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ORIGINAL ARTICLE

Tissue-specific responses to loss of transglutaminase 2

Stephanie Deasey · Shobana Shanmugasundaram · Maria Nurminskaya

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Abstract Of the eight catalytic transglutaminases (TGs), transglutaminase 2 (TG2) has been the most comprehensively studied due to its ubiquitous expression in multiple cell types. Despite the observed critical role for this enzyme in multiple biological processes in vitro, TG2 knockout mouse models have shown no severe developmental phenotypes, suggesting compensation by other TGs. To begin characterization of the compensating mechanisms, we analyzed total transamidating activity and expression patterns of all catalytically active TGs in seven different tissues/organs from wild-type and TG2 knockout mice. Inhibitory analysis with TG2-specific inhibitor KCC-009 suggests that relative contribution of TG2 in total transamidating activity differs in various tissues. Accordingly, our data indicate tissue-specific mechanisms of compensation for the loss of TG2, including transcriptional compensation in heart and liver versus functional compensation in aorta, kidney and skeletal/cartiagenous tissues. On the contrary, no compensation has been detected in skeletal muscle, suggesting a limited role for the TG2-mediated transamidation in normal development of this tissue.

Keywords Transglutaminase · Inhibitors · Cartilage · Heart · Aorta · Liver · Kidney · Muscle · Compensation

Abbreviations

TG	Transglutaminase
FXIIIa	Factor XIIIa
TG1-7	Transglutaminase 1–7
WT	Wild type
$TG^{-/-}$	Transglutaminase 2 null

S. Deasey · S. Shanmugasundaram · M. Nurminskaya (✉)
Department of Biochemistry and Molecular Biology,
University of Maryland School of Medicine,
Baltimore, MD 21201, USA
e-mail: mnurminskaya@som.umaryland.edu

Introduction

The mammalian transglutaminase (TG) protein family consists of nine proteins with eight zymogens/enzymes, designated Factor XIIIa (FXIIIa) and TG1–7 in addition to a structural protein, protein 4.2, which lacks catalytic activity. TG-mediated reactions are essential for multiple biological processes ranging from blood coagulation to skin barrier formation and extracellular matrix assembly (reviewed in Griffin et al. 2002; Lorand and Graham 2003). These enzymes function in a wide range of biological processes by catalyzing three types of posttranslational modifications: transamidation, esterification and hydrolysis (Iismaa et al. 2009). In addition, TG2, TG4 and TG5 can bind and hydrolyze GTP, which inhibits their transamidase catalytic activity (Iismaa et al. 1997; Spina et al. 1999; Candi et al. 2004). Interestingly, while these distinct enzymes are able to recognize the same protein substrate, in vivo they generally exhibit substrate specificity.

TG2 is considered the most fascinating and comprehensively studied of this diverse enzyme family. It is constitutively expressed in many cell types, including, but not limited to, endothelial cells, vascular smooth muscle cells and fibroblasts (Thomazy and Fesus 1989). Additionally, its expression correlates with cell differentiation in some cell lineages, such as the osteochondrogenic lineage (Thomazy and Fesus 1989; Aeschlimann et al. 1993; Nurminsky et al. 2011). In addition to the traditional TG activities, TG2 has been reported to act as a protein kinase (Mishra and Murphy 2004) and a protein disulfide

isomerase (Hasegawa et al. 2003), as well as to facilitate cell–matrix interaction independently from its enzymatic activity (Akimov et al. 2000; Xu et al. 2006; Dardik et al. 2006). TG2 is localized to both the extracellular matrix and multiple cellular compartments, with ample in vitro studies showing a wide range of TG2 functions from cell adhesion to cell death (Griffin et al. 2002; Fesus and Szondy 2005; Iismaa et al. 2009; Nadalutti et al. 2011).

Two mouse knockout models for TG2 were developed simultaneously by different groups to evaluate its in vivo function (De Laurenzi and Melino 2001; Nanda et al. 2001). These models were based on disrupting mouse Tgm2 gene around exon 5 (which encodes part of the catalytic core domain) and both showed absence of TG2 protein in homozygote progeny. However, no obvious developmental phenotype was observed in either of these mouse models despite the previously demonstrated in vitro role for TG2 in multiple biological processes. These phenotypes suggest the common biological phenomenon of backup compensation, which occurs when functionally overlapping proteins compensate for the loss of each other. For example, such rescuing/compensation mechanisms have been described for the family of small leucine-rich proteoglycans (Ameye and Young 2002). In TG2 null ($TG2^{-/-}$) chondrocytes, the compensatory activation of FXIIIa has been observed, resulting in an unchanged level of total transamidase activity (Nurminskaya and Kaartinen 2006; Tanaka et al. 2007). In this study, we analyzed the relative contribution of the TG2-mediated catalytic activity in seven different wild-type (WT) mouse tissues. Next, we examined enzymatic activity in the $TG2^{-/-}$ tissues and analyzed the expression of the eight TGs in the $TG2^{-/-}$ versus wild-type tissues to identify possible tissue-specific compensation mechanisms supporting the $TG2^{-/-}$ phenotype.

Materials and methods

Animals and tissue dissection

Animals used were CB57/B6 and $TG2^{-/-}$ mice (a kind gift from Robert Graham, Victor Chang Cardiovascular Institute, New South Wales, Australia). All procedures were approved by the institutional animal care and use committee at the University of Maryland Medical School and were conducted in compliance with NIH guidelines for the care and use of laboratory animals. The 4-5 week-old mice of each genotype were used to dissect the sternum (designated as non-hypertrophic cartilage), knee joint (designated as ossifying cartilage), skeletal muscle from the limb, aorta, heart, kidney and liver. Tissues from two to three animals were pooled together and total RNA was isolated with Trizol reagent (Invitrogen).

Real-time PCR

Primers for TGs were designed using NCBI primer design software. The real-time PCR was run using first-strand synthesized cDNA as a template on a BIO RAD CFX96 Real-Time System following the manufacturer's instructions for heat activation, amplification and melting curves for 45 cycles. Expression levels were normalized to RLP-19 mRNA with anything showing expression after 35 cycles being disregarded for analysis.

TG activity assay

Total TG cross-linking activity in mouse tissue was assayed by incorporation of the biotinylated pentylamine Ez-link Pentylamine-Biotin (Pierce, IL) into *N,N′*-dimethylcasein (Sigma-Aldrich, MO, USA) in the ELISA-like assay as previously described (Trigwell et al. 2004). The 96-well microtiter plates (Maxisorp NUNC, UK) were incubated overnight with 250 µl of 1 mg/ml *N,N′*-dimethylcasein (Sigma-Aldrich, MO, USA) in 5 mM sodium carbonate (pH 9.8), and blocked with 200 µL of 0.1% bovine serum albumin (BSA) (HyClone, Ut) in 5 mM sodium carbonate (pH 9.8) for 1 h at 37°C. Mouse tissue was lysed in 5 mM Tris–HCl, pH 7.5, 0.25 M sucrose, 0.2 mM $MgSO_4$, 2 mM DTT, 0.4 mM PMSF, 5 µg/ml leupeptine and 0.4% Triton X-100 (lysis buffer), centrifuged and the TG-containing supernatant was used for further assays. Purified guinea pig liver transglutaminase 2 (gplTG2) (Sigma-Aldrich, MO, USA) was used as a standard for activity tests. For inhibitory studies, mouse lysates (20 µg total protein) or purified gplTG2 (75 ng purified protein) was pre-incubated with 30 µM inhibitors for 1 h at 37°C. The reaction was carried out in 100 mM Tris–HCl, pH 8.5, 6.7 mM $CaCl_2$, 13.3 mM DTT and 2.5 mM Ez-link Pentylamine-Biotin (Pierce, IL) for 1 h at 37°C. Incorporated Ez-link Pentylamine-Biotin was detected with 1:5000 ExtrAvidin-Peroxidase (Sigma, MO, USA) and Super AquaBlue ELISA Substrate (eBioscience, CA) followed by reading the absorbance at 405 nm on a Polarstar Optima plate reader.

Data and statistical analysis

Statistical significance was calculated by the student's *t* test ($*P \leq 0.05$; $**P \leq 0.005$); error bars demonstrate the standard error mean.

Results and discussion

Tissue-specific expression of TG family

The expression pattern of all catalytic TG enzymes was analyzed in wild-type (WT) mouse tissues that were chosen

based on previously implicated roles for TG2 in development and pathology. Earlier studies have reported expression of these proteins in various cell types and tissues; however, to our knowledge, a comparative analysis of their expression in various tissues has been limited. The summary of the results is shown in Fig. 1. We were unable to detect the expression of TG7 and TG4 in any of the tissues, in agreement with the previous studies identifying restricted expression of TG4 protein to the prostate (Ho et al. 1992). Expression of FXIIIa varies between the analyzed tissues with lowest expression of FXIIIa observed in the liver and kidney where the regulatory/carrier B subunit of the heterotetrameric plasma coagulation Factor XIII is expressed. Despite FXIIIa's historical identification as a "plasma" transglutaminase, our results combined with previous reports (http://www.ncbi.nlm.nih.gov/UniGene) demonstrate its expression in a vast variety of tissues.

TG1 and TG3 are expressed at similar levels in all analyzed internal tissues which were cleared of skin, where expression of these enzymes is required for stabilization of the cornified cell envelope (Kuramoto et al. 2002; Candi et al. 2002). TG1 identified in the skeletal muscle, aorta and skeletal tissue of mice may function to stabilize adherent junctions, similar to its previously proposed role in the lung epithelium, liver, kidney and endothelium of the myocardial microvasculature (Hiiragi et al. 1999; Baumgartner et al. 2004). However, the biological role of TG3 in these skinless tissues remains largely unknown.

The highest level of TG2 expression was detected in the aorta, correlating with its previously proposed role in vascular remodeling (Bakker et al. 2006). Similarly, the highest expression of TG5 was also detected in the aorta. Expression of both TG2 and TG5 in the liver was significantly lower than that of the other analyzed tissues, while in skeletal muscle and joint expression of TG5 was undetectable. Despite this low level of expression, TG2 still contributed almost 80% of the total transamidating activity in the liver as detected by using the TG2-specific inhibitor KCC-009 (Choi et al. 2005) (Table 1). The tissue-specific expression pattern of TG3 is similar to that of TG2, possibly implicating a common long-range regulatory mechanism for these genes localized on the same chromosome (Grenard et al. 2001). Expression of TG6 was detected in the cardiovascular tissues, both heart and aorta, and also in kidney, but absent from skeletal muscle and joint, adding new sites of expression to the previously described skin, eyes and neurons (http://www.ncbi.nlm.nih.gov/UniGene; Hadjivassiliou et al. 2008). Thus, TG6 seems to be an isoform with a wider distribution than previously believed.

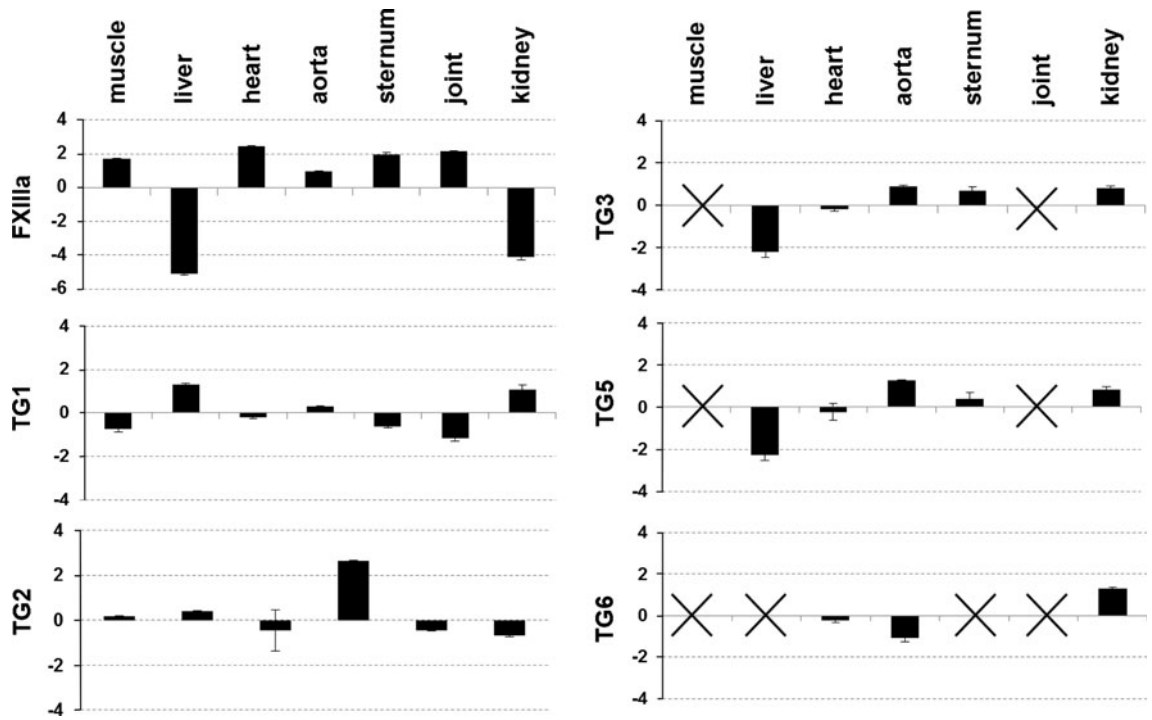

Fig. 1 Expression pattern of TGs in mouse tissues. Quantitative real-time PCR was employed to analyze the expression of each enzyme and the data were normalized to the expression of the housekeeping gene RPL-19. Tissue-specific levels of expression for each enzyme were compared to its average expression between all analyzed tissues, with *crosses* indicating no expression detected above the determined threshold ($P < 0.05$)

Table 1 Percent inhibition of TG activity by KCC-009 in wild-type mouse tissues

Organ/tissue	Activity (U/mg Protein)	Activity (U/mg protein) with 30 μM KCC-009 treatment	Percent inhibition with KCC-009 (%)
Skeletal muscle	7.39 ± 0.34	2.85 ± 0.60	61.37
Liver	46.74 ± 0.19	10.05 ± 0.84	78.50
Heart	29.00 ± 2.52	12.74 ± 0.70	56.06
Aorta	8.11 ± N.A.	6.16 ± N.A.	23.94
Sternum	4.53 ± 1.07	Not detectable	100
Joint/ossifying cartilage	19.80 ± 0.87	7.33 ± 0.55	62.98
Kidney	20.99 ± 1.46	11.80 ± 0.78	43.78

In conclusion, our data revealed wide and varying patterns of expression for many TGs, implicating an additional level of complexity in the biological functions of TGs and potentially different compensation mechanisms for the loss of TG2 in various tissues.

Compensation for TG2 loss in skeletal muscle

Expression of three TGs was identified in the WT skeletal muscle—with TG2 and FXIIIa expressed at relatively high levels and TG1 at much lower levels (Fig. 2a). The TG2 inhibitor KCC-009 inhibited approximately 60% of the total transamidating activity (Table 1), attributing this activity to TG2. Accordingly, genetic ablation of TG2 resulted in a 60% reduction in total transamidating activity in the skeletal muscle (Fig. 2b), corresponding to the portion of TG activity attributed to endogenous TG2. This result indicates a lack of compensation for the loss of TG2 by other TGs, suggesting that TG2 functions in the skeletal muscle independently from its catalytic activity. In agreement with this conclusion, expression of TG1 did not change significantly and expression of FXIIIa was significantly reduced in the $TG2^{-/-}$ versus wild-type muscle (Fig. 2c).

Compensation for TG2 loss in liver

Similar to muscle, the same three TGs—TG1, FXIIIa and TG2—were expressed in the liver (Fig. 3a). Approximately, 80% of total transmidating activity was attributed to TG2 (Table 1); however, the total transamidating activity in the $TG2^{-/-}$ liver was reduced to 42% (Fig. 3b), indicating a possible compensation effect by other TGs. Expression analysis of the $TG^{-/-}$ liver revealed a twofold increase in FXIIIa expression (SEM 0.157, $P < 0.05$) and a 1.6-fold increase in TG1 expression (SEM 0.076, $P < 0.005$) (Fig. 3c). No other TGs were induced in the $TG2^{-/-}$ liver tissue, suggesting transcriptional compensation for the loss of TG2 via increased expression of TG1 and FXIIIa, which are expressed in the WT tissue. Interestingly, the combined activity of TG1 and FXIIIa in the liver constitutes approximately 22% of the total transamidating activity, in contrast to their 40% contribution in skeletal muscle (Table 1). Two possible explanations can be proposed—first, that TG1 and/or FXIIIa, both requiring proteolytic activation, are activated to a higher extent in the muscle than the liver. Alternatively, TG2 in the muscle may be less active than in liver, possibly due to regulation via

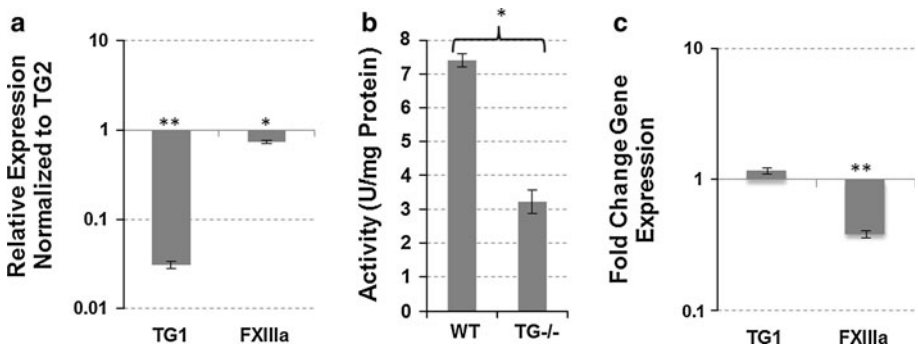

Fig. 2 TG expression and activity in skeletal muscle tissue from wild-type and TG2 knockout mice. **a** Real-time PCR analysis showing expression of TGs compared to TG2 expression in wild-type mouse skeletal muscle. **b** TG cross-linking activity assayed by pentylamine-biotin incorporation into *N,N′*-dimethylcasein. Total protein lysates from wild-type and TG2 knockout mouse skeletal muscle were used. **c** Real-time PCR analysis showing expression of TGs in TG2 knockout mouse skeletal muscle compared to wild-type tissue (*$P \leq 0.05$; **$P \leq 0.005$)

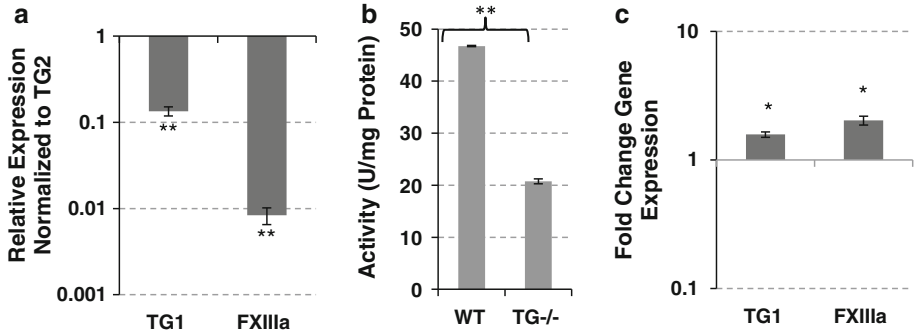

Fig. 3 TG expression and activity in liver tissue from wild-type and TG2 knockout mice. **a** Real-time PCR analysis showing expression of TGs compared to TG2 expression in wild-type mouse liver. **b** TG cross-linking activity assayed by pentylamine-biotin incorporation into *N,N′*-dimethylcasein. Total protein lysates from wild-type and TG2 knockout mouse liver were used. **c** Real-time PCR analysis showing expression of TGs in TG2 knockout mouse liver compared to wild-type liver tissue. (*$P \leq 0.05$; **$P \leq 0.005$)

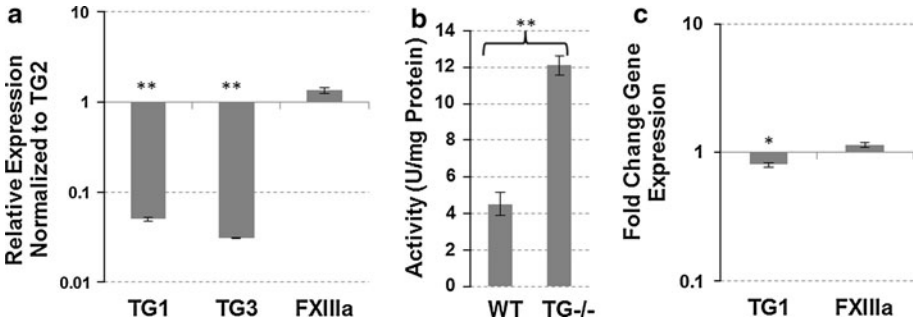

Fig. 4 TG expression and activity in non-hypertrophic cartilage from wild-type and TG2 knockout mice. **a** Real-time PCR analysis showing expression of TGs compared to TG2 expression in wild-type mouse non-hypertrophic cartilage. **b** TG cross-linking activity assayed by pentylamine-biotin incorporation into *N,N′*-dimethylcasein. Total protein lysates from wild-type and TG2 knockout mouse non-hypertrophic cartilage were used. **c** Real-time PCR analysis showing expression of TGs in TG2 knockout mouse non-hypertrophic cartilage compared to wild-type tissue. (*$P \leq 0.05$; **$P \leq 0.005$)

the Ca^{2+}/GTP binding balance. The significantly lower levels of total TG activity in the muscle (Table 1) favor the latter explanation.

Compensation for TG2 loss in non-hypertrophic cartilage

In our previous studies, TG2 was shown to regulate the early stages of chondrogenic differentiation in mesenchymal cells (Nurminsky et al. 2011). However, the cartilaginous tissues in $TG2^{-/-}$ mice have been found to be phenotypically normal, suggesting a compensation mechanism by other TGs. We analyzed the expression of the eight TG enzymes in the sternum cartilage, which is composed of mostly non-hypertrophic chondrocytes. Relative to TG2 expression, FXIIIa was expressed at comparable levels, while TG1 and TG3 were expressed at much lower levels (Fig. 4a). The TG2 inhibitor KCC-009 dramatically decreased total transamidating activity in the wild-type sternum (Table 1), suggesting that TG2 was the major active enzyme in this tissue. Unexpectedly, genetic ablation of TG2 resulted in a significant threefold increase in total TG activity (SEM 0.6, $P < 0.005$) (Fig. 4b). However, no significant change in FXIIIa expression and a slight down-regulation of TG1 was observed (Fig. 4c), and expression of TG3 was reduced to almost undetectable levels (data not shown). These results implicate catalytic rather than transcriptional activation of the FXIIIa, TG1 and/or TG3 in the $TG2^{-/-}$ cartilage, and present an example of functional in contrast to transcriptional compensation for the loss of TG2 that was proposed for liver.

Compensation for TG2 loss in joint/ossifying cartilage

Differentiating chondrocytes of the growth plate have been shown to express both TG2 and FXIIIa (Aeschlimann et al. 1993; Nurminskaya and Linsenmayer 1996). Here, we analyzed the expression of eight TGs in the whole joint, which includes the cartilaginous growth plate, articular cartilage, periosteum and secondary ossification center. All soft tissues, including tendon, muscle, ligament, bursa and synovial sac, were carefully removed at dissection. In addition to the previously described expression of TG2 and FXIIIa, TG1 was expressed in the joint tissues, although at much lower levels (Fig. 5a). Specific inhibition of TG2 with KCC-009

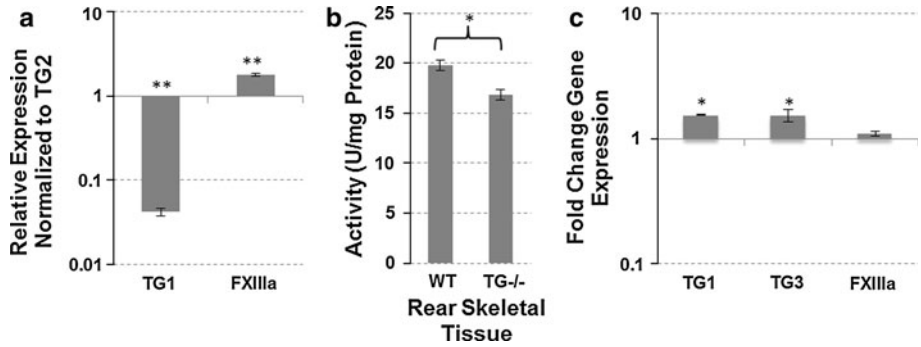

Fig. 5 TG expression and activity in joint/ossifying cartilage from wild-type and TG2 knockout mice. **a** Real-time PCR analysis showing expression of TGs compared to TG2 expression in wild-type mouse joint/ossifying cartilage. **b** TG cross-linking activity assayed by pentylamine-biotin incorporation into *N,N′*-dimethylcasein. Total protein lysates from wild-type and TG2 knockout mouse joint/ossifying cartilage were used. **c** Real-time PCR analysis showing expression of TGs in TG2 knockout mouse joint/ossifying cartilage compared to wild-type tissue. (*$P \leq 0.05$; **$P \leq 0.005$)

significantly inhibited the transamidase activity in the wild-type joint tissue (Table 1). However, genetic ablation of TG2 has minor effect on the transamidase activity (Fig. 5b), indicating that enzymes other than TG2 can support the transamidase activity in the skeletal tissues, in agreement with earlier studies (Nurminskaya et al. 1998; Nurminskaya and Kaartinen 2006; Tanaka et al. 2007). A novel observation of this study was the up-regulation of TG1 and induction of TG3 expression in the $TG2^{-/-}$ joint (Fig. 5c). However, both are expressed at very low levels (average 34 cycle of PCR). In addition to the previously demonstrated proteolytic activation of the FXIIIa proenzyme (Nurminskaya et al. 1998; Tanaka et al. 2007), TG1 and TG3 may also compensate for the loss of TG2 in skeletal tissue. This finding suggests that even a double TG2/FXIIIa knockout model may be insufficient to delineate the role of the TG-mediated protein modifications in skeletal formation.

Compensation for TG2 loss in cardiovascular tissues

Aorta

High levels of TG2 expression have been detected in the aortic tissues, where different cell types express TG2 including endothelial cells, vascular smooth muscle cells and fibroblasts of the adventitia (Greenberg et al. 1991). Additionally, a significant role for TG2 has been implicated in vascular pathologies such as vascular inward remodeling (Bakker et al. 2006; Pistea et al. 2008), and medial calcification (Johnson et al. 2008; our unpublished data). Nevertheless, no phenotypic abnormalities have been reported in the developing vasculature of $TG2^{-/-}$ mice. Endogenous TG activity in the wild-type aortic tissue was much lower than in any of the other analyzed tissues (Table 1), and expression analysis revealed dominant expression of TG2, although several other TGs were expressed at low levels as well (Fig. 6a). Surprisingly, total transamidating activity in the aortic tissue was only slightly inhibited by KCC-009 (Table 1), suggesting that TG2 was mostly present in an inactive (maybe GTP-bound) form in the aorta. However, genetic ablation of TG2 resulted in enhanced total TG activity (Fig. 6b) despite no induction in TG expression (Fig. 6c). Proteolytic activation of the proenzymes expressed in the $TG2^{-/-}$ aortic tissue offers a credible explanation for this observation, but further analysis is needed to elucidate the molecular regulation of this effect. Of note, we did not detect up-regulation of TG5 expression in the fresh $TG2^{-/-}$ aortic tissue, which has

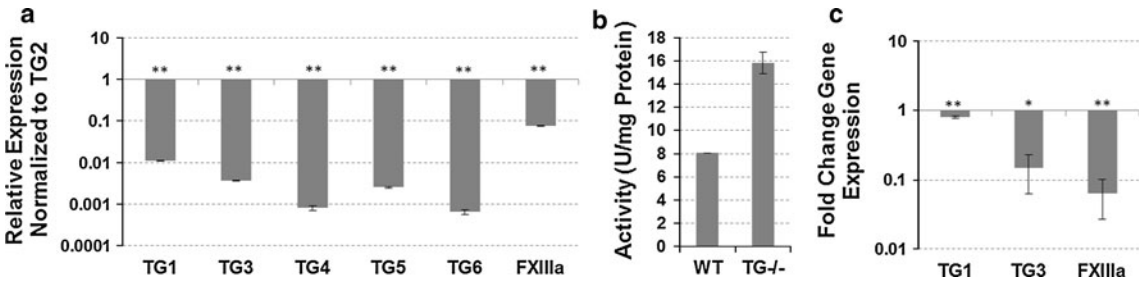

Fig. 6 TG expression and activity in the aorta from wild-type and TG2 knockout mice. **a** Real-time PCR analysis showing expression of TGs compared to TG2 expression in wild-type mouse aorta. **b** TG cross-linking activity assayed by pentylamine-biotin incorporation into *N,N′*-dimethylcasein. Total protein lysates from wild-type and TG2 knockout mouse aorta were used. **c** Real-time PCR analysis showing expression of TGs in TG2 knockout mouse aorta compared to wild-type tissue. (*$P \leq 0.05$; **$P \leq 0.005$)

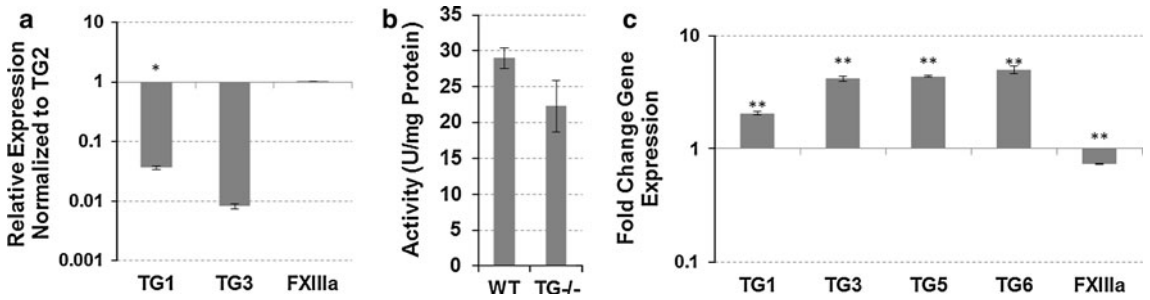

Fig. 7 TG expression and activity in the heart from wild-type and TG2 knockout mice. **a** Real-time PCR analysis showing expression of TGs compared to TG2 expression in wild-type mouse heart. **b** TG cross-linking activity assayed by pentylamine-biotin incorporation into *N,N′*-dimethylcasein. Total protein lysates from wild-type and TG2 knockout mouse heart were used. **c** Real-time PCR analysis showing expression of TGs in TG2 knockout mouse heart compared to expression in the wild-type tissue. (*$P \leq 0.05$; **$P \leq 0.005$)

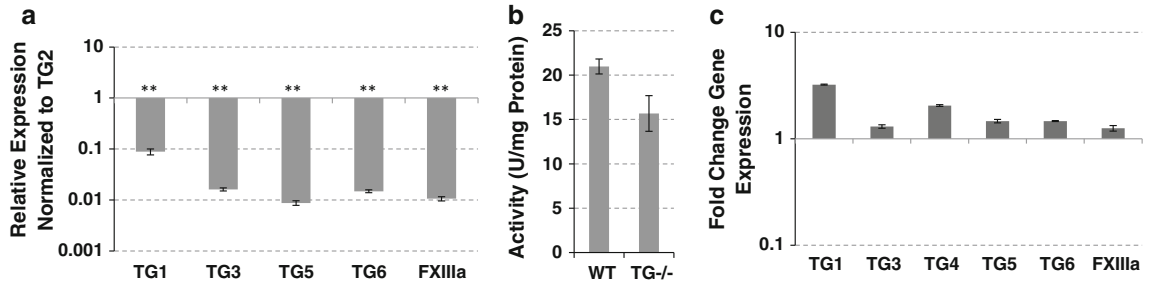

Fig. 8 TG expression and activity in the kidney from wild-type and TG2 knockout mice. **a** Real-time PCR analysis showing expression of TGs compared to TG2 expression in wild-type mouse kidney. **b** TG cross-linking activity assayed by pentylamine-biotin incorporation into *N,N′*-dimethylcasein. Total protein lysates from wild-type and TG2 knockout mouse kidney were used. **c** Real-time PCR analysis showing expression of TGs in TG2 knockout mouse kidney compared to expression in the wild-type tissue. (*$P \leq 0.05$; **$P \leq 0.005$)

been previously reported in the passaged TG2$^{-/-}$ VSMCs and may be an artifact of cell culture (Johnson et al. 2008).

Heart

A role for TG2 in heart biology has been suggested by the finding that its activity is down-regulated in cardiac failure (Hwang et al. 1996) and TG2-induced ventricular remodeling caused by cardiomyocyte-specific transgenic overexpression of TG2 (Small et al. 1999). In addition to TG2, heart tissue expresses FXIIIa at a level similar to TG2 along with lower levels of TG1 and TG3 (Fig. 7a). The TG2-mediated transamidation contributes to almost 60% of total activity as determined with specific inhibitor KCC-009 (Table 1). Nevertheless, in the TG2$^{-/-}$ heart tissue, total TG activity remains practically unchanged suggesting compensation by other TGs (Fig. 7b). In this tissue, transcriptional compensation by TG3, TG5 and TG6 is suggested by the real-time PCR analysis (Fig. 7c). Further studies are needed to identify the cell origin of elevated TG3, TG5 and TG6 expression in the TG2$^{-/-}$ hearts.

Compensation for TG2 loss in the kidney

TG2 has previously been shown to contribute to extracellular matrix accumulation by accelerating matrix deposition of collagens in kidneys (Fisher et al. 2009). In our studies, we found TG2 to be the most abundantly expressed TG in the WT liver, followed by TG1 and low levels of TG3, TG5, TG6 and FXIIIa (Fig. 8a). In the kidney, TG2 contributed to approximately 44% of the transamidase activity as shown by KCC-009 inhibition (Table 1), with only a 25% reduction in transamidase activity in the TG$^{-/-}$ kidney (Fig. 8b). When examining expression of seven other enzymatic TGs, we found that TG1 was significantly up-regulated while other TGases showed non-significant increases (Fig. 8c), indicating that several TGases could be functioning to compensate for the decreased transamidating activity in the TG$^{-/-}$ mice. However, further analysis is required to determine whether compensation is supported by an increase in transcription or proteolytic activation of TG1 and FXIIIa.

Conclusion

In this study, we show a comparative expression analysis of TGs in several tissues from both wild-type and TG2 knockout mice, in which TG2 has been suggested to have a role by in vitro studies. Furthermore, we determined the contribution of TG2 to the total transamidating activity present in each tissue. This acquired information allowed

for preliminary identification of potential tissue-specific mechanisms of compensation for genetic ablation of TG2. Our data suggest translational compensation in the heart and liver, although different TGs were induced for compensation. In contrast, functional compensation in the cartilaginous tissues and aorta is suggested by the presented analysis. Additionally, we found that in skeletal muscle there was no compensation for the loss of TG2. Understanding tissue-specific compensation mechanisms may help in design and generation of the future animal models to understand the biological functions of transglutaminases.

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