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Survivin in cancer diagnosis and therapy – a review

Failure of cancer treatment mainly results from metastasis formation and to a certain extent from local neoplastic infiltration. Numerous genes coding for growth factors and their receptors, adhesion proteins, and cell cycle regulatory proteins are involved in the process of carcinogenesis. Cancer microenvironment is characterized by uncontrolled production of growth factors and cytokines. In tumor specimens, an increased synthesis of the epidermal growth factor (EGF), vascular epidermal growth factor (VEGF), fibroblast growth factor (FBF), insulin-like growth factor (IGF), and various cytokines (e.g. TGF, IL-1, IL-8) have been found (1, 2). Protein overexpression leads to the escape of cancer cells from apoptosis and to autonomic signal transduction stimulating tumor growth, angiogenesis, and eventually metastasis formation.

Caspases, a group of cysteine proteases are involved in apoptosis. The proteins are initiated through different entry sites, such as mitochondria (mitochondrial pathway) and death receptors (receptor pathway); the latter begins with the activation of the TNF receptor that reacts with caspase-8-activating Fadd/Mort-1 protein and forms the DISC complex, which then transduces apoptotic signals and activates caspase-3 (Fig. 1).

The mitochondrial pathway of apoptosis is initiated with cytochrom c release from mitochondria, which normally is localized between an inner and outer mitochodrial membrane. Factors like UV-irradiation and chemotherapy stimulate cytochrome c release into the cytosol; there it reacts with Apaf-1 protein and dATP forming an apoptosome activating pro-caspase-9, followed by caspase-3 activation and finally by cell death.

The third way of apoptotic protein activation is based on caspase-3 activation by granzyme B (serine protease). Caspase-3 links together those three ways of apoptosis induction; activated caspase-3 mediates cleavage of intracellular proteins leading to cell death (Fig. 1).

Mitochondrial and receptor pathways are strictly connected. They are involved in BID protein activation; the protein belongs to the family of Bcl-2 proapoptotic proteins. BID is cleaved and activated by caspase-8 (mitochondrial pathway), and then migrates to mitochondria stimulating cytochrome c release (receptor pathway). Granzyme B also activates BID in the process of proteolysis, and induces apoptosis.

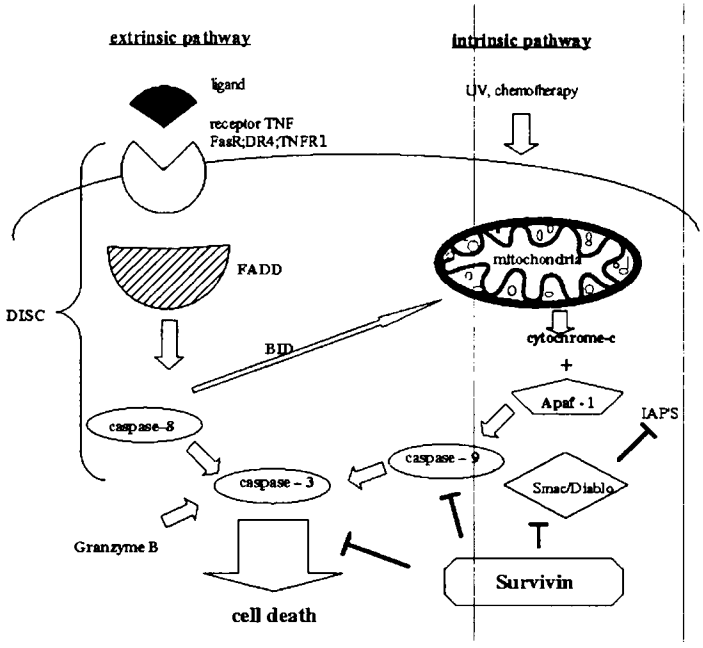

Fig. 1. Mitochondrial and receptor caspase activation

Various mutations of genes coding for apoptotic induction proteins, pro-apoptotic proteins (caspases) as well as those leading to apoptotic inhibitor overexpression allow cancer cells to escape from apoptosis.

Among apoptosis regulating factors involved in neoplastic transformation, Survivin is a key protein of a diagnostic and therapeutic value. Survivin is an apoptotic inhibitor consisting of 142 amino acids with one copy of baculovirus repeat (BIR). The gene coding for Survivin is located on chromosome 17q (3, 4).

Survivin plays an important role not only in apoptosis inhibition but also in the process of mitosis. In murine embryos with Survivin gene knockout, microtubule formation during mitosis is disturbed, and results in polykaryotic cells and eventually cell death (5). Probably the most important diagnostic feature of the protein is its high level in embryo tissues and cancer cells, whereas in adults Survivin is found in placenta, thymus, vessel epithelial cells, bone marrow stem cells and liver, though in much lower concentrations than in cancer cells. Therefore Survivin becomes an interesting research issue because other apoptotic regulatory proteins are also present in mature tissues. The overproduction of Survivin and its mRNA has been found in malignant cancers of the following organs: lung, breast, colon, stomach, esophagus, spleen, liver, uterus, ovary, in leukemia, non-Hodgkin's lymphoma, and melanoma. Protein overproduction in cancer cells is a poor prognostic factor with worse side effects of treatment, higher risk of progression and more frequent resistance to chemotherapy (3, 4, 6–8).

SURVIVIN IN APOPTOSIS INHIBITION

Caspase activity may be restrained by apoptotic inhibitor binding through the BIR domain. Survivin structure has been compared with another member of the protein family of the better-known mechanism of caspase inhibition – XIAP that contains three baculovirus repeats. Caspase-3 and -7

are inhibited by the region between first two BIR domains, whereas caspase-9 – by the third domain. The structure of BIR3 in XIAP shows high homology with the BIR domain in Survivin. Therefore, it is suggested that Survivin inhibits caspase-9, though the mechanism is not entirely understood and it is assumed that the reaction requires an unknown co-factor. It is also thought that Survivin inhibits the activity of caspase-3, although the inhibitor region has not been identified so far. The protein may also inhibit apoptosis indirectly. Survivin deactivates pro-apoptotic molecule Smac/DIABLO, which binds and inhibits IAP function (9, 10).

SURVIVIN AS A PROGNOSTIC FACTOR IN CANCER

Due to the differences in protein expression levels between tumors and normal mature tissues Survivin is regarded as a potential early predictor of neoplasia. The research concerning the level of Survivin in systemic fluids was performed in patients with bladder cancer. The level of apoptotic inhibitor was determined in urine specimens (specimens were filtered through a nitrocellulose membrane) followed by the addition of an anti-Survivin antibody. IAP protein was found in all patients with bladder cancer, and it was absent in healthy controls (11, 12). The protein was also found in oral cavity cancer, although Survivin was expressed already in pre-neoplastic stages (13). Other analyses showed Survivin overexpression in colon polyps, mammary adenoma and Bowen disease suggesting that Survivin may be expressed during neoplastic transformation or it may be a marker of disturbances between cell apoptosis and survival (14).

In summary, Survivin level may be determined in easily accessible systemic fluids and may be not only a marker of cancer but also of other disturbances of the highest risk of neoplastic transformation.

An increased level of Survivin in cancer cells is usually related with an increased number of cell cycles, decreased level of apoptosis, and a higher resistance to chemotherapy and ionizing radiation (15, 16). In research on different types of cancer, the prognostic value of survivin was clearly determined. Both mRNA and protein levels were evaluated. Increased mRNA level correlated with significantly shorter survival time of patients with stomach, colon, urinary system and liver cancer (17–20).

Not only the level of Survivin expression, but also protein location within a cell seem to have a prognostic value. Survivin expression in cell nucleus is an unfavourable prognostic marker in esophagus, liver and ovary cancer (21), whereas in stomach, bladder and breast cancer nuclear protein location correlates with longer survival (22). Research results indicate two different cellular compartments containing the apoptotic inhibitor. Cytoplasmic compartment seems to be involved in the initiation of cancer cell proliferation, while nuclear Survivin may be correlated with longer cell survival (21).

Since there are at least three different splice variants of Survivin, it also makes the protein a good prognostic marker, and the best known Survivin 2B seems to be an interesting factor in cell biology. The research shows that this splice variant of protein fails to inhibit apoptosis and is an antagonist of a full-length survivin. Survivin 2B expression decreases with an increase in tumor mass and tumor malignancy . Other analyses focus on the determination of an exact role of Survivin isoforms in cancer cells (14).

SURVIVIN FUNCTION IN NON-CANCER TISSUES

Since there is a possibility of anti-Survivin therapy in anticancer treatment, the question of potential side effects of such a therapy is raised.

Survivin plays an important role in differentiation and apoptosis inhibition of stem cells. The protein is present in bone marrow of murine embryos, and deletion of the gene coding for the protein results in a rapid death of the embryos, which suggests that the apoptotic inhibitor is essential to normal bone marrow function. Survivin has been determined in CD34+ bone marrow cells, and its expression increases in response to hematopoietic growth factors. In the absence of growth factors, caspase-3 expression is increased, which suggests that Survivin influences normal stem cell proliferation (23). Similarly, during T lymphocyte maturation, after CD3 receptor activation, the level of Survivin expression increases. In liver, the level of Survivin expression decreases due to hypoxia, and increases after partial hepatotectomy. In brain Survivin is detected in embryos as well as in normal cerebral ventricle ependyma and arachnoidea in children. In adults protein expression increases after brain damage (research performed on rat models) and due to hypoxia, which suggests the role of Survivin in cell protection under stressful conditions. Survivin also plays an important role in oogenesis and spermatogenesis, which is suggested by an increase of its activity in testicles and ovaries in response to gonadotropins (24–26).

Stomach mucous membrane undergoes continuous regeneration, and complete cell replacement takes place ever 3–5 days. It has been shown that low Survivin concentrations are present in surface cell nuclei of mucous membrane and the inhibition of protein expression by siRNA increases susceptibility to damage caused by non-steroidal antiinflammatory drugs (28).

The results presented above show that anti-apoptotic function of Survivin may become useful not only in cancer treatment but also in the therapy of stroke due to hypoxia or as a support during gastric ulcer treatment.

Apoptosis is regarded as an important factor regulating blood vessel remodelling both in normal and pathological conditions. The following factors: hypoxia, acidosis, VEGF, bFGF, and IL-8 influence vessel epithelial cells via phosphatidylinositol-3-phosphate cascade activation, which modulates Survivin expression, and eventually results in neo-vascularization (29, 30). Survivin deactivation by antibodies eliminates angiogenic influence of cytokines in cancer cells (30).

Since apoptotic inhibitors are detected mainly in cancer cells and have a prognostic value, they become a potential target of anticancer therapies. The research shows that even though Survivin is present in some normal tissues, protein inhibition is only slightly toxic for an organism (23), but it may reduce tumor growth, increase spontaneous apoptosis and sensitize cancer cells to chemotherapy. The following protein inhibitors have been analyzed: antisense nucleotides, ribozymes, and siRNA.

Antisense nucleotides. One of the therapeutic strategies is to use antisense nucleotides that block mRNA and therefore decrease protein level. Nucleotides block protein synthesis by forming duplexes with cellular mRNA that blocks translation. Nucleotide-mRNA complexes bind with RNaseH, which results in complex dissociation and mRNA deactivation, while the nucleotide binds with another mRNA molecule repeating the process. Treatment efficacy has been proven *in vitro* using lung cancer and mesothelioma cell lines with Survivin overexpression (31), and *in vivo* in gastric cancer cells transfected with plasmids containing gene fragment coding for antisense Survivin; in the latter case a decrease in tumor mass and an increase in apoptosis have been observed (32). Survivin antisense oligonucleotide has now completed a phase I trial in patients with advanced cancers and a phase II trial has been announced.

siRNA. Small interfering RNA molecules are double-stranded RNAs of 21-23 base pairs. They silent the expression of genes with sequence homology. siRNA emerge after double-stranded RNA cleavage into short fragments that bind with a protein of ribonuclease (RISC) activity. Complexes bind complementary mRNA molecules, which results in RNA cleavage and blocks translation. Treatment efficiency in the inhibition of Survivin expression has been proven in cervix cancer cell lines, where

Survivin expression has been blocked already after 60 hours following transfection (33).

Ribozymes. Ribozyme is another agent that inhibits Survivin expression; ribozymes are RNA molecules that catalyze diester-bond hydrolysis in specific RNAs. Treatment efficacy has been confirmed *in vitro* using melanoma, prostate cancer and breast cancer cell lines (34, 35).

CYCLIN-DEPENDENT KINASE INHIBITORS

Another strategy of anticancer therapy uses Survivin antagonists, instead of protein inhibitors. Cyclin-dependent kinases, present in almost all types of cancer cells, are key control proteins of the cell cycle. Apart from apoptosis inhibition, Survivin plays an important role during mitosis binding with microtubules. Survivin phosphorylation, which is essential for its protective function, is regulated by the CDK1 enzyme. Seliciclib, flavopiridol (CDK inhibitor) that are tested on cell lines, stimulate spontaneous mitochondrial apoptosis and increase chemo-susceptibility to taxol (36).

The researches mentioned above point to the efficiency of anticancer therapy that inhibits Survivin expression or function as well as low toxicity for other cells. Low toxicity may result from a significant difference in Survivin expression between cancer cell and a mature normal cell, or from a relatively short time of observation. Potential side effects of anti-Survivin treatment might affect the following proliferating cells: hematopoietic cells, T lymphocytes, gastrointestinal mucous membrane cells, hepatocytes, and neurons. The influence of anti-Survivin therapy on T lymphocytes is particularly disturbing because of their role in cancer cell eradication (37). The treatment also has a toxic effect on stem cells. In that case cell toxicity was relatively low compared with a total inhibition of Survivin expression in tumor cells (38).

The research on the improvement of anticancer treatment efficacy is mainly based on the analysis of differences between cancer cells and normal cells as well as on improving tumor susceptibility to chemo- and radiotherapy. Survivin, which is an apoptotic inhibitor, has both features. Increased protein expression is related with disease progression and higher therapy resistance. The results of research on the inhibition of Survivin expression or function show that protein inhibition improves the efficiency of standard therapy. Moreover the research suggests that Survivin is essential to tumor angiogenesis. Therefore protein inhibition may further improve treatment effectiveness.

Despite its recent discovery in 1997, Survivin has provided unique opportunities for cancer treatment. In a short perniod multiple strategies have entered clinical testing in humans such as vaccine therapy in treating patients with stage IV cutaneous melanoma or Survivin urine mRNA assay risk of bladder cancer study.

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SUMMARY

Survivin plays an important role in cancer development. It is present in most of the types of cancer and is associated with higher resistance to chemo- and radiotherapy. That makes the protein a poor prognostic marker. The research on that apoptotic inhibitor focuses mainly on its value as a target in anticancer treatment. The protein may also become an early marker of a precancerous condition. In the present review survivin importance in diagnostics and therapy of cancer is described.

Surwiwina w diagnostyce i terapii nowotworów – przegląd

Surwiwina odgrywa istotną rolę w rozwoju raka. Jest wykrywana w większości nowotworów i wiąże się z większą opornością na chemio- i radioterapię oraz prognozowaniem krótszego przeżycia. Badania nad tym inhibitorem apoptozy ukierunkowane są obecnie na możliwość zastosowania go jako celu w terapii przeciwnowotworowej oraz jako markera wczesnych zmian przedrakowych. W artykule przedstawiamy możliwości zastosowania wiedzy na temat surwiwiny w diagnostyce i terapii nowotworów.