Therapeutic importance of apoptosis pathways in pancreatic cancer

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Abstract: Recent studies indicate the significant role of apoptosis and the genes that control it in the process of carcinogenesis. Apoptosis or programmed cell death is a complex process that controls cell proliferation and maintenance of accounting for the necessary balance in the body. Disturbances of apoptotic signalling pathways directly lead to the development and progression of cancer. This also applies to pancreatic cancer, which is characterized by poor prognosis and resistance to treatment. In recent years progress has been made concerning the complex pathways of apoptosis, which allowed the development of new therapeutic strategies. This article reviews current knowledge on apoptosis pathways and their role in treatment of pancreatic cancer.

Key words: apoptosis, pancreatic cancer, treatment.

Introduction

Apoptosis or programmed cell death is a physiological process, which leads to elimination from the body of harmful, unnecessary or damaged cells [1, 2]. The balance between the processes of proliferation and apoptosis is essential to maintain homeostasis in the body [3].

Recent studies concerning cancer biology have shown that apoptosis and the genes which control it have a significant influence on the processes of carcinogenesis [4]. In addition, deregulation of apoptosis pathways contribute to the generation of resistance to currently used therapy [5, 6]. Evasion of apoptosis is one of the important characteristics

of cancer, promotes tumor growth and progression of metastases [7]. Therefore, a better understanding of the regulation of apoptosis in cancer cells may contribute to more effective treatments (Fig. 2) [8].

Pancreatic cancer is a cancer of the increasing incidence of survival 5 years not exceeding 5% [9]. Insufficient effectiveness of current treatment a regimen contributes to poor prognosis and is one of the most important challenges in the field of oncology [10].

Currently, considerable progress in clarifying the molecular mechanisms of pancreatic cancer. It was shown that the development of pancreatic cancer is due to the presence of numerous genetic abnormalities and disorders of the genes responsible for the regulation of apoptosis [11]. The accumulation of mutations leading to changes in the morphology of epithelial ductal and tumor development [12]. In pancreatic cancer is well documented high incidence of p53 gene mutations and gene family Bcl-2 (*B-cell lymphoma 2*) [11, 13].

Apoptosis pathways

The main pathways leading to apoptosis can be induced by a signal from the outside, which is associated with activation of membrane death receptors. The second way is called the p53 pathway proceeds with the participation of mitochondria and is referred to as internal [14]. The DNA damaging agents and activating apoptosis replace the thermal stress, oxidative stress, lack or deficiency of growth factors. To internal regulators of apoptosis are included among other proteins belonging to the Bcl-2 family [11].

Activation of the external route begins with the stimulation of receptors "death signal" (Fig. 1) which takes place by attaching to specific ligands [11, 14]. Membrane death receptors belong to the TNF (*tumor necrosis factor*) receptor superfamily, they also include TNF-R1 (*tumor necrosis factor receptor*), Fas (Apo-1/CD95) and receptors for *TRAIL* (*TNF related apoptosis inducing ligand*). These receptors are activated by their natural ligands (*TNFα, FasL, factor TRAIL*) [8, 15]. A unique feature is the presence of death receptor intracellular domain DD (*death domain*). As a result of ligand-binding receptor oligomerization occurs, then it is linked to a protein FADD (*Fas-associated death domain protein*) and procaspase 8 or 10 thereby forming a complex of DISC (*death-inducing signaling complex*) [8, 14, 15]. The consequence of these processes is the activation procaspase 8, which is a direct activator of caspase 3. It is postulated that the role of caspase-10 may be important in cells which lack caspase-8 [16].

In the cells of "type I" apoptosis pathway is exclusively external, but in other "type II" signal amplification occurs through mitochondria. This process is associated with proteolysis of the proapoptotic protein Bcl-2 family, namely protein Bid (*BH3 in- teracting domain*) by caspase 8 [15]. This protein is in an active form *tBid* (*truncated Bid*) moves to the mitochondria, where it comes to its integration with the inner mitochondrial membrane [17–19].
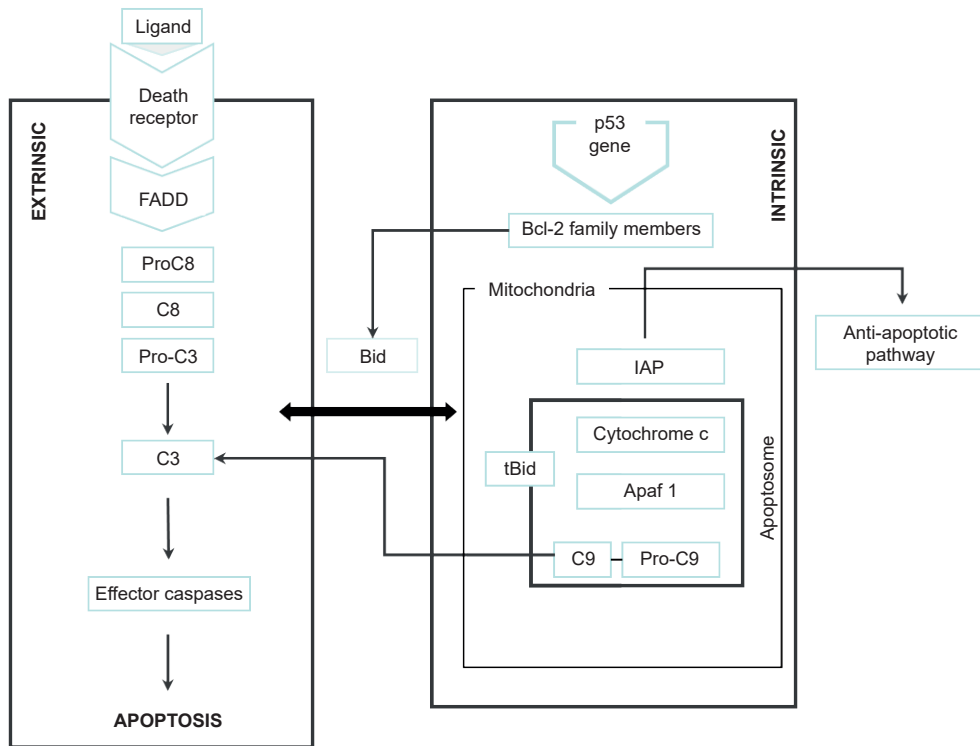
Fig. 1. Simplified diagram of the process of apoptosis in the two pathways connecting by *Bid* protein.

Activation of the internal (Fig. 1) route is associated with the outflow of cytochrome c (*cyt c*), which induces the formation of a complex called apoptosome composed of cyt c, procaspase 9, ATP and the cytosolic protein Apaf 1 (*Apoptotic protease activating factor 1*). The formation of the complex is required for activation procaspase 9, which acts as a direct activator of caspase 3 [14, 15]. The mitochondrial proteins are released by the IAP (*Binding Protein with Low PI*) antagonist inhibitor of apoptosis and protein AIF (*Apoptosis Inducing Factor*) [8].

Morphological processes are described as structural changes in the cell. This leads to loss of intracellular water, the concentration of the cytoplasm and cell size reduction. A characteristic feature of apoptosis is nuclear chromatin condensation and the disappearance of nuclear membrane, and the next stage of the fragmentation of the nucleus [2, 11].

The role of apoptosis pathways in the treatment of pancreatic cancer

Death receptors

One of the factors that trigger the process of apoptotic cell death is death receptor activation. Deregulation of this pathway may contribute to tumor growth and tumor

development [14]. Therefore, these receptors appear to be a natural goal for molecular anticancer drugs [20]. In the case of pancreatic cancer drew attention to the receptor Fas (Apo-1/CD95), TRAIL-R1, TRAIL-R2 and their ligands [15].

Arrangements for the Fas receptor expression in pancreatic cancer are conflicted, which may suggest that cancer cells may avoid apoptosis by downregulation of Fas receptor. In addition, demonstrated that FAP1 (*Fas-associated phosphatase 1*) can block the function of Fas. Cell lines resistant to Fas-dependent apoptosis show a strong overexpression of FAP-1, which was also observed in pancreatic cancer cells. Modulation of the enzymatic activity of FAP-1 can be used in the search for effective treatment of pancreatic cancer [15, 21].

Promising candidate because of its low toxicity is TRAIL, however, most pancreatic cancer cell lines show resistance to it. Sensitivity to TRAIL may be increased by combination therapy with other chemotherapeutic agents. It was observed that TRAIL has a higher antitumor activity in combination with gemcitabine [8, 22]. Because of the resistance to TRAIL is designed for this type of selective receptor variants of recombinant TRAIL. Following an exchange of several amino acids in the native recombinants obtained selectivity for TRAIL-R1 and TRAIL-R2. In this way, managed to obtain a human agonistic monoclonal antibodies specific for TRAIL receptors. Antibodies have proven to be effective as monotherapy and combination therapy [23].

It should be noted that not all receptors of this group have a death domain (DD). Some receptors for TRAIL (TRAIL-R3, TRIAL-R4, DcR3) is a so-called "decoy receptors". Joining factor TRAIL by these receptors does not induce apoptosis pathway activation [22].

In vitro studies have also shown an increased incidence of metastases, suggesting that in addition to the role of pro-apoptotic TRAIL may act as features associated with the mechanisms of tumor cell resistance to apoptosis and activation of a cascade of survival [8, 22].

Inhibitors of apoptosis proteins (IAP)

Protein IAP (*Inhibitor of Apoptosis Proteins*) are important defense against cancer cell death by apoptosis, also perform important functions during cell division. For IAP proteins are: NAIP (*Neural Apoptosis Inhibitor Protein*), XIAP (*X-linked IAP*), survivin, Bruce (Apollon), livin (ML-IAP) and cIAP-1 (*cellular inhibitor of apoptosis-1*)*, cIAP-2 (*cellular inhibitor of apoptosis-2*). Most of them have the ability to directly bind and inhibit caspase 3, 7, and 9 [8, 22, 24]. IAP proteins may also act as ubiquitin ligases and in this way to the degradation of caspases [25]. Membership of a protein depends on the presence of IAP BIR (*baculoviral IAP-repeat*) domains required for interaction with caspases [15].

High expression of IAP proteins (IAP, cIAP-1, cIAP-2, survivin, livin) was observed in pancreatic cancer cells [26]. The IAP protein has the strongest anti-apoptotic activity of XIAP, which has the ability to directly block the caspases. In many cells, including

pancreatic cancer have a reduced level of a negative regulator of XIAP, namely XAF-1 (*XIAP-associated factor 1*), regarded as a tumor suppressor gene product. In multivariate analysis, expression of XAF-1 proved to be a significant predictor of survival among patients. It has been observed since that low levels of XAF-1 correlates with poor prognosis in patients with pancreatic cancer [27].

IAP proteins are negatively controlled at several levels including the mitochondrial Smac/DIABLO (*Second Mitochondria-derived Activator of Caspases/Direct IAP Binding Protein with Low PI*) to prevent inhibition of caspase 3 and 9, which sensitizes cancer XIAP, which binds peptides of Smac/DIABLO can be used as a target for the design of compounds that inhibit the activity of XIAP [28].

For compounds that inhibit XIAP are synthetic, small molecule inhibitors to sensitize cancer treatment. XIAP is an inhibitor of benzoquinone Embelin, a natural compound of plant origin. It was observed that this compound in pancreatic cancer cells induces apoptosis via the TRAIL pathway [29]. The expression level of XIAP may be subject to reduction under the influence of XIAP antisense oligonucleotides and with the participation of RNA interference (RNAi), the phenomenon of silencing on or off gene expression by RNA [22]. XIAP antisense oligonucleotides act on pancreatic cancer cells and cause the start of apoptosis with TRAIL pathway [30]. Acting on XIAP RNAi also can induce apoptosis after TRAIL treatment, anti CD95 or γ radiation [31], and also has the ability to suppress cell growth and proliferation of pancreatic cancer [22, 32]. Reduction of IAP protein expression in some pancreatic cancer cell lines was also observed after treatment with anticancer doxorubicin, cisplatin, gemcitabine [26, 33].

Mitochondrial pathway

Bcl-2 proteins are group of cellular oncogene products that regulate cell death process. The earliest discovered member of this group, from which the name is the anti-apoptotic protein Bcl-2. Proteins of the Bcl-2 family have in common at least one of the four regions of homology domains called Bcl-2 (*Bcl-2 homology domain* — BH): BH1, BH2, BH3 and BH4. These domains determine the structure and function of these proteins [34]. The family of Bcl-2 antiapoptotic proteins include Bcl-2, Bcl-XL (*B-cell lymphoma-extra large*), MCL-1 (*myeloid cell leukemia sequence 1*) and proapoptotic (Bax, Bak, Bad, Bid) [11, 17]. A common cause of lack of efficacy of anticancer therapy is the overexpression of proteins with antiapoptotic properties and reduced expression of proapoptotic proteins (Fig. 2) [17].

High expression of Bcl-2 occurs in many types of cancer, but it does not deal with pancreatic cancer, where expression is normal or even weakly expressed [35]. In pancreatic cancer cells overexpression of Bcl-XL was observed which is the cause of resistance to apoptosis dependent Fas and TRAIL [36]. It was shown also that Bcl-XL expression correlates with survival. Patients with weak expression of Bcl-XL lived significantly longer
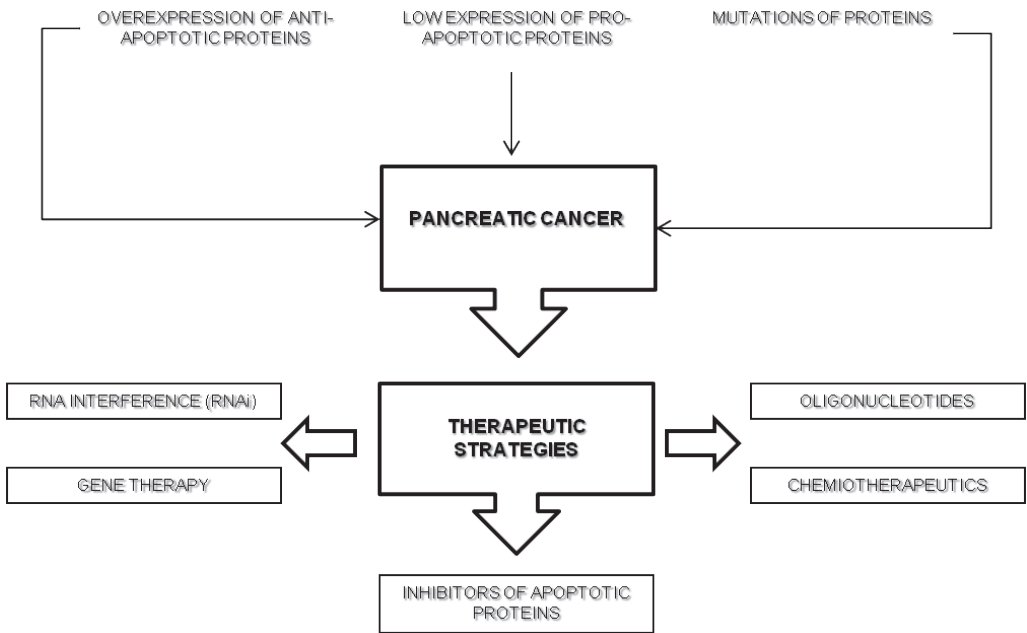
Fig. 2. Effects causing pancreatic cancer and possible treatment options (modified from Westphal and Kalthoff, 2003).

after tumor resection than those in whom there was even a moderate protein expression [37]. Therefore, Bcl-XL may be an ideal target in the treatment of pancreatic cancer [15].

There was a significant role of antisense oligonucleotides Bcl-XL, which inhibit the growth of pancreatic cancer cells, and cause the induction of apoptosis through decrease of Bcl-XL [15]. Bcl-XL antisense oligonucleotides also increase sensitivity to chemotherapeutic agents such as gemcitabine [38].

Proapoptotic proteins play important role in pancreatic cancer therapy. It was shown that pancreatic cancers that overexpress Bax have a greater sensitivity to gemcitabine [39]. Other studies have shown that increased levels of Bax and decrease Bcl-2 expression correlates with the exponentiation inhibitory effect of cisplatin in pancreatic cancer [40]. This means that the overexpression of Bax may be important in increasing the therapeutic efficacy of chemotherapy. It should be stressed that overexpression of Bax in pancreatic cancer had no effect on the rate of apoptosis and the expression of Bcl-2 and Bcl-XL [39, 40].

Another approach to targeting antiapoptotic Bcl-2 is the use of inhibitors. ABT-737 Bcl-2 inhibitor sensitizes pancreatic cancer cells to apoptosis by stimulating the external and internal apoptotic pathways [22, 41]. An inhibitor of Bcl-2 TW-37 inhibits pancreatic cancer cell growth and tumor invasion and induces apoptosis [42].

High affinity for Bcl-XL shows ABT-263 inhibitor recently introduced for clinical trials. Reported data on the efficacy of this measure are promising. It is believed that it will be very effective in cancers characterized by overexpression of Bcl-2 or Bcl-XL [22, 41].

Other factors and signaling pathways could be a target for pancreatic cancer therapy

Nuclear factor of κβ (NF-κβ)

The transcription factor NF-κβ is regarded to be a factor increasing cell survival by activating antiapoptotic proteins. Normally present as an inactive complex in the cytoplasm. The variety of stimuli such as cytokines, oncogenes and stress can lead to its degradation and thus contribute to the activation of antiapoptotic genes and inhibition of apoptosis [43, 15]. It was shown that NF-κβ factor contributes to the inhibition of apoptosis in various tumor types including in patients with pancreatic cancer [15]. Therefore represents an important target in anticancer therapy. It was observed that inhibition of NF-κβ by MG 132 or sulfasalazine sensitizes pancreatic cancer cells to apoptosis induced by etoposide (VP16) or doxorubicin [44]. In addition, inhibition of nuclear factor by derivatives of polyphenols such as quercetin reduces the growth of cancer cells and prevents metastasis [45].

PI3K/Akt pathway

In many tumor types including pancreatic cancers, occur proteins associated with the so-called antiapoptotic routes. Therefore, under the influence of apoptogenic signals responsible for cell survival signal is stronger than death. An example is the route related to the kinase Akt/PKB (PI3K *phosphoinositide 3-kinase/AKT — also known as "protein kinase B", PKB*). The effect of protein kinase Akt/PKB is an inhibition of apoptosis and increased proliferation, including through the induction of NF-κβ, which ultimately leads to overexpression of antiapoptotic genes [14]. It has been shown to inhibit the kinase act through protein phosphorylation of Bad and caspase 9 and outflow of cytochrome c in a manner independent of phosphorylation of Bad. Also acts as a negative regulator of transcription factors of the Forkhead family that can induce gene expression of proapoptotic proteins [46, 47]. Akt to its activation requires phosphorylation by PI3 kinase. The tumor cells present often increased activity of PI3K kinase or Akt kinase [14, 15]. Strategies are implemented, which aim to block the enzymatic activity of PI3K and Akt / PKB. Wortmannin was found to be a potent inhibitor of PI3K. Treatment of cells of this compound led to the inhibition of proliferation and increasing apoptosis. In addition, wortmannin in combination with gemcitabine enhances apoptosis in vitro and in vivo in human pancreatic cancer cells [48].

Mutations in the p53 gene

Important role in the uncontrolled proliferation and resistance to apoptosis in cancer cells, play p53 gene mutations [11]. Product of this gene acts as a regulator apoptotic program and coordinate this process at different levels. During apoptosis, the terms for the activation of p53, a transcription factor that is responsible for the expression of proapoptotic proteins by mitochondrial and by involving the death receptors [47].

Mutations in p53 are observed in 50% of various cancers, and in people healthy, they may serve as a marker of increased cancer risk. Pancreatic cancer cell lines showed mutations in the p53 gene with a frequency of 95%. This affects the development and progression of cancer metastases. Therefore developed a number of methods based on gene therapy to normalize the function of p53 and inhibit the process of carcinogenesis [15].

Conclusions

The mechanisms involved in programmed cell death are crucial in understanding of cancer development and enable discovering of anti-cancer therapy. It is believed that the effectiveness of anticancer therapy is inhibition of cell proliferation and induction of apoptosis. Therefore, a better understanding of molecular mechanisms and signaling pathways that affect apoptosis may lead to the creation of a rational anticancer strategy that will contribute to the effective treatment of cancer patients.

Conflict of interest

None declared.

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