External GTP-bound Transglutaminase 2 Is a Molecular Switch for Chondrocyte Hypertrophic Differentiation and Calcification*

Received for publication, January 26, 2005
Published, JBC Papers in Press, February 3, 2005, DOI 10.1074/jbc.M500962200

Kristen A. Johnson and Robert A. Terkeltaub‡
From the Veterans Affairs Medical Center, University of California, San Diego, La Jolla, California 92161

Chondrocyte maturation to hypertrophy, associated with up-regulated transglutaminase 2 (TG2) expression, mediates not only physiologic growth plate mineralization but also pathologic matrix calcification and dysregulated matrix repair in osteoarthritic articular cartilage. TG2−/− mouse chondrocytes demonstrate markedly inhibited progression to hypertrophic differentiation in response to both retinoic acid and the chemokine CXCL1. Here, our objectives were to test if up-regulated TG2 alone is sufficient to promote chondrocyte hypertrophic differentiation and to identify TG2 molecular determinants and potential downstream signals involved. TG2 activities, regulated by nucleotides and calcium, include cross-linking of cartilage matrix proteins, binding of fibronectin, and hydrolysis of GTP and ATP. Following transfection of TG2 site-directed mutants into chondrocytic cells, we observed that wild type TG2, and TG catalytic site and fibronectin-binding mutants promoted type X collagen expression and matrix calcification consistent with chondrocyte hypertrophic differentiation. In contrast, transfected mutants of TG2 GTP binding (K173L) and externalization (Y274A) sites did not stimulate chondrocyte hypertrophy. Recombinant TG2 treatment of bovine cartilage explants demonstrated that extracellular TG2 induced hypertrophy more robustly in the GTP-bound state, confirming an essential role of TG2 GTP binding. Finally, TG2 treatment induced type X collagen in a β1 integrin-mediated manner, associated with rapid phosphorylation of both Rac1 and p38 kinases that were inhibited by mutation of the TG2 GTP binding site. In conclusion, externalized GTP-bound TG2 serves as a molecular switch for differentiation of chondrocytes to a hypertrophic, calcifying phenotype in a manner that does not require either TG2 transamidation activity or fibronectin binding.

In physiologic endochondral mineralization, the maturation of chondrocytes from resting cells to hypertrophic cells is a critical event in the re-organization of the matrix and enhanced shedding of matrix vesicles that promote calcification (1, 2). The pathologic maturation of chondrocytes to hypertrophy in osteoarthritic (OA) articular cartilage (3, 4) also promotes matrix calcification (5). Chondrocyte hypertrophy is associated not only with expression of the stereotypic marker type X collagen but also with broad alterations in extracellular matrix synthesis, matrix catabolic and calcification-regulatory and angiogenesis-regulating gene expression programs mediating on cartilage beyond calcification (1, 5–8). Genes up-regulated in both hypertrophic growth plate and OA articular chondrocytes include the transglutaminase (TG) isoenzymes TG2 and FXIII A (9–12).

TGs catalyze a calcium-dependent transamidation reaction that catalyzes covalent cross-linking of substrates with available glutamine residues to primary amines (EC 2.3.2.13). Both TG2 and FXIII A, although lacking a signal peptide, are externalized to the pericellular matrix in both growth plate and articular cartilages (10, 13). In both normal and OA cartilage, TG2 appears to be the predominant catalytically active TG in the extracellular matrix (10). Recently, we observed that young TG2−/− mice have a normal skeletal phenotype and that TG2−/− knee articular chondrocytes become hypertrophic in response to the essential physiologic growth plate cartilage differentiation regulator C-type natriuretic peptide (14). However, TG2−/− chondrocytes exhibited markedly inhibited induction of chondrocyte hypertrophy and calcification in response to CXCL1 (15), a proinflammatory chemokine up-regulated in OA cartilages (16). TG2−/− chondrocytes also demonstrated suppressed induction of chondrocyte hypertrophy and calcification in response to retinoic acid (14), an inducer of pathologic calcification in vivo (17). Hence, TG2 is not only a marker of chondrocyte hypertrophy but also a mediator of chondrocyte maturation (14).

TG2 is localized primarily in the cytosol in a catalytically latent form (18). Additionally, TG2 has the potential to translocate to the nucleus, to be externalized, and to ultimately co-localize with proteins in the extracellular matrix or on the extracellular side of the plasma membrane (19). TG2 is one of a minority of TG isoenzymes that binds GTP (20) and TG2 dually functions as a TG and GTPase/ATPase (21). These distinct enzyme activities of TG2 are reciprocally regulated, partly at the level of conformation via the binding of Ca2+ essential for transamidation catalytic “TG activity” (21). Furthermore, binding of guanine nucleotides to TG2 suppresses catalytic TG activity, whereas binding of adenine nucleotides inhibits TG2 GTPase activity without altering TG activity (22). Constitutive cytosolic latency of TG2 is maintained through binding to GDP and ADP, but this state is subject to alterations that can promote TG activity, such as cellular influx of Ca2+ or externalization of TG2, taking TG2 from a high GTP, low calcium to low GTP, high calcium microenvironment (22).

Direct up-regulation of TG2 in chondrocytes by transfection markedly increased matrix calcification (11). TG catalytic ac-

* This work was supported by grants from the Department of Veterans Affairs and the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Veterans Affairs Medical Center, 3350 La Jolla Village Dr., San Diego, CA 92161. Tel.: 858-552-8585 (ext. 3519); Fax: 858-552-7425; E-mail: rterkeltaub@ucsd.edu.
1 The abbreviations used are: OA, osteoarthritis; sTG, soluble recombinant transglutaminase; WT, wild type; TG, transglutaminase; GTP-γS, guanosine 5′-3-O-(thiotriphosphate).
tivity of TG2 has the potential to modulate chondrocyte differentiation and calcification by stimulating increased activity of PLA2 (19) and by promoting conversion of transforming growth factor-β (23) from latency to an active state (12). TG2 can covalently cross-link several collagen subtypes, as well as fibronectin and a variety of calcium-binding and mineralization-regulatory proteins in the pericellular matrix of chondrocytes and osteoelasts (24). Hence, TG2-induced cross-linking of matrix proteins could regulate matrix-cell communication, and TG2 matrix stabilization and cross-linking of calcium-binding matrix proteins such as osteonectin and osteopontin (24, 25) potentially modulates growth of hydroxyapatite crystals (26, 27). However, in seminal work, Nurminskaya et al. (28) observed that hypertrophic chondrocyte-derived or exogenous hepatic TG2 stimulated maturation of preosteoelasts and accelerated mineralization, an effect not dependent on transforming growth factor-β activation and not prevented by the competitive transamination inhibitor putrescine.

TG2, acting at the internal and external faces of the plasma membrane, clearly regulates cell migration and differentiation and wound repair (18). In this context, TG2 can physically interact with the cytosolic tails of certain α integrin subunits (18), and TG2 binds to the gelatin-binding domain of fibronectin in a transamination-independent manner and acts as an integrin co-receptor for fibronectin (19), thereby modulating cell adhesion (29). TG2 may also function as a protein kinase (30). Significant biologic functions of guanine nucleotide-bound TG2 (19) include TG2 GTPase-related effects exerted on cell adhesion (29). TG2 may also function as a protein kinase (30). Significant biologic functions of guanine nucleotide-bound TG2 (19) include TG2 GTPase-related effects exerted on cell adhesion (29). TG2 may also function as a protein kinase (30).

Quantification of Matrix Calcification—To quantify matrix calcification by the transfected CH-8 cells, a previously described Alizarin Red S binding assay was performed, with results further validated in each experiment by direct visual observation of Alizarin Red S staining in each plate (37). Assays of TG2 Transamidation Activity, TG2 Externalization, and TG2-mediated Fibronectin Binding Activity—TG2 transamidation activity was determined through 2 mM biotinylamine incorporation into 20 mg/ml casein, as previously described (14). For qualitative evaluation of TG2 expression in conditioned media, transfected CH-8 cells were placed in serum-free medium for the last 8 h of incubation, and then the conditioned medium was precipitated as described above and separated by SDS-PAGE. TG2 was quantified in the conditioned medium after binding to a Nunc-ImmuNo Module plate (Nunc, Rochester, NY) for detection by direct enzyme-linked immunosorbent assay, using biotin-labeled TG2-specific antibody CUB7402 (38). To assess the ability of CH-8 cells overexpressing specific TG2 mutants to bind to fibronectin, Nunc-ImmuNo Module plates were coated with 0.5 μg/ml fibronectin, blocked with 3% bovine serum albumin, and incubated with aliquots of 25 μg in protein of cell lysates and detected as described (38).

Statistical Analyses—Statistical analyses were performed using the Student's t test (paired 2-sample testing for means) and error bars, where indicated, represent S.D.

RESULTS

Characterization of TG2 Site Mutants—To investigate TG2 structural determinants for regulation of chondrocyte hypertrophy and calcification, we engineered the initial group of TG2 site-directed mutants for study based on the following strategic considerations. First, TG2 Cys-277 is an essential amino acid for transamidation activity (35) and it participates in a catalytic triad conserved in all members of the transglutaminase family (21). Therefore we mutated each of the triad amino acids in TG2 (Cys-277, His-335, and Asp-358). Second, the hydrophobic pocket involved in the capacity of TG2 to bind and hydrolyze guanine nucleotides, includes Phe-174, Val-479, Met-483, Leu-582, and Tyr-583, whereas hydrolysis of GTP involves either Lys-173 or Arg-476 (21).

Materials and Methods

Reagents—All chemicals and other reagents were obtained from Sigma, unless otherwise indicated.

Generation of TG2 cDNA Mutants and Soluble Recombinant Forms of TG2—Human TG2 cDNA in pcDNA4/HisMax was the template for the generation of the following TG2 mutants using the QuikChange II site-directed mutagenesis kit (Strategene, San Diego, CA): fibronectin binding domain mutants TG2 2-3 MUT (A2G and E3A), TG2 7-8-9 MUT (LG7, EA8, and RA9), TG2 DEL5 (LS deleted), the TG2 GTP binding site mutant (K173L) (33), the TG2 mutant of an amino acid required for externalization (Y274A) (34), and three TG2 transamidation active site mutants for study (22). Recombinant forms of His-tagged TG2 were purified from transfected human fibroblastic HEK-293 cells using the Probond Purification Kit (Invitrogen). Where indicated, purified, soluble, recombinant TG2 proteins were used with and without treatment to generate magnesium nucleotide complexes (GTP or GTP-S with TG2) as described previously (22). In brief, 1 mM magnesium acetate was added to the nucleotide complex formation. To do so, MgCl2 was added to 25 μM GTP or GTP-Y-S and incubated on ice for 30 min with 0.1 μg of the soluble TG2 indicated.

Cell Culture Conditions, Transfections, and Isolation of Bovine Cartilage Explants—CH-8 cells, an SV40 immortalized clone of normal chondrocyte (36), were a generous gift of Dr. M. Hiramoto (Nihon University School of Medicine, Tokyo, Japan). CH-8 cells were cultured in Dulbecco's modified Eagle's medium high glucose supplemented with 10% fetal calf serum, 1% glutamine, 100 units/ml penicillin, 50 μg/ml streptomycin, and 50 μg/ml of ascorbic acid) or Medium B (Medium A supplemented with 1 mM sodium phosphate to promote calcification), unless otherwise indicated. Transient transfection of CH-8 cells was performed using Lipofectamine Plus (Invitrogen) according to the manufacturer's instructions, with ~60% transfection efficiency. Soluble TG2 preparations were added to CH-8 cells in 1 ml of Medium A.

For organ culture studies, 2 × 2-mm slices of cartilage were removed from the patellar groove and femoral condyles of normal bovine knees (Animal Technologies, Tyler, TX). The explants were placed in individual wells of a 24-well tissue culture plate in 200 μl of Medium A for 24 h, after which the explants were incubated for an additional 5 days with treatment as indicated. Frozen sections (5 μm) of the bovine cartilage were fixed in cold acetone for 10 min, and sections were immunohistochemically stained using a 1:50 dilution of the type X collagen antibody. Bound antibodies were detected with the avidin-biotin-peroxidase method using the reagents in the Histostain Plus kit (Zymed Laboratories Inc., San Francisco, CA).

SDS-PAGE/Western Blotting and Reverse Transcriptase-PCR—For SDS-PAGE/Western blotting analyses, conditioned media were collected and concentrated with trichloroacetic acid (15% v/v) for 10 min at 4 °C. Protein pellets from the concentrated media were washed in a 1:1 acetone/ethanol mixture and resuspended in 2% SDS, 0.2 M Tris, pH 6.8, and 40% glycerol and protein concentrations were determined with the bicinchoninic acid protein assay (Pierce). Cell lysates were harvested in 10 mM Tris, pH 7.6, 150 mM NaCl, 0.5 mM EDTA, 1 mM EGTA, 1% SDS containing a freshly added Complete Protease Inhibitor Mixture tablet (Roche Diagnostics). Aliquots of 0.01–0.05 μg of protein from each sample were separated by SDS-PAGE under reducing conditions and transferred to nitrocellulose, as described (14). Anti-type X collagen (Calbiochem, San Diego, CA), anti-TG2 (Upstate Biotechnology, Lake Placid, NY), anti-p-Rac1, anti-p-pp38, anti-p-p38 (Cell Signaling, Beverly, MA), and anti-tubulin primary antibodies were used at 1:1000 dilution in Western blotting studies, with luminescent detection employing Supersignal (Pierce) according to the manufacturer's instructions.

GTP-bound TG2 and Chondrocyte Hypertrophy 15005
Mutation of Lys-173 to Leu has been demonstrated to decrease GTP binding and hydrolysis as well as signal transduction regulatory activity of TG2 (33). Therefore, we generated TG2 K173L. Third, Tyr-274 and Cys-277 appear to be critical in mediating the release of TG2 into the extracellular matrix, as recently illustrated by mutagenesis-based studies (34). Hence, we also generated TG2 Y274A to probe for specific effects mediated by externalization of TG2 in chondrocytes but independent of effects on transamidation activity. For evaluation of effects of direct expression of TG2 site mutant cDNAs we chose to employ readily transfectable SV40-immortalized normal articular chondrocytic CH-8 cells. In transient transfection studies, we confirmed that each point mutation in the TG2 catalytic domain triad (C277G, H335A, and D358A) caused a significant decrease in the amount of TG activity when compared with overexpression of the wild type (WT) TG2. There was no significant change in transamidation activity of the GTP binding or externalization site mutants (Fig. 1A). Each TG2 mutant was found to express at comparable levels that were above the observed low background expression level detected in CH-8 cells, as assessed by SDS-PAGE/Western blotting (Fig. 1B).

**TG2 Induction of Matrix Calcification and Type X Collagen Expression Mediated by Externalization and Functional GTP Binding**—Transfection of WT TG2 into CH-8 cells, under pro-mineralizing conditions imparted by addition to the medium of 1 mm sodium phosphate, confirmed (11) the capacity of TG2 to directly promote calcification by chondrocytic cells (Fig. 2A). Mutation of amino acids within the TG catalytic triad did not significantly affect the ability of TG2 to stimulate matrix calcification (Fig. 2A). Conversely, both the externalization site mutant and GTP binding site mutant of TG2 were associated with a lower capacity to induce calcification than WT TG2 (Fig. 2A).

Next, we tested for potential direct TG2 induction of type X collagen expression, the stereotypic marker for chondrocyte hypertrophy (39). Because P promotes chondrocyte hypertrophy (8, 41), we employed conditions where medium was not supplemented with sodium P. Transfection of WT TG2 was discovered to be sufficient to induce type X collagen expression (Fig. 2B). The capacity of TG2 to induce type X collagen was retained by the catalytic site triad mutants but was depressed for the externalization site mutant and absent in the GTP binding site mutant (Fig. 2B).

Qualitative and quantitative assessment of extracellular TG2 levels in the conditioned media were carried out for CH-8 cells transfected with WT TG2 and the panel of TG2 site mutants (Fig. 3, A and B). The most striking observation was the marked diminution of conditioned medium TG2 levels associated with direct expression of the externalization site mutant (Y274A) and the GTP binding site mutant (K173L), in comparison to WT TG2 (Fig. 3, A and B).

In epithelial cells, TG2 externalization has been mechanistically linked with the capacity to bind fibronectin (42). Hence, we concurrently tested the potential role of fibronectin binding in TG2 externalization and induction of type X collagen expression in CH-8 cells. Deletion of the N-terminal seven amino acids of TG2 inhibits binding to fibronectin (42), but poses the risk of marked conformational change. Therefore, we designed point mutants and deletions of limited amino acids in the N-terminal domain of TG2. The first cDNA generated contained a deletion of Leu-5, the second had mutations at Ala-2 and Glu-3, and the third mutations at Leu-7, Glu-8, and Arg-9, because TG2 Glu-8 and Arg-9 have been directly implicated in fibronectin binding (43) (Fig. 4A). Each of these mutants was initially tested for the capacity to bind fibronectin (Fig. 4B). We observed that all three fibronectin domain mutants were significantly impaired in their ability to bind to fibronectin, with the Del 5 construct retaining less than 5% of this function relative to WT TG2. Significantly, all the other TG2 point mutants tested above also had a significant decrease in fibronectin binding, although to a lesser degree than mutants at the N-terminal fibronectin binding domain (Fig. 4B). Next we tested for externalization of the three fibronectin binding domain TG2 mutants after transient transfection into CH-8 cells, as above. Through SDS-PAGE/Western blot analysis, we saw comparable expression levels to the WT TG2 of the fibronectin binding domain mutants, both intracellularly and in the conditioned media (Fig. 4C). Treatment with each of the TG2 fibronectin binding domain mutants also induced type X collagen expression in CH-8 cells (Fig. 4D).

**Extracellular Mg-GTP-bound Forms of TG2 Markedly Induce Type X Collagen Expression**—To complement the preceding experiments based on transfection, and to assess the effects of extracellular TG2 in isolation, we next generated His-tagged WT and mutant TG2 soluble forms (sTG2) to add to chondrocytic cells. First, we observed that WT sTG2 treatment using 0.1 μg/ml protein induced a less robust increase in type X collagen expression in CH-8 cells relative to controls
than was observed in the preceding transfection studies. Additionally, the TG transamidation activity in the cell lysate after transfection with WT sTG2 was 5-fold less than that applied with the 0.1 \text{mM} \text{H9262}\text{g/ml} dose of soluble, extracellular TG2.

As cited above, GTP binding to TG2 induces decreased TG transamidation activity in association with a conformational change in TG2 (22). Based on a previous study demonstrating that Mg-nucleotide complexes are primary substrates for the TG2 GTP binding site (22), we added Mg-GTP or non-hydrolyzable Mg-GTP\text{S} to sTG2. We verified that even in the presence of calcium, both forms of Mg-GTP complexes induced a 5-fold reduction in TG transamidation activity (Fig. 5A). The sTG2 treated with either Mg-GTP or Mg-GTP\text{S} more robustly induced type X collagen than did WT sTG2 in CH-8 cells (Fig. 5B), indicating that hydrolysis of GTP was not necessary for this sTG2 “gain of function.” In parallel studies, simultaneous addition of Mg-ATP or Mg-ATP and Mg-GTP to WT sTG2 to inhibit GTPase activity (22) allowed sTG2 to more robustly induce type X collagen than sTG2 alone (not shown).

Next, we tested the effects of sTG2 in cartilage organ...
culture, to assess modulation of chondrocyte differentiation by TG2 in a more physiologic milieu. To do so, bovine articular cartilage explants were incubated for 5 days in the presence or absence of sTG2 (1 g/ml) with and without Mg-GTP treatment. Frozen sections of cartilage were immunohistochemically stained for type X collagen. Weak type X collagen induction was observed in response to sTG2, with a markedly more robust response demonstrated with sTG2 that was Mg-GTP complexed (Fig. 7). Taken together, these results were consistent with externalized TG2 functioning in the GTP-bound state as a molecular switch for the induction of chondrocyte type X collagen expression.

β1 Integrin Antibody Treatment Impairs TG2 Induction of Type X Collagen Expression—TG2 interacts with the extracellular domains of β1 and β3 integrin subunits and forms 1:1 complexes on the surface of cells (29). Furthermore, collagen deposition and the enlargement of growth plate chondrocytes during hypertrophy are mediated in part by interactions of the extracellular matrix with β1 integrins (41). Hence, we next tested the role of β1 and β3 integrins during TG2-induced hypertrophy. To do so, chondrocytic cells were pretreated for 1 h with 1 μg/ml antibodies to each integrin prior to treatment with 0.1 g/ml recombinant WT TG2 or the TG2 K173L mutant for 3 days. SDS-PAGE/Western blotting of the cell lysates revealed inhibition of WT TG2-induced type X collagen expression when the cells were treated with the β1 integrin antibody, but not after treatment with the β3 integrin (Fig. 8). TG2 mediates cell adhesion and migration partly by acting...
as an integrin co-receptor for fibronectin (45), and both integrin signaling and certain effects of TG2 on cells are mediated by focal adhesion kinase signaling (30). During modulation of cell-matrix interactions, focal adhesion kinase has been demonstrated to act through small GTPases, such as Rac1 (46), which is also known to be critical in regulation of cardiac hypertrophy (47). Significantly, p38 mitogen-activated protein kinase, one of the downstream mediators of integrin signaling (46) is an essential transducer of chondrocyte hypertrophy in vitro (48). We observed that Rac1 phosphorylation was induced within 5 min of exposure to 0.1 μg/ml WT sTG2 (Fig. 9) and p38 phosphorylation was induced within 10 min of treatment with WT sTG2 (Fig. 9). But stimulation of chondrocytic cells with 0.1 μg/ml of the K173L mutant sTG2 revealed that the soluble TG2 GTP binding site mutant had a diminished capacity to induce Rac1 and p38 phosphorylation relative to WT sTG2 (Fig. 9). Therefore, soluble extracellular TG2 induced rapid signal transduction in chondrocytes, as well as type X collagen expression and calcification, in a manner dependent on integrity of the guanine nucleotide binding site.

**DISCUSSION**

TG2 expression has been implicated as a major, direct mediator of not only osteoblast maturation (28) but also chondrocyte maturation to hypertrophy associated with matrix calcification in response to retinoic acid and CXCR2-binding chemokines (14, 15). Here, we discovered that the ability of extracellular TG2 to bind GTP was central to inductive effects of a gain of function of TG2 on chondrocyte type X collagen expression and calcification. Significantly, these alterations of chondrocyte differentiation were not dependent upon TG2 transamidation activity or on binding of fibronectin.

Although externalization of TG2 was required to induce type X collagen and calcification it was not sufficient to do so, as illustrated by the failure of exogenous treatment with the TG2 GTP binding site mutant Lys-173 to induce these effects in chondrocytic cells. These results were buttressed by the markedly augmented induction of type X collagen by WT TG2 treated with Mg-GTP. Furthermore, treatment of chondrocytic cells with a soluble form of the poorly externalized Tyr-274 mutant of TG2 also robustly induced type X collagen expression, which was not the case in cells transfected with the Tyr-274 mutant. TG2 can hydrolyze GTP to GDP at a rate comparable with that of other G proteins (22), but based on TG2 crystal structure, it has been proposed that TG2 binding of GDP, or exchange with GTP, are particularly avid (21). Hence, the results of this study suggest that the guanine nucleotide-bound form of TG2 may stably assume an ideal conformation for externalization by chondrocytic cells and also trigger type X collagen expression and calcification selectively in response to specific agonists in the chondrocyte. It will be of interest to determine whether such a mechanism mediates our observations that interleukin-8/CXCL8, which is up-regulated in OA articular cartilages (16, 49) and stimulates chondrocyte hypertrophy, increases TG2 translocation from the cytosol to the conditioned medium in CH-8 cells, an activity not shared by
C-type natriuretic peptide, which induces cultured chondrocyte hypertrophy in a TG2-independent manner (14). In the growth plate, TG2 has been observed to remain intracellular in situ during growth plate chondrocyte proliferation and maturation, and to become externalized in the hypertrophic zone (9). Moreover, externalized TG2 has been detected in OA articular cartilages (10). Therefore, our current observations underscore the functional importance of TG2 release by the chondrocyte. TG2 lacks a signal peptide and has not been observed to transition into a cellular secretory pathway through the Golgi. Externalization is mediated by TG2 conformation, regulated in part by the Tyr-274 and Cys-277 residues (34), and possibly proceeds by a process similar to that proposed for translocation of TG2 into the nucleus (19). It will be of interest to further understand how chondrocytes externalize TG2, and whether chondrocytes express a tissue-selective chaperone for TG2 release.

In this study, TG2 GTPase activity was not required to switch on chondrocyte type X collagen expression, as demonstrated by results of cell treatment with non-hydrolyzable GTP bound to sTG2. Moreover, inhibition of GTPase activity of WT TG2 (through Mg-ATP and Mg-GTP addition) was associated with retention of the capacity to promote the induction of type X collagen. We determined that although the fibronectin binding domain of TG2 was not critical for induction of chondrocyte hypertrophy, the interaction between TG2 and β1 integrins may at least partially mediate the induction of hypertrophy in chondrocytic cells. Our exploratory results of signal transduction in response to WT sTG2 as opposed to K173L sTG2 lead us to speculate that conversion of TG2 to a GTP-bound conformational state allows TG2 to be directly recognized at the level of the chondrocyte plasma membrane. Alternatively, conversion of TG2 to a GTP-bound conformational state may allow TG2 to complex with one or more extracellular matrix proteins and external domains in integrins to induce rapid signaling events including phosphorylation of Rac1 or p38 mitogen-activated protein kinase. Another possibility is that intercalation of secreted GTP-bound TG2 into the extracellular matrix may indirectly regulate how matrix constituents such as collagens interact with the chondrocyte to stimulate signal transduction and affect cell differentiation.

TG2 interconversion between guanine nucleotide-bound transamidation-latent and calcium-bound transamidation active forms has the corresponding capacity to switch off and

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switch on a variety of variation-related TG2 functions (22). In the maintenance of vascular tone and remodeling of arteries, early steps appear to be mediated in part by smooth muscle cell activation through TG2 effects on protein cross-linking, whereas downstream events may require TG2 G-protein functions (50). As such, our results with clonal chondrocytic cells in fixed culture conditions should not be interpreted to exclude a role for TG2 transamination activity in modulating chondrocyte differentiation or calcification in the growth plate or in OA cartilage in situ (9, 51).

Significantly, TG2 binding to GTP or GDP renders TG2 highly resistant to proteolytic degradation (22). As such, steady release of bound guanine nucleotides from TG2 outside the cell could help preserve and fine-tune the TG2 functional potential in the protease-rich chondrocyte extracellular environment for the prolonged time intervals needed for chondrocytes to mature as well as to modify and calcify their matrix. The known increase in ectonucleotide pyrophosphatase pyrophosphohydrolase activity (EC 3.6.1.8) in articular cartilages with chondrocalcinosis and/or OA (23) also provides the theoretic potential to promote increased transamination activity of extracellular TG2 via enhanced extracellular GTP hydrolysis to GMP and PPi, in articular cartilages. Indeed, we observed that total TG transamination activity was increased on average greater than 40-fold in human knee meniscal fibrocartilages with late stage OA relative to normal menisci (11). In this context, it also is noteworthy that interleukin-1, which markedly suppresses chondrocyte ectonucleotide pyrophosphatase pyrophosphohydrolase expression (23), induces TG2-dependent calcification of chondrocyte ectonucleotide pyrophosphatase pyrophosphohydrolase (11). In this context, it also is noteworthy that interleukin-1, which markedly suppresses chondrocyte ectonucleotide pyrophosphatase pyrophosphohydrolase expression (23), induces TG2-dependent calcification in association with a TG2-dependent rise in TG activity in chondrocytes, yet interleukin-1 does not induce chondrocyte hypertrophy in vitro (14). It will be of interest to discern the mechanisms that dissociate TG2-mediated hypertrophy from matrix calcification.

An important component of this study was the confirmation of the induction of type X collagen expression by Mg-GTP bound sTG2 using cartilage organ culture. However, limitations of this study included the primary utilization of SV40-immortalized chondrocytic CH-8 cells. The CH-8 cells were chosen for study because of their human origin and because they maintained a chondrocytic phenotype in culture. Additionally they allowed efficient transfection and useful comparisons of transfected cells and cells treated with exogenous TG2. But CH-8 cells in monolayer culture cannot be held to be a precise surrogate for primary chondrocytes in three-dimensional non-adherent culture, nor for cartilage in organ culture or in situ. Additional limitations of this study included administration to chondrocytic cells of recombinant exogenous TG2 prepared from fibroblastic cells, thereby possibly imposing a lack of chondrocyte-specific post-translational processing, secretion, and proteolysis of TG2. CH-8 cells express both FXIIIa and FXIIIA and proteolytic activity was increased on average greater than 40-fold in CH-8 cells in monolayer culture cannot be held to be a precise surrogate for primary chondrocytes in three-dimensional non-adherent culture, nor for cartilage in organ culture or in situ. Additional limitations of this study included administration to chondrocytic cells of recombinant exogenous TG2 prepared from fibroblastic cells, thereby possibly imposing a lack of chondrocyte-specific post-translational processing, secretion, and proteolysis of TG2. CH-8 cells express both FXIIIa and TG2 constitutively, effects that could have modulated ob-
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