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Distribution of transglutaminase family members in mouse whole body sections

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ABSTRACT

Transglutaminases (TGs) comprise a protein family in which the members catalyze the formation of isopeptide bonds between glutamine and lysine residues in various proteins. Eight enzymes have been identified and designated as factor XIII (FXIII) and TG1-7. Expression studies of four major members, i.e., FXIII, TG1, TG2, and TG3, have been performed in a relatively large number of mammalian tissues in comparison with those on the other isozymes. The structural and biochemical characteristics of these individual isozymes and expression analyses of TG family in some tissue extracts have been reported, but there have been no simultaneous comparative analyses of both their mRNA and protein expression patterns in tissues distributions. Thus, we developed novel experimental systems for in situ hybridization using cryofilm attached to whole body sections of neonatal mice, thereby obtaining data regarding the tissue distributions of the major TG isozymes. In this study, we performed the first detailed comparative analysis of the mRNA and protein distribution studies of TG family members in a wide range of mouse tissues. These data will be helpful for elucidating the unknown physiological and pathological functions of TGs.

1. Introduction

Transglutaminases (TGs) comprise a protein family of eight isozymes designated as factor XIII (FXIII), TG1, TG2, TG3, TG4, TG5, TG6, and TG7, which catalyze the formation of isopeptide bonds between glutamine and lysine residues in substrate proteins in Ca^{2+}-dependent posttranslational modifying reactions [1–3]. TGs are widely distributed and each isozyme is involved in multiple biological processes in which they catalyze the formation of isopeptide bonds and the incorporation of a primary amine [2,4,5]. Among the eight family members, FXIII (plasma-type), TG1 (keratinocyte-type), TG2 (tissue-type), and TG3 (epidermal-type) are the major isozymes, and are known to be expressed in the plasma, in keratinocytes, ubiquitously, and in the epidermis, respectively.

FXIII is a zymogen and a tetrameric protein made of two catalytic A (FXIIIa) and two carrier/inhibitory B subunits [6]. Cellular FXIIIa is believed to exist as a homodimer with two A subunits and is found in the cytoplasm of megakaryocytes/platelets. FXIIIa can be detected in macrophage populations associated with wound healing and tumor progression [7] as well as in other cell types such as chondrocytes and osteoblasts [8]. FXIIIa-deficient mice exhibit a phenotype with reduced survival during pregnancy due to the occurrence of spontaneous hemorrhaging and early abortion [9].

TG1 is synthesized as an inactive zymogen of 106 kDa. It is expressed at low levels in proliferating keratinocytes and is induced progressively during the differentiation of keratinocytes [10]. In addition, TG1 also localizes to the adherens junctions of epithelial cells in the lung, kidney, and liver [11] as well as in myocardial microvascular endothelial cells [12]. TG1 is mostly bound to the plasma membrane and is proteolytically processed into a highly active complex of 10/67/33-kDa during terminal differentiation [10]. A previous study showed that $TG1^{-/-}$ neonates were smaller than the wild-type neonates, and they became progressively dehydrated and died within 4–5 h after birth due to the impaired formation of the cornified envelope [13].

Abbreviations used in text: FXIII, factor FXIII; HE, hematoxylin-eosin; IHC, immunohistochemistry; ISH, in situ hybridization; PB, phosphate buffer; PBS, phosphate buffered saline; SSC, standard saline citrate; TG, transglutaminase.

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TG2 is perhaps the most intriguing member of the TG family. It is found throughout the body, with constitutive expression in endothelial cells, smooth muscle cells, and fibroblasts, as well as in a number of organ-specific cell types [14,15]. The transamidation activity of TG2 plays both intracellular and extracellular roles, including in apoptosis, several signal-transduction pathways, matrix stabilization, wound healing, angiogenesis, and bone remodeling [1]. In addition to its transamidase activity, it has many other functions such as the exertion of GTPase activity involved in intracellular G-protein signaling, scaffold activity to stabilize fibronectin binding by integrins, intracellular serine/threonine kinase activity, and extracellular protein disulfide isomerase activity [1]. Transgenic mouse models overexpressing human TG2 in the brain and heart exhibited significant hippocampal neuron apoptosis [16] and cardiac hypertrophy [17], respectively. TG2 is expressed ubiquitously throughout the whole body and has multifunctional roles; however, in previous studies, $TG2^{-/-}$ mice did not exhibit any obviously abnormal phenotype [18,19]. However, further biomechanical studies and injury models have demonstrated the diverse biological functions of TG2, such as the induction of hepatocyte apoptosis [20–22], clearance of apoptotic cells [23,24], tumor progression [25], and kidney fibrosis [26–28].

TG3 is synthesized as an inactive zymogen, which is proteolytically processed by cathepsin L into an active complex that is responsible for the formation of the epidermis [29–31]. TG1 and TG3 are believed to act cooperatively in the crosslinking of several proteins, such as involucrin, loricrin, and small proline-rich proteins, during the differentiation of keratinocyte in the skin. In hair follicles, TG3 catalyzes the crosslinking of trichohyalin and keratin intermediate filaments to harden the root sheath of hair follicles [32,33]. $TG3^{-/-}$ mice exhibit impaired hair development with a reduced skin barrier function [34].

Individual studies of the TG isozymes have determined their structural and biochemical characteristics, but their tissue and cellular distributions have not been assessed in simultaneous comparative analyses due to the lack of a suitable technique and specific antibodies against the isozymes. To address these problems, in addition to our previous technology for immunohistochemistry (IHC) [35], we developed novel experimental systems for in situ hybridization (ISH) using cryofilm attached to whole body sections of neonatal mice, thereby elucidating the tissue distributions of the major TG isozymes, including FXIIIa, TG1, TG2, and TG3.

Thus, we compared the mRNA and protein distributions of these enzymes in a wide range of mouse tissues and cells. These data will contribute to elucidate the functions of TG family members in various physiological and pathological processes.

2. Materials and methods

2.1. Materials

Chemical reagents were purchased from WAKO chemicals (Osaka, Japan) and Nacalai Tesk (Osaka, Japan). Rabbit polyclonal anti-FXIIIa antibody (PAB0059) was purchased from AXXORA LLC. (Farmingdale, NY). Rabbit polyclonal anti-TG1, TG2, and TG3 [36] sera were made by Japan Lamb (Hiroshima, Japan) using each of recombinant TGases as an antigen that was produced in our laboratory. IgG was affinity purified using NHS-activated Sepharose 4 Fast Flow, which was immobilized with recombinant protein (GE Healthcare Ltd.; Buckinghamshire, UK). No cross-reactivity of these antibodies against other isozymes were confirmed by western blot (*data not shown*).

2.2. Preparation of tissue sections

ICR neonatal mice were purchased from Japan SLC Inc. and processed for preparation according to the method described by Dr. Kawamoto [37]. Whole body sections were produced using a multipurpose cryosection preparation kit (Section Lab.; Hiroshima, Japan). Briefly, mouse was fixed and frozen in cold hexane and freeze-embedded with Super Cryoembedding Medium (SCEM) from Leica Microsystems (Wetzlar, Germany). Ten-μm thick sections were prepared with a cryomicrotome (Leica Microsystems, CM3050S) from the frozen specimen block and collected with cryofilm.

All animal experiments were carried out according to the guideline of Nagoya University.

2.3. ISH in whole mouse section

ISH was performed according to the standard protocol according to manufacturer's instruction (Roche Diagnostics; Mannheim, Germany). Briefly, sagittal sections of neonatal ICR mouse was hybridized to digoxigenin (DIG)-labeled 400 nt sense and antisense RNA probes corresponding to the transcript regions encoding the each mouse TG isozyme (nt 1748–2148 for FXIIIa, nt 1–403 for TG1, nt 1–403 for TG2, and nt 2–401 for TG3). Sections were fixed, and digested by proteinase K (1 μg/ml). Following acetylation, the sections were incubated overnight at 37 °C (for FXIIIa and TG3), 42 °C (for TG1) and 50 °C (for TG2) in hybridization buffer containing

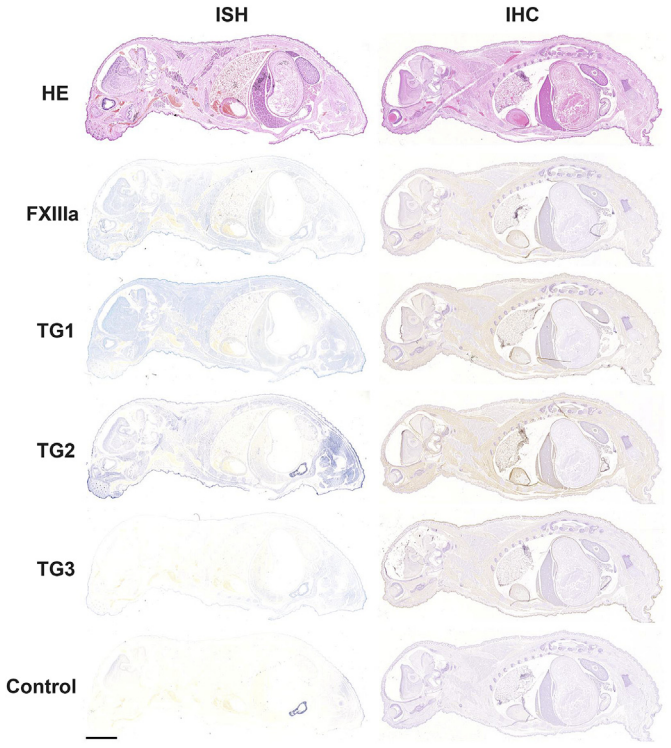

Fig. 1. Tissue distributions of mRNA and protein of the major TG isozymes in whole mouse sections. Neonatal-mouse sagittal-plane sections were subjected to in situ hybridization (ISH) and immunohistochemistry (IHC) using RNA probes and polyclonal antibodies, respectively, for FXIIIa, TG1, TG2, and TG3. Sense RNA probes for each TG isozyme were used as negative controls for ISH (representative results obtained using the sense probe for TG2 are shown). For the IHC analyses, the same amounts of non-immunized rabbit immunoglobulin-G were used as the negative control instead of the primary antibody. The sections were counterstained with hematoxylin (blue color). Bars = 3 mm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).