Expression of survivin in fetal and adult normal tissues of rat

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Abstract

Survivin is an inhibitor of apoptosis protein and regulates the cell cycle in the G2/M phase. Survivin is expressed during embryonic and fetal development, selectively over-expressed in common human cancers and completely down-regulated in normal adult tissue. This work was aimed at studying the expression of the survivin homologues and their subcellular distribution in fetal and normal adult tissues of rat. Survivin expression was evaluated by immunohistochemistry in formalin-fixed, paraffin-embedded tissue sections of fetal and normal adult tissues of rat using the polyclonal serum SUR12A-CFI. This serum demonstrated intense positive survivin staining in adult kidney, ovary and oviduct, and a variable expression in different fetal organs, with particularly intense expression detected in the adrenal gland, liver, stomach, small intestine, colon, kidney and skin. In both fetal and adult tissues, the expression was predominantly cytoplasmic. It was concluded that survivin was abundantly and prominently expressed during fetal development in rat and that the polyclonal anti-human survivin antibody SUR12A-CFI is reactive with rat survivin.

Key words: Survivin expression, fetal development, immunohistochemistry.

INTRODUCTION

Apoptosis or programmed cell death is an essential physiological process in almost all tissues and is critical for normal embryonic development. However, no cell death was found during normal development of early embryos, and apoptosis could only be detected under abnormal conditions to eliminate defective embryonic cells. The primary function of survivin is its anti-apoptotic activity, in addition to the role it plays in chromosome segregation and cytokinesis. Targeting with antisense oligonucleotide to suppress survivin expression in embryos caused a development arrest at the blastocyst stage, and these embryos had diverse-sized and lobulated nuclei. Survivin was also observed in an immunohistochemistry assay in oocytes through hatched blastocysts and was predominantly localised in the cytoplasm. Survivin has also been reported to be localized in microtubules and/or kinetochores or it may be detected in the cytosol of tumour-derived cell lines and non-neoplastic cells.

Survivin of rat (Rattus norvegicus) is a 142 amino acid sequence protein with a molecular weight 16.7 kDa (Fig. 1). In the present study, the expression and subcellular localization of survivin in normal actively dividing cells were performed using an anti-human survivin antibody.

MATERIALS AND METHODS

Preparation of pregnant rats

The work utilized 3 Sprague Dawley female rats purchased from the Animal House, School of Medical Sciences, University of Science of Malaysia. Pro-estrus female rats (n=3) were selected and were allowed to mate, and gestation was confirmed by examination of the vaginal secretions.

Preparation of rat fetal and adult tissues for survivin staining

Pregnant rats at gestational ages in day 18 post-coitus were weighed and sacrificed by CO₂. Selected fetal and adult organs were removed, blotted dry and placed in a container of 10% formalin. The selected organs and tissues included adrenal glands, liver, stomach, small intestine and colon, kidneys, skin and ovary. Furthermore, whole fetuses were taken and processed in order to study the overall expression of survivin and its subcellular localization.
Tissues were subjected to a series of processing steps, which included fixation, dehydration with ethanol, clearing with xylene and wax impregnation with paraffin in Tissue Tek®, an automated closed system (Sakura, Japan). Tissues were then embedded in paraffin as the final process of making tissue blocks. Tissue blocks were then trimmed and sectioned with a microtome (Leica, Germany) to obtain 4 μm sections. The ribbons of sections were floated in a 50 °C water bath (Tissue Prep™ Floatation Bath Model 135, Fisher) and fished and mounted onto the poly-L-lysine slides. The sections were then deparaffinized on a 60 °C hot plate. This was followed by the hydration process which included immersion in xylene for 2 minutes and a series of steps of decreasing ethanol concentrations beginning with absolute ethanol (2 minutes), 95% ethanol (2 min), and 80% ethanol (2 min). Then the slides were dipped in 3% hydrogen peroxide for 15 min.

**Antigen retrieval and immunohistochemical staining for the tissue detection of survivin.**

The slides with the tissue sections were put through the antigen retrieval process using the pressure cooker method at 120 °C for 20 min. in Tris-EDTA buffer, pH 9.0. They were then cooled down in cold water for 15 min. Bovine serum albumin was added to block the non-specific binding sites. The anti-survivin polyclonal antibody SUR12A-CFI was added at the dilution of 1:1280 and left overnight. The reaction was probed with a secondary antibody.

| Human Sequence | MGAPTLPAW QPFLKDHRS TFKNWPFLGC ACTPERMAE AGFIHCPTEN 50 |
| Rat Sequence   | MGATALPPIW QMYLDHRIY TFKNPWFLIRD CACTPERMAE AGFIHCPTEN 50 |

**FIG. 1:** The amino acid sequences of human and rat survivin molecules. The sequences used to produce SUR12A-CFI are underlined.

| TABLE 1. The intensity of the survivin expression in selected rat organs. |
|-----------------------------|----------------|
| Intensity                  |               |
| Positive control           | +++           |
| Negative control           | 0             |
| Fetal Tissues              |               |
| Adrenal gland              | ++            |
| Liver                      | ++            |
| Stomach                    | +++           |
| Small intestine            | ++            |
| Colon                      | ++            |
| Kidney                     | ++            |
| Skin                       | +++           |
| Adults Tissues             |               |
| Ovary                      | +++           |
| Kidney                     | ++            |
| Stomach, small intestine, colon and liver | + |

Key: Negative= 0, Weak intensity = +; moderate intensity = ++; high intensity = +++
an anti-rabbit IgG, and visualized by the avidin-biotin-enhanced horseradish peroxidase method (DAKO) using diaminobenzidine (DAB)(Sigma). Staining with a light-blue nuclear counter-stain with Gill’s hematoxylin then followed. Finally the sections were dehydrated by immersion in increasing concentrations of alcohol beginning with 90% ethanol (2 min), and 95% ethanol, then in increasing concentrations of xylene. The slides were examined microscopically using an established scoring system. A known survivin positive breast cancer served as positive control. Substitution of primary and secondary antibodies utilized in the assay by pre-immune rabbit sera served as negative control steps, to assure that no non-specific “reactivity” or “stickiness” occurred. The rat adult tissues which were negative for survivin also served as internal negative controls.

RESULTS
An overall positive immunostaining for survivin was visualized macroscopically in whole body slides of the rat fetus. Obvious differences in staining intensity were demonstrated in different parts of the body (Fig. 2). Selected organs from other rat fetuses of the same mother demonstrated very intense survivin expression in skin epidermal cells. Actively dividing cells in the germinal layer adjacent to the dermis which undergo maturation changes concerned with the production of keratin, demonstrated very intense survivin staining (+++). Positive immunostaining was detected in the adrenal gland, liver, stomach, small intestine, colon, and kidney of the developing fetal rat (Table 1). The staining was intense and predominantly cytoplasmic (Fig. 3).

In adult rat normal tissues, positive immunostaining for survivin was detected in the kidney (Fig. 4) and ovary (Fig. 5). Survivin was also expressed abundantly in the oviduct of the normal adult rat. The staining was intense, predominantly cytoplasmic and evenly distributed among all cells. Most other tissues and organs were negative except for a very weak expression of survivin detected in the stomach, small intestine, colon and liver.

DISCUSSION
In the present study, the polyclonal anti-human survivin serum, designated as SUR12A-CFI, demonstrated reactivity with rat survivin in formalin-fixed, paraffin-embedded tissue sections by immunohistochemistry. Rat survivin is homologous to human survivin. The polyclonal serum SUR12A-CFI was raised against the C-terminal and the N-terminal regions of the survivin molecule. The sequence homology between human and rat survivin molecules exceeds 70%. The tissues investigated included developing or growing tissues (fetal) and tissues from adult rats. In general, the pattern of expression of survivin correlated with cell division. Survivin was detected in virtually all fetal tissues. However, there were clear variations in the expressions, in that it was expressed strongly in some tissues, and weakly in others. In adult tissues, a similar picture was obtained. It was highly expressed in the kidney, ovary and
However, it was also weakly expressed in the stomach, small intestine, colon and liver, which are tissues containing dividing cells. It was not expressed in brain tissue, which is differentiated and not dividing.

Furthermore, it was demonstrated that in normal fetal and adult cells with positive immunostaining, survivin is normally expressed predominantly in the cytoplasm. The cytoplasmic localization of survivin reflected the normal functional activity of the molecule. In contrast to this, relatively increased nuclear expression of survivin has been reported in tumour cell lines and in aggressive tumours, and especially correlated with bad prognoses in breast cancer patients.13

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FIG. 3: Photomicrograph showing positive immunostaining for survivin in formalin-fixed, paraffin embedded section of day 18 fetal adrenal tissue (arrows) (A) Magnification x 100   (B) Inset: Magnification x 400

FIG. 4: Positive staining for survivin in formalin-fixed, paraffin embedded section of adult rat kidney. The staining is predominantly in the proximal tubular cell cytoplasm (arrow)(A) Magnification x 100  (B) Inset: Magnification x 400
SURVIVIN IN RAT TISSUES

FIG. 5: positive immunostaining for survivin in a formalin-fixed, paraffin embedded section of normal adult rat ovary. Magnification x 2.5

REFERENCES