UPREGULATION OF TRANSGLUTAMINASE IN
THE GOLDFISH RETINA DURING OPTIC
NERVE REGENERATION

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1. SUMMARY

To elucidate the molecular involvement of transglutaminase (TG) in central nervous system (CNS) regeneration, we cloned a full-length cDNA for neural TG (TG_N) from axotomized goldfish retinas and produced a recombinant TG_N protein from this cDNA. The levels of TG_N mRNA and protein were increased at 10-30 days after optic nerve transection, and this increase in TG_N was only localized in the ganglion cells in goldfish retinas. In retinal explant cultures, the recombinant TG_N protein induced a drastic enhancement of neurite outgrowth, while TG_N-specific RNAi significantly suppressed this neurite outgrowth. Taken together, these data strongly indicate that TG_N is a key regulatory molecule for CNS regeneration.

2. INTRODUCTION

Transglutaminase (TG), a protein cross-linking enzyme, is widely distributed in mammalian cells and tissues. Neural TG (TG_N), which is expressed in neural tissue, rapidly increased in rat sciatic nerves¹ and superior cervical ganglia² after nerve injury. In the central nerve system, the TG_N activity of goldfish optic nerve increased, whereas that of rat optic nerve decreased after optic nerve crush.³ Fish can successfully regenerate the optic axons and eventually function after nerve injury, whereas rat cannot regenerate their optic axons. Therefore, to elucidate a functional role of TG_N on CNS regeneration in genetic level, we first isolated a full-length cDNA clone for TG_N from a cDNA library prepared from axotomized goldfish retinas. In addition, we produced a recombinant TG_N protein and anti-TG_N

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antiserum. We also investigated the expression and localization of the TGₕ protein by immunohistochemical staining. Moreover, we made a TGₕ specific small interference RNA to estimate the effect of TGₕ on neurite outgrowth in explant culture system. In the present study, we showed a novel functional role of TGₕ on axonal elongation of goldfish optic nerve after injury.

3. METHODS

3.1. Animals

Adult common goldfish (Carassius auratus; body length about 6-8cm) were used throughout this study. Goldfish were anesthetized with ice-cold water. The optic nerve was sectioned 1mm away from the posterior of the eyeball with scissors. After surgery the goldfish were kept in water tanks at 22°C ± 1°C for 1-40 days.

3.2. Cloning of Goldfish Neural Transglutaminase (TGₕ)

A cDNA library was constructed from poly (A)⁺ RNA (5µg) from goldfish retinas of which optic nerve had been transected 5 days before as described previously.⁴ Tissue-type transglutaminase (tTG) cDNA from red sea bream (Pagrus major) liver⁵ (gift from Dr. Yasueda, Ajinomoto Co.) was labeled with [³²P] dCTP and 2 × 10⁵ colonies were screened with this probe. Five positive clones were subcloned into pBK-CMV phagemid and sequenced using DNA sequencer. Two independent clones were obtained. The 5’ TGₕ mRNA was cloned by the RACE method.

3.3. Purification of Recombinant TGₕ

A full-length TGₕ cDNA clone was inserted into the expression vector pFLAG-CMV-1, and the constructs were transfected into HEK 293 cells using Lipofectamine. For the control, only the pFLAG-CMV-1 vector was transfected to create mock cells. All cells were maintained in Dulbecco’s MEM containing 10% fetal calf serum in a 5% CO₂ humidified incubator for 48h at 37°C. The cells were then harvested, lysed and centrifuged for 30min at 15,000g. The FLAG-tagged enzyme was purified using ANTI-FLAG M2 affinity gel.

3.4. Immunohistochemistry

A rabbit antiserum against TGₕ was obtained by subcutaneous injection of purified TGₕ. Tissue fixation and cryosectioning were carried out as described previously.⁶ Retinal sections were autoclaved at 121°C for 15min in 10mM citrate buffer. After washing and blocking, the sections were incubated with the rabbit polyclonal anti-TGₕ antibody (1:100 dilution) overnight at 4°C. Following incubation with a biotinylated secondary antibody for 2h at room temperature, the bound antibodies were detected using horseradish peroxidase-conjugated streptavidin and 3-amino-9-ethylcarbazole.