Brain ischemia occurs when insufficient blood flows to the brain, leading to an alternative oxygen supply or cerebral hypoxia (Weinachter et al., 1990). Ischemia eventually leads to brain death due to the poor oxygen/ATP supply. Hypothermia is a strong neuroprotectant that reduces ischemic brain injury, but the detailed mechanisms of its protective effects are not entirely understood (Darwazeh and Yan, 2013). We showed previously that hypothermia increases the beta-catenin-interacting protein 1 (CTNNBIP1) gene expression, which may provide clues to develop treatments to recover from and diagnose ischemia.

**Key Words:** Hypothermia, Beta-catenin-interacting protein 1 (CTNNBIP1), PC12 cells
could be effective in ischemia (Kwon et al., 2014). In this study, we present the results of CTNNBIP1 gene expression under various hypothermic conditions including lithium chloride treatment in vitro.

PC12 cells were cultured on collagen-coated flasks in 85% RPMI 1640 supplemented with 25 mM HEPES buffer, 10% heat-inactivated horse serum, 5% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 1 g/l D(+)-glucose, 25 μg/ml streptomycin and 25 U/ml penicillin at 37 °C in a 5% CO2 atmosphere. The cells were incubated under control conditions (37 °C) or hypothermia (32 °C). The expression of the cold-induced RNA-binding protein (CIRBP) was used as a positive control (Al-Fageeh and Smales, 2009) in all experiments. Total RNA from cultured PC12 cells was extracted using an RNA isolation reagent (TRI-Reagent Ambion, Austin, TX, USA). A reverse transcription-polymerase chain reaction analysis was performed using the forward primer F (5’-ATGAACCGTGAGGAGGAC-3’) and R (5’-GATCTGGAAACGCCATCAGC-3’) for CTNNBIP1; F (5’-TCAGCTTCGACACCAATGAG-3’) and R (5’-GTATCCTGGGACC CGTTAT-3’) for CIRBP. The conditions were: 30 cycles [94 °C for 30 s; 58 °C for 30 s; and 72 °C for 1 min (final cycle of 10 min)] using Taq DNA polymerase.

CTNNBIP1 mRNA expression was increased gradually about two-fold in a time-dependent manner until 7 h compared to that of the control (Fig. 1A). However, expression decreased by about half during days 1–3 of hypothermia exposure (Fig. 1B). These results show that hypothermia increased CTNNBIP1 gene expression only in the short-term period (≤1 day). We also tested the effect of 1, 3, and 5 h exposure to normal temperature after 2 days of hypothermic treatment on CTNNBIP1 gene expression (Fig. 2A). The resulting CTNNBIP1 gene expression was not repaired until its control. As shown in Fig. 1A, short-term hypothermic treatment increased CTNNBIP1 gene expression.

To know, in this time, repeat treatments both 2 h normal temperature and 2 h hypothermia, which how to effect for the CTNNBIP1 gene expression (Fig. 2B). These results show that CTNNBIP1 gene expression increased about four-fold after two treatments. We also examined the effects of lithium chloride (LiCl), a well-known GSK-3β inhibitor, on CTNNBIP1 gene expression during hypothermic treatment (Fig. 3). LiCl reduces hypothermia through Tau hyperphosphorylation and enhances β-catenin protein expression (Meffre et al., 2015). A 2-day LiCl treatment (37 °C) resulted in downregulation of CTNNBIP1 gene expression. However, LiCl treatment under hypothermic conditions enhanced CTNNBIP1 gene expression, supporting the suggestion that hypothermia affects how LiCl interacts with unidentified intracellular thermal factors.

In summary, this is the first study to demonstrate that short-term hypothermic treatment upregulates CTNNBIP1 gene expression and that repeated-hypothermic treatment
induces relatively higher gene expression. LiCl upregulated CTNNBIP1 gene expression under hypothermic conditions. These in vitro findings indicate that hypothermia can be used to control CTNNBIP1 gene expression. An in vivo experiment must be conducted to verify these results and provide the possibility to develop brain ischemia recovery treatment and diagnostic method.

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Conflict of interest
The authors declare that there is no conflict of interests regarding the publication of this article.

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