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Telomerase induction in astrocytes of Sprague–Dawley rat after ischemic brain injury

Sunkyung Baek[a], Youngmin Bu[a], HwaSu Kim[b], Hocheol Kim[a,*]

[a]*Department of Herbal Pharmacology, Graduate School of East-West Medical Science, Kyung Hee University, Seoul 130-701, South Korea*
[b]*Department of Life Science, College of Natural Science, Chung-Ang University, 221 Heuksuk-Dong, Dongjak-Ku, Seoul 156-756, South Korea*

Abstract

Telomerase, a reverse transcriptase, consists of an RNA template and protein polymerase. This ribonucleoprotein protects the linearized chromosomal end region and elongates the telomere during chromosomal replication. Telomerase is not expressed in adult somatic cells but it shows high activity in most cells during embryonic development. We report, by RT-PCR and immunohistochemical results, that the induction of telomerase protein catalytic subunit (TERT) in transient middle cerebral artery occlusion induced brain injury. TERT mRNA emerged 24 h after ischemia. We examined which brain cell expressed TERT in the penumbra region of injured brain. The expression of TERT began from 24 h and remained until 5 days after ischemia. We identified that TERT was co-localized with the astrocyte marker, GFAP, at 3 days after ischemia. This is strong evidence that TERT is induced in astrocytes when the brain is damaged by ischemia, and that this enzyme may play an important role in ischemic brain injury.

Keywords: Telomerase; TERT; Ischemia; Middle cerebral artery occlusion; Astrocyte

Telomerase, a ribonucleoprotein, is critical for the replication of the extreme end of linearized chromosomes [8]. The RNA subunit of this enzyme provides the template for the addition of the telomere nucleotide sequence [1] and the protein catalytic subunit (TERT) is responsible for telomerase activity [14]. Sequence comparison of TERT placed this enzyme in the reverse transcriptase family but revealed that it was distinguished from retroviral and retrotransposon relatives. In spite of that difference, the telomerase catalytic subunits isolated from ciliate, yeast, mouse, and human are phylogenetically conserved and represent a close relationship in the evolution of reverse transcriptase [10]. Recently, there have been a number of breakthroughs in telomerase research. The identifications of the RNA subunits and the catalytic subunits from ciliate to human have been reported [4,10] and studies of their functions are in progress [16]. Telomerase is active in germ line cells while this enzyme is not expressed in most human somatic tissues [9] and telomere length is significantly shorter than germ line cells [2]. Telomerase inactivation induces telomere shortening and as a result, cells undergo senescence. The re-activation of telomerase had not been reported in normal somatic cells except cancer cells [2]. However, recent studies suggest that telomerase can be induced in some types of normal somatic cells in adults. In addition, the recent discovery of neural progenitors in adult mammals raises the possibility of repairing damaged tissue by recruiting their latent regenerative potential [13]. Moreover, it was reported that telomerase could protect developing neurons against DNA damage-induced apoptosis [12] and protect mature neurons against amyloid beta peptide-induced apoptosis [18]. These results show that telomerase might assist in repairing the damaged tissue of brain ischemia. Recently, it was reported that telomerase was induced in microglia in response to excitotoxic and traumatic brain injury [5]. These studies have aroused our interest in exploring the induction of telomerase in ischemic brain injury. The present study was designed to investigate the induction of telomerase in rat brain cells after ischemia.

All surgical procedures were conducted according to the rat welfare guidelines of NIH and the Korean Academy of Medical Sciences. Rats were housed under controlled temperature (20 ± 2 °C) and lighting (07:00–19:00 h) conditions, with food and water available ad libitum. Focal ischemia-reperfusion was produced by the method described by Longa et al. [11]. Briefly, male Sprague–Dawley rats (290–310 g, Taconic) were fasted overnight but had free access to water. Rats were anesthetized with 1–2% isoflurane in an N_2O and O_2 (70:30) mixture. The rectal temperature was controlled at 37 ± 0.5 °C throughout the experiment with a heating lamp and homeothermic blanket

* Corresponding author. Tel.: +82-2-961-0419; fax: +82-2-964-0325.
E-mail address: hckim@khu.ac.kr (H. Kim).

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system (Harvard Apparatus). The right carotid bifurcation was exposed through a midline neck incision. A filament, which had a round tip and a distal cylinder of silicon rubber (0.30 mm in diameter), was introduced into the external carotid artery. Correct placement of the suture was established when the suture was inserted at least 19–20 mm from the carotid bifurcation. After 90 min of occlusion, the suture was withdrawn to allow reperfusion. Rats were sacrificed at 6 h to 7 days after occlusion with an overdose of chloral hydrate.

To investigate TERT mRNA expression, we performed RT-PCR. PCR product was directly synthesized from 2 μg of total RNA isolated from the ipsilateral hemisphere with the SuperScript one step RT-PCR system with Platinum Taq (Invitrogen) using gene specific primers and following recommendations provided by the supplier. Reaction mixtures consisting of 2 μg of total RNA, 2 × reaction mix (Invitrogen), and 5 pmol primers were incubated at 45 °C for cDNA synthesis and then denatured at 94 °C for 5 min, subjected to 40 PCR cycles (30 s at 94 °C, 30 s at 45 °C, and 1 min at 70 °C), and then elongated at 70 °C for 10 min. PCR products were analyzed by agarose gel electrophoresis (1.3%) followed by staining with ethidium bromide and scanning with a Gel-Doc (Bio-RAD). The primers used in this study were as follows: TERT forward primer, 5′-CAGGGTAAGCTGGTGGA-3′; TERT reverse primer, 5′-GCAGGAAGTGCAGGAAG-3′; GAPDH forward primer, 5′-GTGATGGGTGTGAACCACGAG-3′; and GAPDH reverse primer, 5′-CAGTGAGCTTCCCGTTCAGCT-3′. Values for TERT mRNA levels were normalized to the level of GAPDH mRNA in the same sample. In preliminary studies, we established that the RT-PCR products of the correct size corresponded to TERT mRNA by excising the band from the gels and sequencing it. We also performed preliminary analysis to determine the optimum PCR conditions. To determine that TERT could be induced by ischemic injury, we performed immunohistochemical study. Briefly, brain tissues were fixed for 30 min in a solution of 4% paraformaldehyde in PBS and incubated for 5 min in 0.2% Triton X-100 in PBS. Tissues were then incubated for 1 h in PBS containing 3% goat serum and either TERT antibody (diluted 1:1000; Santa Cruz Biotechnology Inc.) or GFAP (diluted 1:100, Sigma) was added. Tissues conjugated with GFAP antibody were then incubated overnight at 4 °C, washed with PBS, and incubated for 1 h at room temperature in the presence of a 1:200 dilution of FITC labeled rabbit anti-goat IgG (Vector Laboratories) in PBS. Tissues conjugated with TERT antibody were further processed using an ABC kit (diluted 1:50; Vector Laboratories) with diaminobenzidine (Sigma) as substrate. Tissues were visualized and photographed under BX50 Olympus microscopy (Olympus).

To identify whether TERT was induced in ischemic brain injury, we examined the levels of TERT mRNA using an RT-PCR approach (Fig. 1). The result of RT-PCR using RNA isolated from the ipsilateral hemisphere indicated that

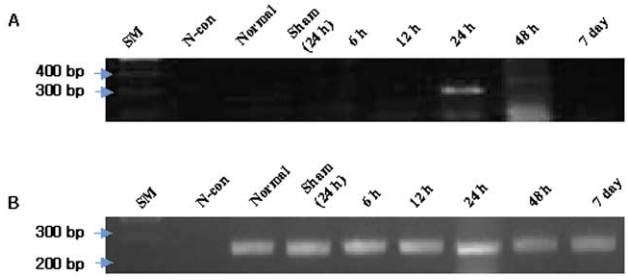

Fig. 1. Time dependent expression of TERT and GAPDH mRNA in MCAo rat brain. Total RNA was isolated from the injured hemisphere and RT-PCR analysis was performed. The induction of TERT mRNA (A) and the expression of GAPDH mRNA (B) were determined by RT-PCR analysis. It can be seen that TERT mRNA was expressed at 24 h after brain injury and vanished after that.

TERT mRNA emerged at 24 h after occlusion, and then vanished. In the contralateral hemisphere, TERT mRNA was not expressed (data not shown). Based on the result of TERT mRNA expression, we performed immunohistochemical study to confirm TERT expression. TERT was not expressed in the penumbra region of sham and normal rat brain (Fig. 2A). TERT expression began from 24 h after occlusion and it remained until 5 days in the penumbra region of injured rat brain (Fig. 2C–E). In the contralateral hemisphere, TERT was not expressed, similar to sham and normal control (data not shown). With these results, we examined which brain cell expressed TERT in the penumbra region of damaged brain. By double label immunohistochemistry, TERT was expressed in astrocytes from 3 days after middle cerebral artery occlusion (MCAo) (Fig. 3). The TERT expression was co-localized with the astrocyte marker, GFAP, indicating that TERT is expressed in astrocytes (Fig. 3B).

In normal conditions, it has been reported that telomerase was expressed in prenatal embryos [2,16] but many studies have failed to detect any activity in the adult rat brain. As for in pathological states like cancer or traumatic brain injury, telomerase was expressed in the brain or other tissue [2,3,5].

Our study demonstrated that cerebral ischemic damage could induce TERT expression in adult brain. TERT mRNA

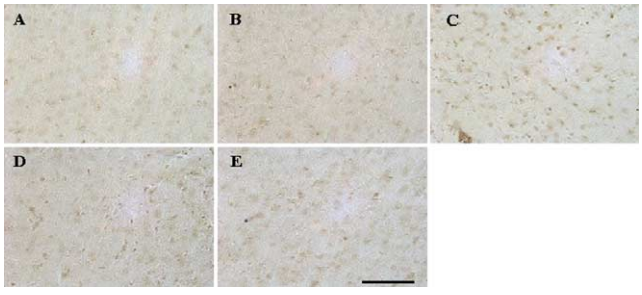

Fig. 2. Immunohistochemical detection of TERT in the penumbral region. TERT expression was investigated 1 day (B), 2 days (C), 3 days (D), and 5 days (E) after MCAo injury. In the non-treated control brain TERT was not detected (A). Intense TERT immunoreactivity in the penumbra was shown 2 days after injury and the expression of TERT remained until 5 days after injury. Scale bar, 100 μm.

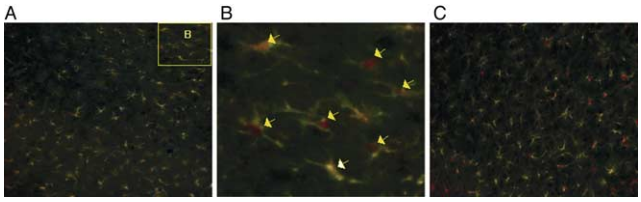

Fig. 3. Co-localization of TERT with the astrocyte marker, GFAP. Double labeled immunostaining with antibodies against TERT (brown) and GFAP (green) in the penumbra region at 3 days after ischemia (A), the amplified region of A (B), and at 5 days (C). The arrow shows co-localized TERT with GFAP in astrocytes.

was not expressed in the normal, sham operated rat brain or even in the contralateral hemisphere of MCAo rat brain. These results indicated that TERT mRNA was induced only in damaged tissue. The time pattern of TERT mRNA emerged at 24 h and then vanished after 24 h after MCAo.

The TERT expression began from 1 day after occlusion, remained until 5 days and co-localized with the GFAP reactive astrocyte at 3 and 5 days. After MCAo, astrocytes play a major role in inflammation, neuron protection and gliosis [6,7,15]. Telomerase induction might assist those functions of astrocytes at their precise working time. Moreover, induction of TERT at 24 h and 2 days after injury suggests that expression of this enzyme could protect neuronal cells against ischemic injury as it was previously reported that TERT-induced cells were resistant to apoptotic injury [12,16]. According to a recent study, reactive astrocytes expressed protein tyrosine phosphatase at 3 days after permanent MCAo [17]. This report suggested that the protein induced astrocytes, thereby limiting astrocyte proliferation, which acts to limit the extent of the astroglial scar that is formed following ischemic damage. Compared to this result, TERT expression at 3 and 5 days indicates that this enzyme might be related to astrocyte proliferation for gliosis or regulation for cell activation.

In summary, expression of TERT mRNA commenced at 24 h after ischemia and the protein emerged at 2 days in the penumbra region of the injured hemisphere. In our results, the brain cells that expressed TERT after ischemic injury were astrocytes. Compared with the previous finding that TERT was expressed in microglial cells after brain injury [5], induction of TERT in astrocytes at 3 days after ischemia might be related to astrocyte proliferation or regulation. However, the function of telomerase in the injured brain has not been clearly defined. Further research is required to investigate the exact mechanism of telomerase in astrocyte function after brain ischemia.

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