Expression Levels of BIRC5 Isoforms in Different Neuroblastoma Cell Lines

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ABSTRACT

Neuroblastoma, a neuroendocrine tumor, is the most common extracranial solid cancer in infancy and childhood. BIRC5 is a member of inhibitor apoptosis gene family and its expression level may be related to neuroblastoma. There are several isoforms of BIRC5. In this study, mRNA forms of this gene were detected in two types of neuroblastoma cell lines using RT-PCR. There was no significant change in the ratio of these two isoform in the two cell lines.

INTRODUCTION

BIRC5 (also called Survivin) is a member of inhibitor of apoptosis (IAP) gene family that exhibits differential expression in nearly all human cancers but not in most normal tissues (Altieri, 2003). The human BIRC5 gene, spanning 14.7 kb on telomeric position of chromosome 17, produces a 16.5 kDa protein (Ambrosini *et al*., 1998). This protein has one N-terminal baculoviral IAP repeat (BIR) domain, a long C-terminal α-helix coiled region, and a dimeric arrangement (Verdecia *et al*., 2000).

BIRC5 exists in at least three isoforms as the result of alternatively spliced transcripts (Mahotka *et al*., 1999). These isoforms include full-length BIRC5, BIRC5-2B, and BIRC5-ΔEx-3. They may have differential localization, expression, and function in tumor cells (Kappler *et al*., 2001; Krieg *et al*., 2002). The BIRC5 gene has a three-intron, four-exon structure. The BIRC5-2B transcript results from the retention of a portion of intron 2, whereas BIRC5-ΔEx-3 transcript results from the removal of exon 3 (Fig 1).

The sequence alteration produced from the splice variants result in marked changes in the corresponding protein structure and differences in their ability to inhibit apoptosis (Mahotka *et al*., 2002). In this study, the ratio of different isoforms of BIRC5 is tested in SH-EP1 and SK-N-ER cell lines by RT-PCR.

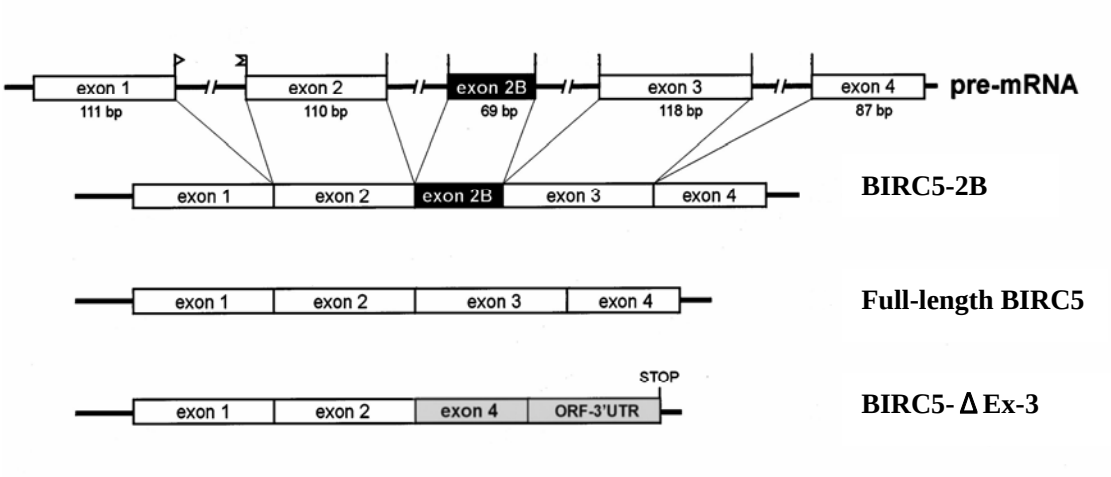

Figure 1 Structure of different isoforms of BIRC5. The full-length BIRC5 has four exons. BIRC5-2B has an additional exon (exon 2B) inserted between exons 2 and 3, and BIRC5-ΔEx-3. (Adapted from Mahotka *et al.*, 1999)

MATERIALS AND METHODS

Cell lines

Human fibroblast SH-EP1 and SK-N-ER cell lines were kindly provided by Leleesha Samaraweera.

Total RNA extraction

Total RNA from SH-EP1 and SK-N-ER cell lines was prepared using the RNAqueous kit purchased from Ambion separately.

Primers

Primers specific to BIRC5 were designed as: 5'-TGCTTCAAGGAGCTGGAAGG-3' (forward primer) and 5'-AGAAGCACCTCT GGTGCCAC-3' (reverse primer). The forward and reverse primers were located in exon 2 and exon 4, respectively.

RT-PCR

RT-PCR was performed using the QIAGEN OneStep RT-PCR Kit. For each set of reactions, a master mix was prepared using 3 µl of 5x RT-PCR buffer, 0.6 µl of dNTP Mix, 0.6 µl of RT-PCR Enzyme Mix, and 5.3 µl of RNase-free water per reaction sample. 9.5 µl of the master mix was dispensed into each PCR tubes. 7.5pM of forward primer and 7.5pM of reverse primer were added to each tube. 20ng total RNA from SK-N-ER or from SH-EP1 was added as template. Additional RNase-free water was added to make the reaction volume 15 µl. 15 µl of mineral oil was added to each tube to overlay the reaction components.

One-step RT-PCR was performed according to the following protocol: RT-- 50℃

for 30min, 95℃ for 15min, PCR-- 94℃ for 30sec, 60℃ for 30sec, 72℃ for 30sec, 40 cycles, followed by 72℃ for 7min.

Gel Extraction

The RT-PCR products in each reaction were analyzed in 1% agarose gel. After electrophoresis, all the bands were excised and extracted using QIAquick Gel Extraction Kit.

Sequencing

Gel extraction products were sequenced by Genewiz.Inc.

RESULT

BIRC5 mRNA levels in two cell lines

The presence of the BIRC5 in two cell lines, SH-EP1 and SK-N-ER, was detected by RT-PCR. Two main isoforms of BIRC5, full-length BIRC5 and BIRC5-ΔEx-3, were clear in agarose gel. There was no significant change in the ratio of these two isoform in the two cell lines (Fig 2)

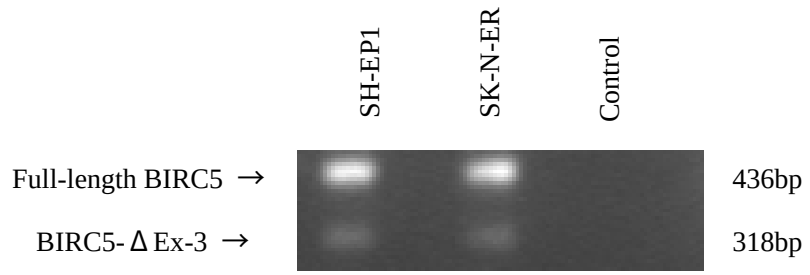

Figure 2 RT-PCR analysis of different isoforms expression of BIRC5. Primers were designed and RT-PCR was performed according to materials and methods.

Sequencing

Sequence analysis was also performed to confirm that RT-PCR products were different isoforms of BIRC5 (Fig 3).

Figure 3 Sequence alignment of the RT-PCR product in reference with full-length BIRC5 and BIRC5-ΔEx-3 NCBI reference sequence.

DISCUSSION

Neuroblastoma is composed of three cell types differing in their biochemical characteristics as well as growth and differentiation potentials: neuroblasts (N cells), substrate-adherent cells (S cells), and more primitive stem cells (I cells) (Ross, 2003). They have different abilities to grow as tumors. I-type is the most malignant, N-type is moderately tumorigenic, and S-type is unable to form tumors. SH-EP1 cell line is S-type while SK-N-ER cell line is I-type.

It was reported that only wild-type BIRC5 and BIRC5-ΔEx-3 are associated with protection against apoptosis (Krieg *et al.*, 2002). Insertion of exon 2B in BIRC5-2B interrupts the essential BIR domain, leading to the loss of cytoprotection of this isoform. The removal of exon 3 in BIRC5-ΔEx-3 also interrupts the BIR domain. However, this isoform of the protein retains its ability to suppress apoptosis (Mahotka *et al.*, 1999). Loss of this exon also results in a frame shift in exon 4, generating a novel COOH-terminal, which contains a bipartite nuclear localization signal potentially controlling selective accumulation of this isoform in the nucleus

(Rodriguez *et al.*, 2002).

Because full-length BIRC5 and BIRC5-ΔEx-3 are involved in protection against apoptosis, they were expected to be main isoforms of this gene in tumor cells. The result of this work was consistent with this expectation. There was no significant difference in the ratio of these two isoforms in SH-EP1 and SK-N-ER. There are some possible explanations to it. Full-length BIRC5 and BIRC5-ΔEx-3 may have similar ability to suppress apoptosis, or neither of them is the critical factor to suppress apoptosis in neuroblastoma. Further study in more types of cells may give a more detailed pattern of different isoforms of BIRC5 in different cell lines.

In addition, this work was focusing on the mRNA level instead of protein expression level. So the possibility that posttranscriptional decoration also regulates the expression level of different isoforms of BIRC5 was not excluded. Immunological methods such as western blot can be used in further studies.

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