joint mice resembling those observed in human OA (Fig. 2) and availability of genetically modified mice strains allow to explore the contribution of single proteins and enzymes to the pathophysiological processes occurring in tissues and organs. Blot analysis confirms the increased TG2 expression also in OA mouse cartilage after the surgical induction of mouse joint knee instability (Fig. 3). The surgical induction of severe knee instability in knockout mice allows observing that OA is established also in the absence of TG2 tissue availability (Orlandi et al. 2009). This indicates the existence of alternative and TG2-independent pathways sustaining OA joint tissue remodeling. Stimulation in vitro of TG2 knockout mice chondrocytes results in the strong induction of other

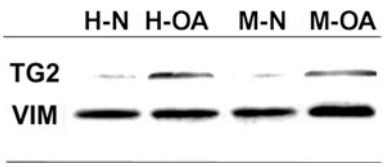


**Fig. 3** Translutaminase 2 (TG2) expression in normal and osteoarthritic cartilage. Representative blots showing the increase of TG2 protein content in human (H-OA) and mouse osteoarthritic cartilage (M-OA) compared to human (H–N) and mouse normal cartilage (M–N); vimentin (VIM) is used as control of protein loading

TGs, such as factor XIIIA (Johnson et al. 2003). Since TG2 knockout mice show a normal musculoskeletal apparatus (De Laurenzi and Melino 2001), it is likely that TG2-independent pathways sustain osteocartilaginous growth during bone development and growth and consequently, also during the OA process. The existence of redundant TG2-dependent and TG2-independent mechanisms for chondrocyte hypertrophy well explains why normal TG2 knockout mice demonstrate no gross phenotypic abnormalities in their developmental growth plates (Tarantino et al. 2009). Moreover, thrombin treatment in chondrocyte cultures increases FXIIIA mRNA and protein without affecting TG2 levels (Rosenthal et al. 2004). In the model of surgically induced severe OA, cartilage destruction was reduced whereas osteophyte formation was increased in TG2 knockout mice compared to wild-type mice (Orlandi et al. 2009). These data are in line with a distinct role of TG2 during the different phases of tissue remodelling that characterizes the osteoarthritic process, i.e., the maintenance of cartilage integrity and/or subchondral sclerosis and the development of extra-plate osteophytes. It has been previously reported that joint TG catalytic activity is diminished by 50% in unstimulated TG2 knockout mice (Johnson et al. 2003). It is possible to hypothesize a different role of TG2 in hypertrophied chondrocytes and osteoblasts during OA. IL-1$\beta$ is a contributor to the pathogenesis of OA (Attur et al. 1998). Since the chondrocyte response to IL-1$\beta$ in TG2 knockout mice appears reduced (Johnson et al. 2003), terminal chondrocyte differentiation and cartilage matrix calcification process may be impaired. It has been reported that human osteoblasts from sclerotic subchondral bone show increased TG2 gene expression and reduced matrix mineralization compared with non-sclerotic osteoblasts in vitro (Sanchez et al. 2008). In parallel, protein synthesis of TGF-$\beta1$ is higher in sclerotic than in non-sclerotic osteoblasts, while IL-1$\beta$ production is similar (Sanchez et al. 2008). TGF- $\beta1$ expression characterizes chondrocytes, osteoblasts and osteocytes and affects many aspects of bone formation (Janssens et al. 2005). In experimentally induced OA, reduced cartilage destruction in TG2 knockout mice compared to wild-type mice associates to the increase of TGF-$\beta1$ expression (Orlandi et al. 2009). Since endogenous TGF-$\beta1$ represents a crucial factor in the process of osteophyte formation, a compensatory overexpression of TGF-$\beta1$ may have an important function during osteophyte development. It is noteworthy that blocking of TGF-$\beta1$ receptor II prevents osteophyte formation (Scharstuhl et al. 2002). TGF-$\beta1$ influences TGs-catalyzed linkage to the matrix (Nunes et al. 1997), although other studies seem to suggest that the mechanism of TG2-induced mineralization does not involve TGF-$\beta1$ (Nurminskaya et al. 2003). Overexpression of TGF-$\beta1$ is also likely to maintain chondrocyte hypertrophy and sustain cartilage repair.

Moreover, lack of TGF-$\beta$1 results in osteoarthritis-like cartilage degeneration (Blaney Davidson et al. 2007). TGF-$\beta$1 is also able to induce osteophytes similar to those found in osteoarthritis and TGF-$\beta$ is highly expressed in osteophytes themselves (Janssens et al. 2005; Blaney Davidson et al. 2007). Altogether, these findings suggest that TGF-$\beta$1 overexpression in osteoarticular and non-cartilaginous tissues in TG2 knockout mice may favor cartilage integrity maintenance by diffusion but also promotes excessive extra-plate osteophyte formation.

## Transglutaminase 2 as a marker of osteoarthritis

Secreted biomarkers in the joint fluid, serum or urine, may reflect different stages of OA, so acquiring a potential role in the stratifying the risk of progression and/or monitoring the disease course, and therapeutic efficacy (Gray et al. 2008; Bauer et al. 2006; Poole 2003; Lohmander and Eyre 2005). In human joints, TG2 expression is increased in cartilage and non-cartilaginous tissues (Johnson et al. 2001; Summey et al. 2002). Some authors described TG2 as a biomarker of OA severity in Hartley guinea pig knees (Johnson et al. 2004). In the latter, both TG2 synovial fluid protein and knee cartilage mRNA levels correlated with the histological grade of OA (Johnson et al. 2004). Nevertheless, non-specific synovitis may also contribute to increase the amount of TG2 levels in synovial fluid, so participating to the synovial fluid TG2 pool in the OA disease in a non-specific manner. In this sense, additional studies are needed to explore and quantify the different contribute of cartilaginous and non-cartilaginous tissues to the increase bioavailability of TG2 in OA human joints and an accurate correlation with different phases of OA. As matter of fact, glucosamine, a potential OA therapeutic (Sawitzke et al. 2008), binds TG2 and (at low millimolar concentrations) inhibits TG2 at very low concentration (Kim et al. 2009).

## Conclusions

Histomorphometrical and biomolecular investigations document increased TG2 expression in human and experimental osteoarthritis. A schematic flow chart resuming main events leading to tissue remodeling characteristic of OA is reported in Fig. 4. TG2 is likely to mediate the hypertrophic differentiation of joint chondrocytes and interleukin-1-induced calcification. The increased level of TG2 in human and experimental OA suggests that TG2 joint tissue and synovial fluid expression may represent an additional marker to monitor the severity and/or progression of human OA. In addition, experimental models suggest that TG2-regulated pathways are differently modulated in hypertrophic chondrocytes of damaged cartilage and in areas of ossification during OA. An improved knowledge of the interplay between TG2 and inflammatory mediators may help to establish targeted therapy to better counteract OA tissue remodelling.

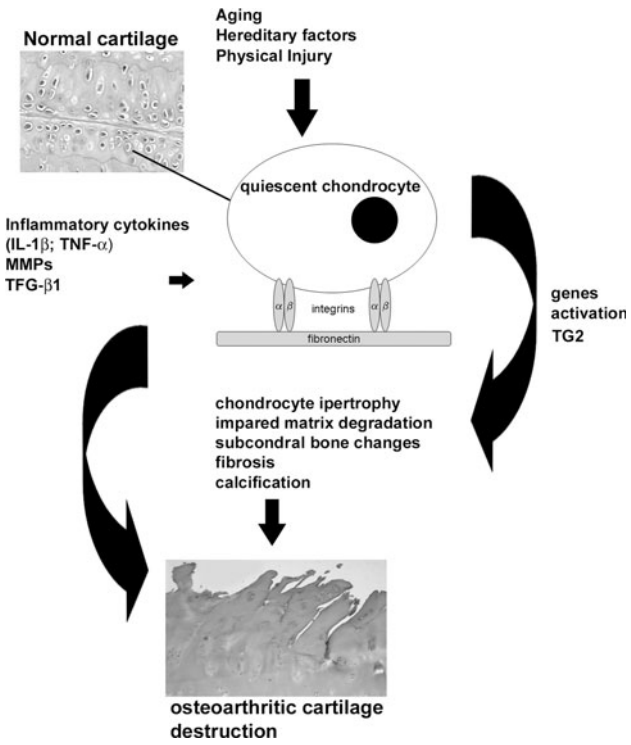


**Fig. 4** Schematic flow chart resuming the main events leading to chondrocyte activation and tissue remodeling occurring during osteoarthritis

**Conflict of interest** The authors declare that they have no conflict of interest.

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