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EXPERIMENTAL STUDY

Mutation detection in the promoter region of survivin gene on N-methyl-N-nitrosourea induced colon tumor model in experiment

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Abstract: Survivin (also known as BIRC5) is one of the first reported inhibitors of apoptosis proteins (IAPs), which is an important family of proteins that regulate apoptosis. It is developmentally regulated and expressed during cell differentiation in humans, mice and rat. Survivin is expressed in a series of human cancers and it has been widely accepted that survivin is strongly related to the onset and development of cancer. In the present study, we tried to determine differences in the promoter region of survivin gene in colon tissue samples from N-methyl-N-nitrosourea (MNU) induced rat colon tumor model and control group. Polymerase chain reaction (PCR) – single strand conformation polymorphism (SSCP) analysis was used for this aim. No significant differences were found in the promoter region of survivin gene between the normal and tumor tissues *(Tab. 2, Fig. 1, Ref. 16)*. Text in PDF *www.elis.sk.*
Key words: survivin, promoter, single strand conformational polymorphism (SSCP), rat.

Programmed cell death is also called apoptosis or physiological cell death, plays diverse roles in embryogenesis and normal homeostasis, as well as in tumorigenesis (1, 2). Apoptotic processes are regulated by a few factors, which have inhibitory or stimulatory effects. Survivin is one of these factors. It is a member of the IAP family, which is situated on chromosome 17q25, is a unique bifunctional protein that inhibits apoptosis by suppressing caspase-3 and caspase-7, and modulating the G2/M phase of the cell cycle by associating with the mitotic spindle microtubules. Inhibitors of apoptosis proteins are a family of negative regulators of apoptosis. Survivin plays a key role in the regulation of apoptosis and cell division (3, 4). Survivin is expressed in a cell cycle-regulated manner with a peak in the G2/M phase of the cell cycle, and a rapid down regulation level, and mediated by cell cycle-dependent elements (CDEs) and cell cycle homology regions (CHRs) located in the proximal region of the survivin promoter (5–6).

It is developmentally regulated and expressed during cell differentiation in humans, mice and rats. Survivin has been reported to be expressed in most human malignant tumors but not in normal differentiated tissues of adult human, with the exception of thymus, basal colonic epithelium, endothelial cells, and neural stem cells during angiogenesis. High survivin expression has been found in the majority of human cancers (7–10).

Survivin may act both as a mitotic regulator and a cytoprotective factor at cell division, a pathway potentially exploited in cancer where the survivin gene is broadly up-regulated (11–13). Our aim in this study was to observe any difference in promoter region of survivin gene in N-methyl-N-nitrosourea (MNU) induced rat colon tumor model using polymerase chain reaction (PCR)- single strand conformation polymorphism (SSCP) analysis.

Materials and methods

Samples

We used 28 formalin fixed paraffin embedded Wistar albino rat colon tissues that were obtained from a previous research. Briefly, animals were divided in 2 groups (Tab. 1), MNU and control group. Preparation of the rat colon tumor model, the paraffin embedding procedure is described in a previous research (14).

DNA Extraction

Paraffin embedded colon tissues were cut in to 4μm thick slices and placed on microscope slides. Then microscope slides were kept in the clean xylene for 10 minutes twice. To remove the residual xylene, the samples were washed twice with absolute ethanol for ten minutes. After the deparaffinization step slides

Tab. 1. Experimental groups.

Groups	Sample Codes	Sample (n)
Control Group	M1-M10	10
MNU Group	M41-M58	18

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were kept in the room temperature for drying. Qiagen Qiamp DNA FFPE Tissue Kit (Venlo, Netherlands) was used according to the manufacturer's protocol for the extraction of DNA.

PCR amplification

DNA was amplified with primers designed using the web site http://frodo.wi.mit.edu/

to produce a 280 bp product of proximal region of the survivin promoter. The primers forward; 5' - AAGGCGACTTTTTC-CAGAGG- 3' and reverse; 5'-TTAAGGTACAGCTGCCAG-GTC-3' were included. Each 50 µl reaction mixture contained 10X DreamTaq™ Green Buffer 5 µl, dNTP Mix 2 mM each 5 µl and primers 0.5 µM, Template DNA 1 µg, DreamTaq™ DNA Polymerase 1.25 U (Fermentase – Lithuania) with nuclease-free water to 50 µl. PCR was conducted as follows: 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 second, 57° for 90 second, 72 °C for 90 second, completed with a final elongation step at 72 °C for 5 minute. PCR products were subjected to electrophoresis on a 2 % agarose gel and stained with ethidium bromide.

Single-strand conformation polymorphism (SSCP)

SSCP was used to observe any difference inthe promoter region of survivin between MNU and control groups. Genomic DNA was isolated as described above. A volume of 8–10 µl of PCR product diluted with 10 µl denaturating buffer (95 % formamide, 10 mM NaOH, 0.25 % bromphenol blue, and 0.25 % xylene cyanol) was denatured by heating at 95 °C for 10 minutes and immediately placed in ice. The denatured PCR samples were run on 12 % acrylamide/bis gel in 0.5X TBE buffer for 3.5 hours at 200 V. Ice cooled water circulation with electric pump was applied to buffer cooling of the SSCP system. Gels were silver stained to visualize DNA bands (5).

Results

PCR amplifications of the total of six samples from all groups failed so we discarded them from consideration. Final distribution of sample count in the groups is shown in Table 2. SSCP results can be seen in the Figure1.

Discussion

Previous studies showed the importance of survivin overexpression in animal tumor models. Xiao-Dong Zhu and et al used N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and high dose sodium-chloride diet to induce rat gastric tumor model. In this model they tried to show survivin expression in gastric carcinoma model of rats like in human gastric carcinoma. According to their results, survivin expression in glandular stomachs of normal rats, of rats in middle induction period, in adenocarsinomas and tissues adjacent to tumor were 0 %, 40.0 %, 78.3 % and 38.9 %, respectively (15). Iskandar and Al-Joudi detected the expression of the survivin homogues and their subcellular distribution in formalin-fixed paraffin-embedded tissue sections of fetal and normal adult tissues of rat in their studies by using immunohistochemistry. Their results showed that was survivin abundantly and prominently expressed during fetal development in rats (12). In a similar study Lin Fan et al. showed that overexpression of survivin promoted mesenchymal stem cell (MSCs) survival in the infarcted myocardium and also enhanced the secretion effect of MSCs for vascular endothelium growth factor in vitro and in vivo in a rat model. This led to angiogenesis in the infarcted myocardium and ultimately reduced the infarct size, inhibited myocardial remodeling, and resulted in substantial recovery of cardiac function after myocardial infarction (16). In another study, Chen et al investigated the protein and mRNA expression of survivin, as well as the methylation status of the CpG sites in exon 1 of the survivin gene for 7,12 dimethylbenz[a]anthracene (DMBA)-induced hamster buccal- pouch squamous-cell carcinomas. Cytoplasmic stainings of survivin protein and mRNA were detected in all of the hamster buccal-pouch tissue specimens treated with DMBA, whereas neither survivin protein nor survivin mRNA were noted for all of the untreated and mineral oil-treated hamster buccal-pouch tissue specimens. Furthermore, all the untreated and mineral-oil treated samples had a survivin-methylated allele, whereas the DMBA-treated cancerous tissues showed no evidence of survivin methylation. Their results suggest that survivin may play an important role in DMBA-induced hamster buccal-pouch carcinomas, and that the gene expression may be modulated by an epigenetic mechanism (7).

Survivin shows differential expression in cancer and interconnects multiple pathways required for tumor maintenance, which makes it a valuable marker in cancer genetics. The goal of our study was to determine the status of promoter region of survivin

Tab. 2. PCR amplified samples.

Groups	Sample (n)
Control Group	7
MNU Group	14

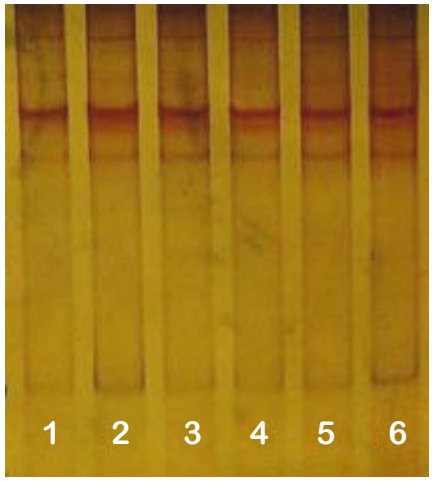

Fig. 1. Analysis of survivin promoter genotypes by single – strand conformation polymorphism. Line 1, 2 are control groups, Line 3 – 6 are MNU induced rat colon tumors.

gene in MNU induced colon tumor model in rat. We did not see any difference in the proximal region of the survivin promoter between the control group and MNU group. We are planning to further investigate other sections of survivin gene in this model.

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