REVIEW

Apoptosis in oncology

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ABSTRACT

Apoptosis is a complex process involving a large array of genes and mutation of any of these genes may lead to malignancy formation. Re-acquirement of FasL by tumor cells may enable them to evade the surveillance of immune system and thus contributes to the growth of tumor. Apart from traditional therapies, inducing apoptosis of tumor cell by new methods employing death receptor ligands and making use of Fas counterattack is also being developed.

Key words: *Apoptosis, mitochondria, death receptor, death receptor ligand, tumor therapy.*

INTRODUCTION

Apoptosis, also known as programmed cell death, is a highly orchestrated form of cell death in which cells neatly commit suicide by chopping themselves into membrane-packaged bits. It is critical not only to the development but also to the homeostasis and normal functioning of the adult for a multiple cellular organism. The malfunctioning of apoptosis during the development will lead to abortion or abnormalities, while failure of DNA-damaged cells to kill themselves via apoptosis may result in malignancy formation. Apoptosis is also one of the weapons immune system employs to eliminate virus-infected cells or transformed cells, but unfortunately tumor cells may also employ the weapon to counterattack immune system and they can even gain a super arm in the combat. It is also by inducing apoptosis of target cells that irradiation and some chemotherapeutic drugs play their anti-cancer roles. However, mutations of apoptosis-related genes often make these therapies futile. Understanding the mechanisms of apoptosis and apoptosis of cancer cells attract the attention of scientists worldwide and the present results and bright prospect are well worth their efforts. Herein we briefly discuss the two general pathways of apoptotic signal transduction followed by detailed description of progress in its application in oncology.

General pathways of apoptotic signal transduction

A large variety of stimuli, including the ligation of death receptors and DNA injury, may induce the apoptosis of the target cells. Generally speaking, there are two pathways for the death signal transduction, which are called death receptor pathway and mitochondria pathway, respectively. Most death receptors/ligands belong to TNFR/TNF superfamily. Up to the end of 1999, there have been identified approximately 25 pairs of TNF/TNFR superfamily members (some are orphan receptors or ligands), among which best characterized are Fas/FasL and TNFR/TNF[1]. FADD and TRADD play important regulatory roles in their respective death signal transduction[2],[3]. As to the mitochondria pathway, Bcl-2 family members are important apoptosis modulatory proteins and they play their regulatory roles largely by keeping and altering the concentration rate of the pro- and anti-apoptotic members, especially that of Bcl-2 and Bax. Other important regulatory proteins involved in mitochondria pathway include Apaf-1, AIF (apoptosis inducing factor), and cytoc et al.

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Despite the multiple apoptotic stimuli and the different pathways, the apoptotic cells often show similar morphological and biochemical changes, such as DNA fragmentation, chromatin condensation, membrane blebbing, cell shrinkage, and membrane-enclosed vesicles (apoptotic bodies), suggesting that different pathways lead to the activation of common proteinases, which are now defined as caspases.

Caspases may execute cells by several means. One role of caspases is to inactivate proteins that protect living cells from apoptosis[4],[5]. In fact, cleavage of these proteins not only inactivates their activity, but also produces a fragment that promotes apoptosis. Besides, caspases also contribute to apoptosis through directly disassembling and indirectly re-organizing cell structures[6-10]

Apoptosis in tumorigenesis

Mutations of apoptosis-related genes lead to tumor formation

Since apoptosis is a complex process involving a large array of genes, and any mutation of apoptosis-related genes may lead to the failure of transformed cells' death, which results in tumor formation at last. The most common mutated genes found in cancer cells include *p53*, *c-myc*, Bcl-2 family members and ndm23. And the Fas-related genes mutation such as deletion or down-regulation of Fas, truncation of Fas in its cytoplastic domain and up-regulation of negative regulatory proteins also abrogate the Fas-mediated cell death. These genes interact each other to decide the fate of both the normal and transformed cells.

P53 is a very important regulatory protein targeting many other apoptosis regulatory genes such as Bcl-2 and Bax. Wild-type p53 can down-regulate the expression of Bcl-2 and up-regulate that of Bax, altering the balance of the couple genes in favor of apoptosis. Many kinds of human tumors such as colorectal carcinoma, brain and lung cancer, mammary carcinoma, skin and bladder carcinomas are found to be p53 mutated and the mutated p53 loses the function of binding DNA and exerting control over the expression of target genes. What's more, wide-type p53 will be deprived of the ability to bind DNA and activate target gene expression when complexed with mu-

tated p53, which is termed dominant negative [11]. Thus cells with DNA damages, which should have been checked by p53 on stage G1 and compulsory to be repaired, will progress into S stage and in some circumstances further deteriorate into malignancies. Large T antigen, E1B of adenovirus and E6 of human herpes viruses et al also encode proteins that directly or indirectly inhibit the function of p53, making the normal p53 unable to transactivate the target genes specifically.

Bcl-2 family members are expressed in most normal human tissues, although in low quantity. Bcl-2 is significantly overexpressed in acute myoleukia and Bcl-2 in M1 and M2 surpasses that in M3, M4, or M5. It is also worth noticing that the expression is related to the cell differentiation, i. e. the expression of Bcl-2 is in a positive proportion to its degree of malignancy and negative proportion to its clinical responses[12]. In acute B lymphocyte leukemia, the life of lymphocyte with high expression of Bcl-2 could be postponed more than one week, even after the bone marrow culture was removed, the cells still remained alive[13]. And it is also found that Bcl-2 over-expression and down-expression of Bax exist in 1/3 mammary carcinomas.

Fas counterattack-a model of malignant cells evading the immune surveillance

FasL is one of the weapons the immune system employs to kill Fas-expressing tumor cells. Unfortunately, many tumor cells express high level of FasL too, with which they can fight back. Melanoma, pulmonary carcinoma, colorectal carcinoma, glioma and hepatoma are five typical examples. Shiraki K et al makes investigations into the expression of FasL in human colorectal carcinoma and liver metastasis foci. The result showed that among the 7 cases of the primary foci, 2 expressed FasL and in all of the 4 patients with liver metastasis, the FasL expression of metastasis was found positive, suggesting that the FasL expressing tumor cells may be tougher[14]. The explanation is that FasL-negative tumor cells are more likely to be attacked by the activated T lymphocytes while FasL expressing tumor cells may escape the killing of lymphocytes by fighting back. And also they may induce the apoptosis of neighboring liver cells to facilitate the formation of

neoplasm.

Someone argues that since both tumor cells and activated CTLs (cytotoxic T lymphocyte) express FasL, how can it be that CTLs are always killed or prohibited rather than vise versa? Surely there is experiment demonstrating that when tumor cells and antigen-specific CTLs express both Fas and FasL, bi-direction interactions can lead to killing in both cell types[15]. There is, however, an important difference between the conditions used for measuring cytotoxicity in vitro and those present at the tumor site. The number of effector cells (E) used in the standard cytotoxicity assay always exceeds that of tumor targets (T), while the E: T ratios in the established tumor, almost invariably are in favor of tumor cells, even when considerable TIL infiltrates are present. Therefore, it is likely that, in vivo, FasL+ tumor will always have an advantage in perpetrating the death of Fas+ immune cells. Besides, FasL expressing tumor cells are often Fas down-regulated or nullified because of mutation of genes involved in Fas-mediated death signal transduction.

Immune effector cells nullified by FasL expressed on the surface of tumor cells include not only activated T lymphocytes that express Fas but also NK cells. A recent report demonstrated that murine AK-5 tumor cells transiently upregulated FasL when grown in peritoneal cavity of syngeneic mice, and that this FasL upregulation coincided with depletion of the intraperitoneal NK cell population[16]. Depletion of NK cells was local to the tumor microenvironment, as splenic and peripheral NK cells were unaffected. The same AK-5 tumor cells did not express FasL when injected subcutaneously. FasL-negative, subcutaneous AK-5 tumors showed about 70% regression, mediated largely by NK cells, whereas FasL-expressing intraperitoneal tumors grew successfully, always resulting in death of host. These findings suggest that FasL-mediated counterattack against anti-tumor NK cells may contribute to the successful immune evasion of FasL-expressing tumors in vivo.

The high prevalence of expression of FasL in several diverse tumor types suggests that FasL is a general, perhaps essential factor in the inhibition of anti-tumor responses[17]. As such, the Fas counterattack might be a rational target for therapeutic intervention.

However, there are also some experiments inconsistent with the Fas counter-attack model[18]. Hiroshi Arai et al. found that FasL expression on the surface of tumor cells promotes tumor regression through apoptosis or inflammation rather than enhanced tumor growth through its effects on immune suppression[19]. The mechanism of antitumor effect was dependent on Fas expression of tumor cell. In the case of Fas+ tumors, there appeared to be direct induction of apoptosis. In cells resistant to lysis by FasL, tumor regression was induced through an independent mechanism involving FasL-induced inflammation and a potential ``by-stander effect''.

Apoptosis in cancer treatment

Apoptosis and traditional tumor therapy

(1) Traditional tumor therapies down-regulate the threshold of apoptosis

Since some malignancy formation is caused by the hampering of apoptosis, down-regulating its apoptotic threshold may be an ideal way to treat cancer[20]. In fact, it is one of the mechanisms by which traditional radiotherapy and chemotherapy take effect. Sakakua C et al examined the histological response, rate of apoptosis, DNA fragmentation and p53 status in tumors from 28 patients with rectal cancer undergoing HCR therapy (combined hyperthermia, chemotherapy and radiation) before surgery and from 22 patients who did not have preoperative treatment. The therapeutic effect of HCR therapy was closely correlated with the rate of apoptosis and related to the p53 status of the tumors to some extent[21].

However, mutations of apoptosis- related genes or any factors influencing their activity may endow tumor cells the ability to decline being executed. For example, the overexpression of Bcl-2 results in many tumor cells' resistance against therapeutic drugs and further experiments confirmed this phenomenon has no direct relation with the expression of multiple drug resistance gene following drugs accumulation[22]. However, leukemia cells transduced with antisense oligonucleotide of Bcl-2 will regain the sensitivity

towards chemotherapeutic drugs[23]. Estrogen can up-regulate the expression of Bcl-2 in mammary carcinoma cells, so the use of Tamoxifen, which can act as an antagonist against estrogen, helps the cells reacquire the sensitivity to amycin by down- regulating the expression of Bcl-2[24].

Cell death induced by some certain chemotherapeutic drugs are p53-dependent, thus p53 mutation will lead to drug resistance in some circumstances. For those cells whose apoptosis may be p53 dependent and at the same time p53 deficiency does exist, it may be advisable to transduce wide type p53 gene into these cells to re-endow them with sensitivity to chemotherapy. Experiments showed that p53 transduced vectors can induce the apoptosis of p53 mutated non-small-cell lung carcinoma, squamous epithelial carcinoma and transplanted human mammary carcinoma in mude mice and sometime even inhibit their metastasis[25-28].

(2) Chemotherapeutic drugs and death recepor/ligand

Certain chemotherapeutic drugs have been noted to induce apoptosis by altering surface expression of death receptors. For example, results from our and other labs both showed doxorubicin can up-regulate the Fas expression of tumor cells, suggesting that chemotherapy may have a role in regulating responsiveness of tumors to Fas/FasL-mediated apoptosis[29]. And incubation of cell lines with doxorubicin or 5-fluorouracil significantly augmented TRAIL-induced apoptosis in most breast cell lines as well[30]. Beltinger showed that TK/GCV treatment increase cell surface of CD95 (Fas) and TNFR besides inducing p53 accumulation. TK/GCV-induced apoptosis involves CD95-L-independent CD95 (Fas) aggregation of FADD and caspase-8-containing, death-inducing signaling complex [31]. However, our unpublished data also intriguingly showed that some chemotherapeutic drugs such as bleomycin can up-regulate the expression of FasL on the surface of some human hepatoma cell line as well as primary liver cancer cells, which may be a share of explanation for drug-resistance or drug tolerance.

Death receptor ligand in cancer treatment

The idea of targeting specific death receptors to induce apoptosis in tumors is attractive, because death receptors have direct access to the caspase machinery. Moreover, unlike many chemotherapeutic agents or radiation therapy, death receptors initiate apoptosis independently of p53 tumor suppressor gene, which is inactivated by mutation in more than half of human cancers. Despite these advantages, the clinical utility of both TNF and FasL has been hampered by toxic side effects. Systematic administration of certain TNF doses causes a severe inflammatory response syndrome that resemble septic shock; this is believed to be mediated mainly by induction of proinflammatory genes in macrophages and endothelial cells through NF-κB activation. Injection of agonistic antibody to Fas in tumor-bearing mice can be lethal, apparently because of apoptosis induction in hepatocytes, which express abundant Fas.

In 1996, Robert M. Pitti et al isolated a new member of TNF superfamily via an expressed sequence tag, designated it as Apo-2 ligand (Apo-2L) because of its structural and functional similarity to Fas/Apo-1L[32]. Several differences between Apo2L and TNF or Fas suggest that Apo2L may be a safer agent. First, although DR4 and DR5 can activate NF-κB upon overexpression, Apo2L itself induces this response only weakly, and activation requires doses that are considerably higher than doses of TNF that activate a strong NF-κB response. Second, Apo2L mRNA is expressed in many tissues, suggesting that the ligand may be nontoxic to normal cells. Repeated intravenous injections of Apo2L in nonhuman primates did not cause detectable toxicity to tissues and organs examined. Third, DR4 and DR5 are expressed in normal tissues and in many types of tumor cells, whereas DcR1 and DcR2, which lack the cytoplasmic region and has a truncated death domain respectively[33], are expressed frequently in normal tissues but infrequently in tumor cells. This differential expression of death and decoy receptors might enable Apo2L to induce apoptosis in tumors while sparing normal cells. What's more, Apo2L cooperated synergistically with the chemotherapeutic drugs 5-fluorouracil or CPT-11, causing substantial tumor regression or complete tumor ablation[34].

Despite of the promising preclinical study in multiple tumors such as melanoma[35-37], human mammary adenocarcinoma cell line MDA-231[38], human brain tumors[39] and malignant gliomas[40], a report by Jo et al[41] indicates that human primary hepatocytes are also efficiently killed by TRAIL, an alarming finding that should warn researchers to proceed with great caution into clinical trials involving TRAIL.

Therapeutic implications from Fas counterattack

If the Fas counterattack is finally confirmed to be the case in patients with cancer, new therapeutic strategies may be employed making most use of what we have had at hands. Applicable ways include depriving the tumor cells of their newly acquired FasL, grinding the edge of weapon of FasL on the surface of CTLs, re-endowing tumor cells with sensitivity to Fas-mediated cell death, and strengthening CTLs to be tolerant of the Fas-mediated apoptosis.

In more details, the use of neutralizing antibodies specific to FasL or their fragments on the surface of tumor cells might be helpful in removing their fighting back ability. The use of the soluble form of Fas, such as Fas-Fc, targeted to the tumor cells, could result in blocking the ligand and thus preventing its binding to receptors on lymphocytes. In tumors that are Fas-sensitive, agonistic anti-Fas antibodies, which are able to induce apoptosis, could be of use. Immune cells may also be protected from Fas-mediated apoptosis by administration of antagonistic anti-Fas antibodies directed to the Fas antigen on the surface of immune cells, antisense oligonucleotides specific for Fas, transducing lymphocytes with an "apoptosis-resistance" gene and treating the patient with cytokines that selectively protect immune cells from apoptosis, typical of which are IL-2, IL-12, and IL-15[42]. To modify Fas expression or increase sensitivity to Fas- mediated signals in tumor cells, administration to cancer patients with cytokines such as IFN γ is feasible, and also possible is genetic engineering through transduction of tumor cells with genes such as Fas or Bax to up-regulate their expression.

Perspective

Although substantial progress has been made in understanding the mechanism of apoptosis and much effort has been done to treat cancer by inducing the death of tumor cells, still much remains to be elucidated. Examples include the final establishment of Fas counterattack, how the death receptors and decoy receptors interact to decide the destiny of TRAIL-engaged cells, how the mitochondria and death receptor pathways get related to each other, and how to apply the newly identified death receptor ligands such as THANK and LIGHT[43] in cancer treatment. In addition, other means may be combined with apoptosis-inducing methods to kill cancer cells more effectively. For example, patients with cancer can be treated with tumor vaccines and the produced cytotoxic T lymphocytes (CTL) then be returned to the patients after treated with Fas-Fc, which can block the engagement of Fas on activated T cells by the FasL on the surface of some tumor cells. The goal of future research lies in further study on the detailed regulation of apoptotic signal transduction pathways and how to apply the in vitro and in vivo study in clinical trial.

Conclusion

In summary, apoptosis is a complex process essential to normal development and functioning of multiple cellular organisms. Its great significance has appealed to numerous distinguished scientists worldwide and the overall picture of the two general pathways of apoptotic signal transduction has been outlined. With more and more evidences accumulating that apoptosis plays an important role in not only elimination of transformed cells but also in actively evading the immune surveillance, much work has also been done to treat cancer by inducing cell death via apoptosis. As our understanding of apoptosis further deepens, surely more effective methods will emerge and be used to treat malignant tumors.

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