Chapter 95

Reciprocal changes in factor XIII and retinal transglutaminase expressions in the fish retina during optic nerve regeneration

Kayo Sugitani[1], Kazuhiro Ogai[1], Yoshiki Koriyama[2], Satoru Kato[2]

[1]Division of Health Sciences, Graduate School of Medical Science, Kanazawa University、Kanazawa, Japan

[2]Department of Molecular Neurobiology, Graduate School of Medicine, Kanazawa University, Kanazawa, Japan

Corresponding author: Kayo Sugitani, Division of Health Sciences, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa 920-0942, Japan, Phone: +81 76 265-2599; E-mail: sugitani@staff.kanazawa-u.ac.jp

Keywords: cellular Factor XIII, TGR, optic nerve regeneration, retinal ganglion cells, wound healing, transglutaminase, neurite sprouting, neurite elongation

Abbreviations: FXIII-A, factor XIII A subunit; cFXIII, cellular factor XIII; CNS, central nervous system; RGC, retinal ganglion cell; TG, transglutaminase; TG_R, retinal transglutaminase

Abstract

Unlike mammals, fish retinal ganglion cells have the capacity to repair their axons even after optic nerve transection. In the process of fish optic nerve regeneration, a large number of genes have been described as regeneration-associated molecules. Using molecular cloning techniques, we identified two types of cDNA clones belonging to the transglutaminase (TG) family which were upregulation genes; one is cellular factor XIII (cFXIII) and the other is a tissue type TG named TG_R. cFXIII mRNA started to increase in the retinal ganglion cells at 1–2 days, peaked at 5–7 days, and returned to the control level by 20 days post optic nerve injury. In contrast, TG_R mRNA started to increase at day 5–10, peaked at day 20, and then gradually decreased by day 40 after nerve injury. To elucidate the molecular involvement of these TGs in optic nerve regeneration, we studied the effects of recombinant TG_R protein or overexpression of cFXIII using a retinal explant culture system. cFXIII effectively induced neurite outgrowth only from naïve (intact) retinas. In contrast, the TG_R protein significantly enhanced neurite outgrowth only from primed retinas, in which the optic nerve had been crushed 5–7 days previously. These reciprocal expressions of cFXIII and TG_R suggest that these two types of TGs are important for the neurite sprouting and axonal elongation processes, respectively, during optic nerve regeneration processes.

95.1. Introduction

Fish retinal ganglion cells (RGCs) have the capacity to repair their axons even after optic nerve transection. We screened for regeneration-associated genes using axotomized fish retinas to identify the molecules for the rescue and repair of mammalian CNS neurons. In our previous study [1-3] , four periods of goldfish optic nerve regeneration were reported: (i) the first period, preparation for neurite sprouting (0–6 days after nerve injury); (ii) the second, axon elongation (1–6 weeks after nerve injury); (iii) the third, synaptic refinement in the tectum (2−5 months after nerve injury); and (iv) the last, restoration of visual function (6 months after nerve injury). To identify the genes whose expression was specifically upregulated in each optic nerve regeneration period, a cDNA library was prepared from goldfish optic nerves and retinas after nerve injury. Out of many candidate molecules, we cloned two types of transglutaminase (TG), protein cross-linking enzymes, were identified as upregulation molecules. Factor XIII-A was upregulated mainly in the first stage of regeneration [3, 4], and TG_R increased and had axon elongation effects only in the second period of optic nerve regeneration after nerve injury [5].

95.2. Cellular Factor XIII (cFXIII)

Factor XIII (FXIII) was originally identified as a plasma transglutaminase (TG) heterotetramer (A_2B_2) consisting of two catalytic A subunits and two non-catalytic B subunits. It promotes clot stability by catalyzing the formation of covalent cross-linking reactions in polymerized fibrin as a blood coagulation factor [6]. Cellular FXIII (cFXIII) consists of a homodimer of A subunits (A_2); it exists as an intracellular form of FXIII in various tissues, platelet, monocytes, macrophages and megakaryocytes [7, 8]. By the screening of a cDNA library prepared from goldfish retina at 1 day after optic nerve injury, a positive clone with a 2,560 bps fragment was

identified as the full-length cDNA clone of FXIII-A (DNA Data Bank of Japan; Accession No. AB622931) encoding a protein of 744 amino acid residues with a predicted molecular mass of 83.8 kDa [3]. FXIII-A mRNA signals in the retina started to increase at day 1 and peaked at 3-7 days, then had decreased by 20 days after optic nerve injury (Table 95.1). The distribution of FXIII-A was confined to the RGC layers [3, 4] during the period of neurite sprouting in the optic nerve regeneration process.

To investigate the functional role of upregulation of cFXIII in optic nerve regeneration, we induced overexpression of the FXIII-A gene using retinal explant cultures by lipofection. Neurite outgrowth was assessed as the ratio of the outgrowth in the control culture and the FXIII-A overexpression culture (Fig.95.1). In this study, we compared the effect of neurite outgrowth using two types of retinas; one was an unprimed retina (naïve retina without optic nerve lesion), and the other was a primed one in which the optic nerve had been injured 5 days previously. As seen in Fig. 95.1, the FXIII-A overexpression experiments showed that cFXIII induced neurite outgrowth only from unprimed (naïve) retinas but not from primed retinas. Fig.95.1b shows that these inductive effects of neurite outgrowth were lost in the primed retina. The levels of endogenous cFXIII in the primed retina had already increased in RGCs at this time.

←Fig.95.1

On the other hand, a large number of FXIII-A positive cells accumulated in the area surrounding the optic nerve injury site within a few hours after nerve injury [3]. Nuclear staining of the crushed optic nerve sections with DAPI showed that DAPI-positive glial cells merged with FXIII-A expressing cells. These cFXIII positive cells in the injured optic nerves were identified as astrocytes/microglial cells by immunohistochemical study using the antiserum of some glial marker proteins. The increased level of FXIII-A mRNA was maintained for 1-40 days in the optic nerve after optic nerve injury [3].

95.3. Retinal transglutaminase (TG_R)

Following the end of increasing cFXIII expression in the damaged retina, retinal transglutaminase (TG_R), a different type of TG, was identified as an upregulated gene (DNA Data Bank of Japan; Accession No. AB198723) encoding 678 amino acid residues, with a predicted molecular mass of 75.9 kDa [5]. The levels of TG_R mRNA and protein increased only in the RGCs during the period 5-40 days, and peaked at 20 days after optic nerve injury (Table 95.1). This corresponds to the period of axonal elongation during the optic nerve regeneration process.

To investigate the functional role of upregulation of TG_R in optic nerve regeneration, we prepared retinal explant cultures in the presence of recombinant TG_R protein. The percentage of explants showing positive neurite outgrowth was compared under various culture conditions. As seen in Fig. 95.1, the TG_R protein induced neurite outgrowth from the primed retina but not from the unprimed retina. The addition of recombinant TG_R to the retinal culture also induced striking neurite outgrowth from adult rat RGCs [5]. These molecular and cellular data strongly suggest that TG_R promotes axonal elongation at the surface of injured RGCs after optic nerve injury.

←Table.95.1

95.4. Conclusions

The TG family catalyzes post-translational, covalent protein cross-linking reactions in diverse processes in nervous systems [9]. During goldfish optic nerve regeneration, we observed expression of two types of TG gene, FXIII-A and TG_R, in the RGCs (Table 95.1). However, these different types of TG are upregulated at different stages in optic nerve regeneration (Fig. 95.2). TG_R is upregulated in the second stage (1–6 weeks after injury), which corresponds to the

←Fig.95.2

period of axonal elongation to the target and the beginning of synaptic connection in the tectum [5]. On the other hand, FXIII-A is upregulated in the first stage, which corresponds to the period of preparation for axon regrowth [3]. In our culture study, we clearly demonstrated that the endogenous FXIII-A protein (cFXIII) in RGCs induced neurite outgrowth from naïve retinas, but not from primed retinas. In contrast, recombinant TG_R protein induced a drastic extension of long and thick neurites only from primed retinas in which the optic nerve had been injured 5-7 days previously. These results correspond to the peak period of upregulation for these two types of TGs. Additionally, it is strongly suggested that cFXIII produced by non-neuronal cells also induce the elongation of regenerating axons. These reciprocal changes in the expression of cFXIII and TG_R in the RGCs in the early stage of regeneration suggest that the both types of TGs are important for neurite sprouting and axonal elongation, respectively, during optic nerve regeneration.

Acknowledgements This work was supported by Grants-in-Aid for Scientific Research to K.S. (No. 23618006) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1. Kato S, Koriyama Y, Matsukawa T, Sugitani K. Optic nerve regeneration ingoldfish. In: Becker, C.G., Becker, T. (Eds.), Model Organisms in Spinal Cord Regeneration. Wiley-VCH, Germany, 2007; pp. 355–372.
2. Matsukawa T, Arai K, Koriyama Y, Liu Z, Kato S. Axonal regeneration of fish optic nerve after injury. Biol Pharm Bull 2004; 27:445-451.
3. Sugitani K, Ogai K, Hitomi K, Nakamura-Yonehara K, Shintani T, Noda M, Koriyama Y, Tanii H, Matsukawa T, Kato S. A distinct effect of transient and sustained upregulation of cellular factor XIII in the goldfish retina and optic nerve on optic nerve regeneration. Neurochem Int 2012; 61:423-32.
4. Sugitani K, Ogai K, Nakashima H, Kato S. Factor XIIIA induction in the retina and optic nerve after optic nerve lesion in goldfish. Adv Exp Med Biol 2012; 723:443-8.
5. Sugitani K, Matsukawa T, Koriyama Y, Shintani T, Nakamura T, Noda M, Kato S. Upregulation of retinal transglutaminase during the axonal elongation stage of goldfish optic nerve regeneration. Neuroscience 2006; 142:1081-92.
6. Schwartz ML, Pizzo SV, Hill RL, McKee PA. Human Factor XIII from plasma and platelets. Molecular weights, subunit structures, proteolytic activation, and cross-linking of fibrinogen and fibrin. J Biol Chem 1973; 248:1395-407.
7. Derrick EK, Barker JN, Khan A, Price ML, Macdonald DM. The tissue distribution of factor XIIIa positive cells. Histopathology 1993; 22: 157–62.
8. Quatresooz P, Paquet P, Hermanns-Lê T, Piérard GE. Molecular mapping of factor XIIIa-enriched dendrocytes in the skin. Int J Mol Med 2008; 22: 403–9.

9. Lesort M, Tucholski J, Miller ML, Johnson GV. Tissue transglutaminase: a possible role in neurodegenerative diseases. Prog Neurobiol 2000; 61:439-63.

Figure Legends

Fig. 95.1. Neurite outgrowth effects of cFXIII or TG_R protein in goldfish retinal explant cultures.

Neurite outgrowth for 5 days of culture in the presence of FXIII-A or TG_R protein in unprimed (naïve) goldfish retinal explants (a) and primed retinal explants (b) compared with controls (no addition).

a) Overexpression of FXIII-A protein increased the number of unprimed explants with neurite outgrowth compared with the controls and TG_R (***P*<0.01 increased relative to control).

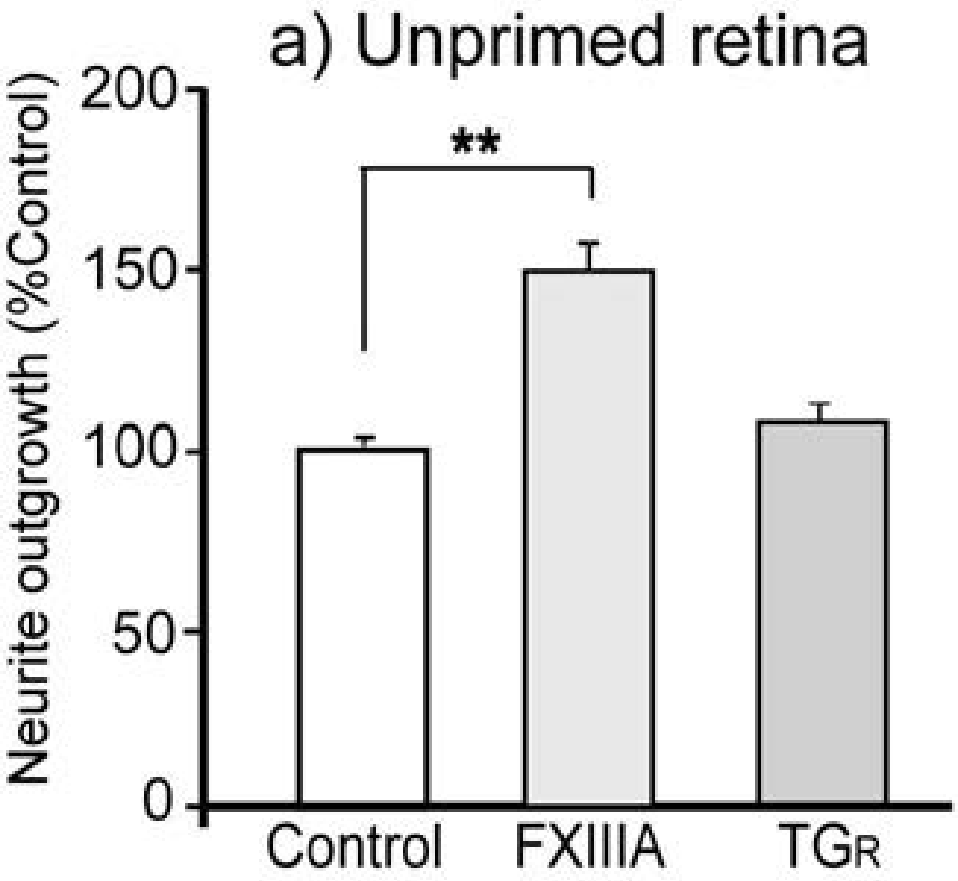
b) Recombinant TG_R protein (0.01U/ml) increased the number of primed explants with neurite outgrowth compared with the controls (***P*<0.01 increased relative to control).

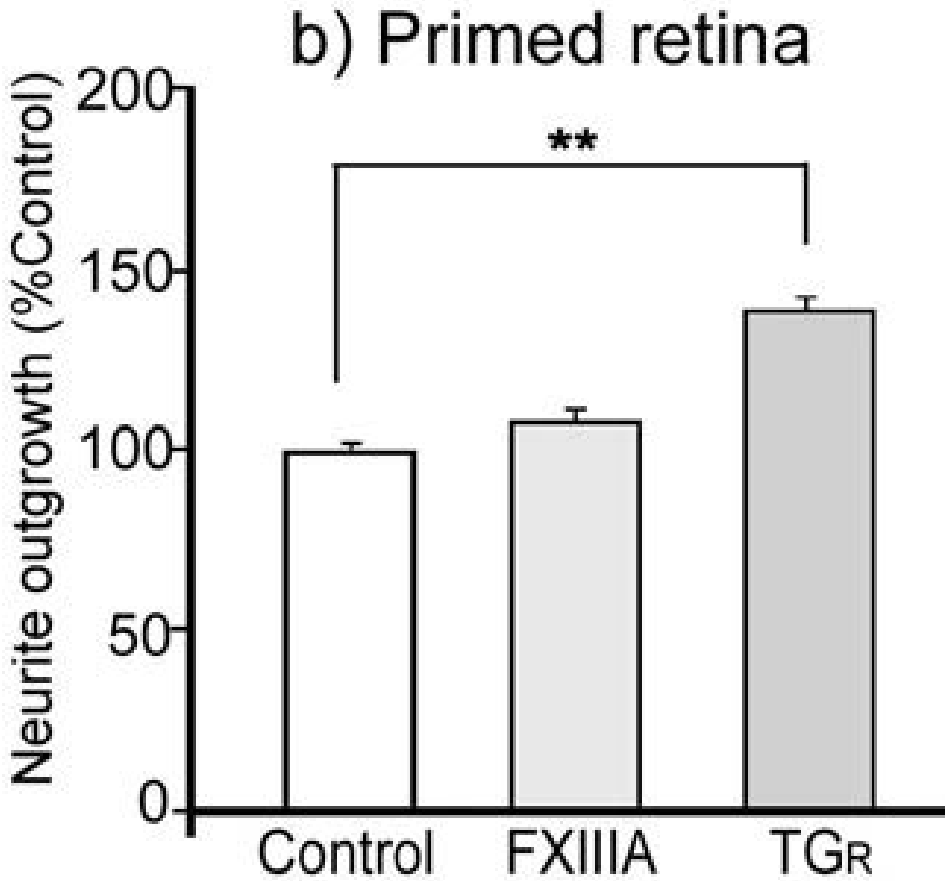
Fig. 95.2 Pattern diagram of cFXIII and TG_R expression in the goldfish retina during optic nerve regeneration

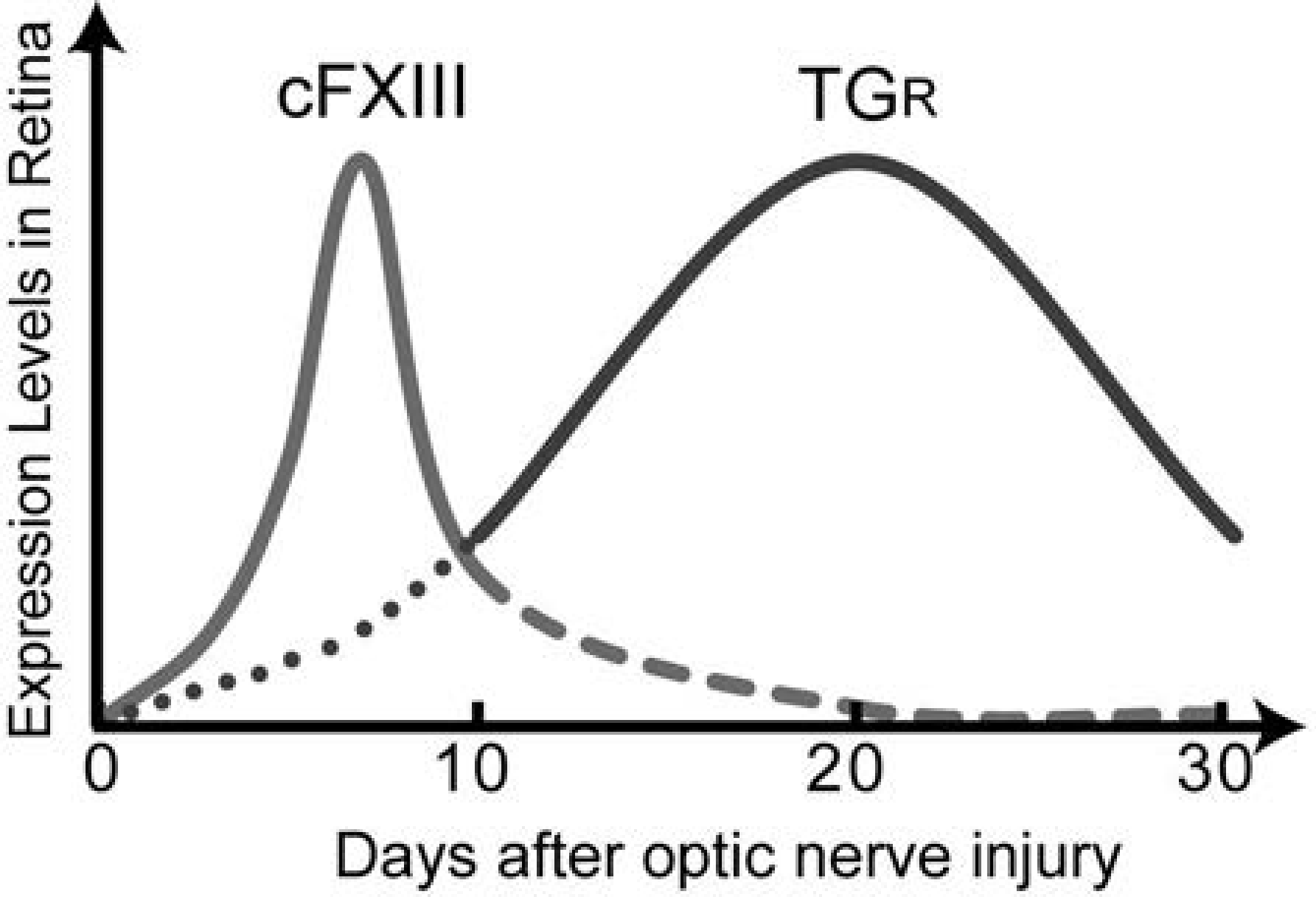
Table Legend

Table 95. 1 Two types of TG expression in goldfish after optic nerve injury

	Period of increase after optic nerve injury	Localization of protein	Function
Cellular Factor XIII (cFXIII)	1 - 10 days	RGC	Neurite sprouting
	2,3 hours ~	Optic nerve	Wound healing?
	~ 40 days	Optic nerve	Axonal elongation
Retinal TG (TG_R)	5 – 40 days	RGC	Axonal elongation


a) Unprimed retina
Neurite outgrowth (%Control)
200
150
100
50
0
**
Control
FXIIIA
TGR


b) Primed retina
Neurite outgrowth (%Control)
200
150
100
50
0
**
Control
FXIIIA
TGR


cFXIII
TGR
Expression Levels in Retina
0
10
20
30
Days after optic nerve injury