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Pregledni članak
*Review article**

ROLE OF NITRIC OXIDE AND PEROXYNITRITE IN APOPTOSIS–RELATION TO ENDONUCLEASE ACTIVITY

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Summary: Apoptosis is a form of cell death utilized physiologically to maintain tissue homeostasis, as well as in response to various toxic and inflamatory stimuli or anticancer drugs. Since the process of apoptosis is followed by phagocytosis, the cleavage of DNA to low mollecular weight material may serve as a protective function limiting the probability of gene transfer to the nuclei of viable neighbour cells. Many different endonucleases have been proposed as candidates responsible for the internucleosomal cleavage of the genomic DNA observed during apoptosis. The main effect was attributed to the alkaline DNase I (Mg^{2+} and caspase-dependent) and acid-DNase II. It was also documented that both of them contain a potential protease (caspase) cleavage site, but they can be also activated upon the influence of other »fragmentation factors«, including nitric oxide (NO). The complexity of biological effects induced by NO may be the result of the cell redox state changes, due to its potential interaction with superoxide. The apoptotic effect of both, nitric oxide (NO) and peroxynitrite (ONOO) are dose-dependent and cell-specific may point out the existence of possible »inducible« form of endonuclease.

Key words: apoptosis, nitric oxide (NO), peroxynitrite (ONOO), endonucleases-DNases

Apoptosis – morphological events

The integrity of multicellular organisms depends on the body ability to produce new cells and to destroy old ones by controlled cell death. The growth of any tissue, whether normal or malignant, is also determined by the quantitative relationship between the rate of cell proliferation and the rate of cell death. Programmed cell death (PCD), universal in multicellular organisms, has been first established in single cell organisms (1). As a widespread phenomenon, PCD occurs normally at different stages of morphogenesis. Changes in cell survival contribute to the pathogenesis of various disorders such as cancer, viral infections, neuropathies and immunopathies (2, 3). The understanding of apoptosis forms the basis for development of new therapeutic strategies aimed to influence malignant proliferation.

In PCD, a cell undergoes an active, energy-dependent process of cellular death initiated by specific signals, genetically invoked. It may be prevented or detected before being irreversible. There are endogenous, tissue-specific agents (glucocorticoids, absence of a tissue-specific survival factor) and exogenous, cell-damaging treatments (chemicals, radiation and viruses) (4, 5). Cells that degenerate as a result of necrosis have a passive role in initiation of the process, occurring as a result of the loss of membrane integrity, cellular oedema, followed by osmotic lysis (6). Apoptosis was originally defined by Kerr et al. (7) as the specific sequence of structural changes resulting in the death of the cell, which could not be prevented. The usual sequences of events are characterized by chromatin aggregation, nuclear and cytoplasmic condensation, and fragmentation of the dying cell into a cluster of membrane-bound (apoptotic) bodies containing morphologically intact organelles. Plasma membrane blebbing and cellular fragmentation occur subsequently to the nuclear fragmentation. The cell rounds, condenses and fragments into large blebs, while the plasmalemma becomes osmotically impermeable. The cell shrinks or fragments, and relatively impermeable membranes surround cells and their organelles. Opposite to necrosis mitochondria do not

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swell and lose their function, since functionally active mitochondria are often contained in apoptotic bodies. The cytoplasm is, to the light microscopic eye, unremarkable. Lysosomes increase in number and redistribute in the cytoplasm with large autophagic vacuoles. The morphological changes may occur in about 20 minutes. Very late in the process, when most of the cytoplasm is filled with vacuoles or has been pinched off, the chromatin coalesces and finally a nucleosomal ladder may be detected along the nuclear membrane. When formed, apoptotic bodies are rapidly phagocytized by macrophages or by adjacent cells without causing inflammation (8, 9).

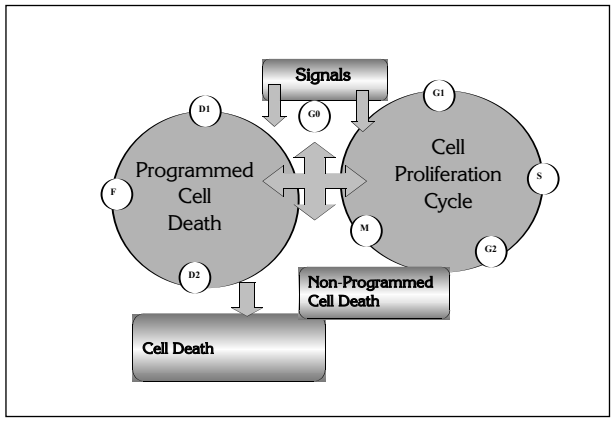

Figure 1. Relationship between cell proliferation and cell death cycle

Regulation of cell cycle and apoptosis

Cell death is a processes opposite to mitosis, but equal in complexity and regulation. The cell can be metabolically active but not undergoing either proliferation or death (G_0 cell); cell can go to proliferation (G_0 -> G_1 -> S -> G_2 -> mitosis), or to death by either the programmed pathway (D_1 -> F -> D_2 apoptotic cellular fragmentation) or the nonprogrammed (necrotic) pathway *(Figure 1)*.

During the D_1 phase of PCD, the cell undergoes epigenetic reprogramming in which certain genes that were previously expressed are now repressed, while previously repressed genes now become expressed (10). It finally results in the activation of double-stranded DNA fragmentation during the F phase. During this F phase, the nuclear morphology dramatically changes, while the plasma and lysosomal membranes are intact and the mitochondria are still functional. Proteases are activated during the D_2 phase, including the interleukin-1 beta-converting enzyme (ICE)-like proteases that hydrolyze poly(ADP-ribose) polymerase (PARP). The other ICE-like proteases degrade the lamins in the nuclear membrane, when the nucleus undergoes fragmentation. It was demonstrated that proliferating cells can be induced to undergo PCD at any stage of the proliferative cell cycle (G_0, G_1, S, G_2, M). The examples are immature thymocytes that can undergo PCD when they are proliferatively quiescent (G_0) or androgen-dependent prostatic glandular cells following androgen ablation (G_0) without entrance into the cell cycle (11,12). Based on the use of protein synthesis inhibitors, it is established that the enzymatic machinery responsible for PCD is already present within cells, but the signal transduction needed to activate this pre-existing death machinery sometimes requires new protein synthesis. The three types of gene products involved in the process of PCD were proposed. The first is involved in generation of the signal transduction for activation of the death process in healthy, undamaged cells (»physiological cell death«). They are highly linked to the particular cell type (during differentiation). Often the same gene product can have both the ability to stimulate cell proliferation and PCD, depending on the cell differentiation stage (transforming growth factor-beta 1 can stimulate proliferation of mesenchymal cells and the death of some types of epithelial cells) (13). Genetic changes followed by the loss of function of these type-I genes make the affected cells resistant to specific induction of physiological cell death (for example, glucocorticoids activate PCD in certain thymus cells, but the mutation in their glucocorticoid receptor makes them resistant to glucocorticoid-induced PCD). The second type of PCD genes encodes proteins responsible for the sensitivity to activation of PCD during pathological damage of the cell (radiation, viral infection, chemicals). This type includes p53 and *bcl*-2 genes. p53 gene is involved in cell cycle arrest during the G_1 to S phase transition, DNA repair, control of genome integrity and apoptosis (11, 14). The p53 protein is an antiproliferative transcription factor that enhances the rate of gene transcription important for p53-dependent functions. The mutation of p53 gene is an important factor for cancerogenesis in a variety of tissues, including epithelial and nervous tissue. DNA damage (induced by UV irradiation) and growth factor withdrawal lead to p53-dependent apoptosis. Genetic alteration leading to the loss of function of this type-II gene decreases the sensitivity of the affected cells to pathological (damage-induced) PCD (from radiation, chemotherapy, viral infection). In contrast, *bcl*-2 is a gene whose product

Table I Types of genes involved in programmed cell death

Type 1 (Signal transduction)	Type 2 (Sensitivity)	Type 3 (Machinery)
growth factors	p53	endonucleases
androgenes	*bcl*-2	proteases
estrogenes	*bcl*-x	ICE family
interleukins	*bax*	calpain
growth factor receptors		
c-*myc*		

decreases the sensitivity to the activation of PCD induced by the same types of damaging agents. In this way genetic alterations followed by the loss of function of this type of gene increases the sensitivity of the affected cells to pathological PCD. The third group of genes encode proteins that are of critical importance for the process of cell death itself, induced by all treatment, both physiological and pathological (*Table I*). Genetic alteration of third type of gene by the loss of function can prevent the cell's ability to undergo PCD (10, 14).

Biochemical events of apoptosis

Once initiated, PCD leads to a cascade of biochemical events that result in irreversible degradation and fragmentation of the genomic DNA. The central events, effectors and regulators are very similar among different cell types, conserved also over a very long period of evolution, transferred from bacteria to eucaryotes. A central role is played by proteases and nucleases that catalyze specific reactions critical for the entire process of cell death. The biochemical road to cell death involves two distinct routes the 'extrinsic' and 'intrinsic' pathways, which proceed through death receptors or through mitochondrial events. Apoptosis may be induced by lack of anti-apoptotic stimulation, leading to loss of normal mitochondrion structure and release of cytochrome c, or by ligands binding to death-receptors. These pathways finaly converge at the level of effector caspases. During the cell death overall protein synthesis collapses (15).

Analogous to growth regulation (by growth factors and receptors), a complementary process exists to regulate cell death, by death factors and death receptors. Current studies support the role of these death ligands and corresponding receptors not only in the regulation of physiological cell death, but also in many human disease states. Death receptors are members of the Fas and tumour necrosis factor receptors 1, 2 (TNF-R1 and TNF-R2), 3, as well as tumor-necrosis factor related apoptosis inducing ligand (TRIAL) receptors 1, 2, 3 and 4. They usually possess a 60 80 amino acid death domain in the cytoplasmic side of the receptor, reffered as death domain. It has a function to recruit adapter molecules important for death signal downstream initiation. Fas, (APO-1 or CD95), is a 45-kDa type I membrane integral glycoprotein and belongs to the TNF/nerve growth factor (NGF) receptor family. This family is composed of TNF receptors (type I, 55 kDa; type II, 75 kDa), low affinity NGF receptor, the B cell antigen CD40, as well as the T cell antigens CD27, CD30, OX40, and 4-1BB. Most ligands of the TNF/NGF receptor family belong to the TNF family, which includes TNF, lymphotoxin, Fas ligand (FasL) and CD40 ligand. As a cell surface protein Fas is expressed in the lymphocytes, thymus, liver, heart, lung, small intestine, kidney, testis and ovary. Fas ligand (FasL) is synthesized as a type II membrane protein with a molecular mass of 40 kDa, and under certain conditions, a trimeric soluble form of FasL is produced by proteolytic cleavage. The membrane-bound form of FasL, the soluble form of human FasL, or the agonistic antibodies against Fas induce apoptosis by binding to Fas in Fas-bearing cells (3, 16).

Regardless of the origin of the apoptotic stimulus, apoptosis occurs through the activation of caspases, a family of cysteine proteases present in normal cells as inactive precursors (17). At least 13 caspase family members have been identified in mammals, and these can be divided into three groups (18). Caspases in the first group are mainly involved in the production of cytokines, whereas caspases in the second and third group play a direct role in apoptosis. Among the caspases involved in apoptosis, caspases 8, 9, and 10 carry a long prodomain at the N terminus, and are activated by oligomerization at the plasma membrane or by apoptosomes at mitochondria, and thus function as initiator elements. Caspases 3, 6, and 7, carrying a short prodomain, are activated by the initiator caspases, and are responsible for cleaving at least 60 death-related substrates. Initiator caspases 8, 9 and 10 are capable of activating effector caspases 3,6 and 7. The initiator caspases are activated by forming heteromeric complexes with acceptor molecules. In this way, caspase 8 combines with Fas and FADD to form the DISC (death-inducing signaling complex), while caspase 9 can be complexed with cytochrome c and Apaf1 by an energy-dependent binding in a structure known as the apoptosome. The release of cytochrome c from the intermembrane space is induced by dysregulation of the mitochondrial potential and permeability transition. This is followed by the cleavage and subsequent activation of downstream caspases 3 and 6. Activated caspases, in turn, cleave multiple cytosolar and nuclear substrates, among them DNA-degrading enzyme(s). Cleavage of a selected group of substrates by caspases is responsible for the morphological and biochemical changes of apoptotic cell death: cell membrane disruption and blebbing, cytoskeletal rearrangement, nuclear condensation, and DNA fragmentation (19). Although it appears that caspases and caspase-activated nuclease play a key role in apoptosis, a number of other proteins, exerting similar function were isolated. Maybe, the most interesting and intriguing thing of apoptosis is the universal property of some proteins to exert dual function - to protect from proteolysis and maintain normal structure and function of normal cells or to contribute to protein or chromatin cleavage (being free from inhibitory complex or conformation) in apoptosis (9).

Endonuclease activity and apoptosis

The degradation of nuclear DNA into nucleosomal units is one of the best-characterized biochemical features of apoptotic cell death (20). Several molecules that might be responsible for this endonucleoly-

tic activity have been detected and characterized from various sources. The cleavage of DNA may serve as a protective function, limiting the probability of gene transfer in a potentially active site from dying cells to the nuclei of other, viable neighboring cells. The identification of endonuclease involved in genome degradation pathway may have a crucial importance in finding the possible mechanisms of PCD modulation, probably used as therapeutic targets against disease. It is possible that various endonucleases exert DNA degrading activity as well as that many proteins can receive DNA degrading properties upon changed pH conditions (acidification) through yet unrecognized pathway. The initial phase of DNA digestion is the formation of 300 500 kb fragments, followed by a subsequent degradation to 180 kb fragments. The enzymes responsible for chromatin degradation have been differentiated based on their ionic sensitivity. Up to now, three different endonucleases have been involved in DNA fragmentation leading to nucleosomal appearance. Some authors postulated that the well characterized DNase, I 30 kDa nuclease, was constitutively expressed in cells of tissues potentially primed for apoptosis Another endonuclease, cation-independent, with optimal activity at pH 5 was also identified, proposed to be DNase II. The enzymes Ca^{2+}/Mg^{2+} dependent DNase and acidic DNase are Zn-ion sensitive, because the enhancement of the activity was achieved by using Zn chelators. This finding supports the role of zinc in the modulation of PCD, offering some protective effects. Finally, a lower molecular weight nuclease, related to cyclophilin as well as Mg^{2+}-dependent caspase-activated endonuclease CAD were well documented (21). The relationship between caspase activation and nuclear DNA fragmentation was elucidated by the purification and cloning of a caspase-activated DNase (CAD), and its inhibitor (ICAD). According to the recent data, CAD is the major DNase responsible for the cell-autonomous nuclear DNA fragmentation that occurs in apoptotic cells. It cleaves DNA leaving 5'-phosphate-3'-hydroxyl ends. The DNase inhibitor (ICAD-L), known as a DNA fragmentation factor (DFF-45), is a 45kD protein composed of 331 amino acids. CAD has an intrinsic DNase activity which can be only expressed after caspase 3-mediated cleavage of ICAD at amino acids Asp-117 and Asp-224 (22, 23).

The nuclease activity in apoptosis is documented to be inherent also to cyclophilins (A, B and C), independent on their peptidylprolyl cis-trans isomerase activity, which is involved in protein folding. The active protein arises as a result of proteolytic activation of 34 kDa and/or 74 kDa precursor via serine protease. Cylophilin C is active in the presence of only Mg^{2+} ions. Their optimal pH is very close to the DNase I optimal pH, in the range of 7.5 9.5. As the enzymes with endonucleolytic activity they have ability to degrade supercoiled DNA, single stranded and double stranded DNA. Recombinant cyclophilin B induced DNA degradation of naked nuclei, but did not induce internucleosomal DNA degradation. The products formed (50kB pairs) are almost identical to those identified during apoptosis (24, 25).

DNase II is known to be present in various mammalian tissues, isolated directly from lysosomes of the spleen, rat and monkey liver, Chinese hamster ovary cells, human gastric mucosa, cervix, and urine as well as during lens fiber differentiation. DNA fragmentation in apoptotic thymocytes caused by macrophage coculture was inhibited by agents that block the acidification of lysosomes, suggesting that acid DNase in lysosomes of phagocytic cells can also be responsible for DNA fragmentation. The cleavage of DNA by DNase II generates 5'-hydroxyl and 3'-phosphate ends. Lysosomes are rich in acid phosphatases, which can remove the phosphate group from the 3' end of DNA fragments. It seems that DNase II, in combination with acid phosphatase in lysosomes can also degarde DNA. Since nuclear extracts contain a cation-independent endonuclease with identical pH-dependent activity, the specific involvement of DNase II in physiological nuclear degradation was also demonstrated, where nuclear DNase II can mediate internucleosomal DNA digestion during apoptosis. Leucocyte DNase II is a 35 kDa protein. It was suggested that the hog splenic DNase II may have a dimeric structure composed of two similarly sized subunits, while the bovine hepatic enzyme appeared to consist of two non-identical subunits, one of them is a 27 kDa polypeptide. DNase II is synthesized as a proenzyme, but following the release of a signal peptide, proteolytic processing transforms the single chain into the two-chain enzyme. This acidic endonuclease is activated in intact cells by reducing intracellular pH values below 7 with a proton ionophore. A His residue at the active site was reported to be involved in its catalytic function. Later, the amino-acid sequence surrounding this His was determined: Ser-Thr-Glu-Asp-His-Ser-Lys-Trp. Very important sites of its regulation are two potential protein kinase C phosphorylation sites, found at positions 109 and 301, one potential tyrosine kinase phosphorylation site at position 233, and three potential N-nitrosylation sites at positions 40, 261, and 323. This structure is very similar to those of the cathepsins B and D. There is no significant sequence homology between DNase I and II (26, 27).

It was indicated that leucocyte-DNase II is derived from leucocyte-elastase inhibitor (LEI) by a posttranslational modification. The soluble factors present in spleen extract facilitate this transition at pH 6, suggesting that this process likely occurs in apoptotic cells after decrease in intracellular pH. Three hypotheses were proposed to explain the nature of posttranslational modification leading to production of L-DNase II from LEI, such as »relaxed« and »stressed« states. The transition from LEI to L-DNase II may be the result of a proteolytic cleavage of N- or C-terminal regions, because the elastase releases a peptide at the N-terminal end of the protein similar to that seen after the exposure to acidic pH. The transition from p42 to p27 takes place the most probably in two steps. First of them is induced by acidification followed by the formation of an inter-

mediate form, p35. The second step, induced by DNA, gives final modification to p27 L-DNase II, making it an element of proteolytic cascade activated in apoptosis. It seems that LEI exerts a double function: it prevents the proteolytic cascade of apoptosis in living cells and via the transition to DNase induces nuclear degradation in apoptotic cells. It means that L-DNase II activity is not present in the normal cell when protease activities are inhibited by the LEI anti-protease action (28).

The cascade involvement of endonucleases was well explained during lymphocyte clonal maturation and deletion. During apoptosis in thymocytes, cleavage of DNA occurs, most probably, at two main steps: the first, Mg^{2+}-dependent process, involves the formation of large fragments (about 30 kilobase pairs); the second involves the internucleosomal cleavage of DNA, depending on the presence of both Ca^{2+} and Mg^{2+}. The identified nucleases exert different molecular weight, 18 kDa (NUC-18) and 30 40 kDa, released from cytosol/nuclear fraction (25). Active form of caspase-dependent DNase is released from its inhibitor (DFF40/CAD) upon proteolytic cleavage by caspase 3 and induces nuclear condensation and further DNA fragmentation. Simultaneously, cyclophilin B localised in endoplasmic reticulum is released into cytosol, translocated into nuclei, which finally degrades CAD-fragmented chromosomal DNA into small pieces. Wu et al. (29) reported that the apoptotic DNA degradation in *C. elegans* proceeds in three steps: first of them is yet unidentified CAD-like DNase; followed by DNase II. Finally, DNA is degraded completely by the enzyme(s) provided by surrounding cells. The DNase II can also be provided from surrounding cells.

Nitric oxide and apoptosis

In 1980, the physiological importance of the endothelium ability to release a diffusible vasodilator, later identified as nitric oxide was reported, whereas the 1998 Nobel Prize was awarded for nitric oxide (NO) research (30). NO is a key transducer of the vasodilator message from the endothelium to vascular cells, called »endothelium-derived relaxing factor« (EDRF) of blood vessels (31). It is a constituent in central and peripheral neuronal transmission. It also participates in the nonspecific immune defense as the main cytostatic and cytotoxic effector of the cellular immunity system. NO exerts both autocrine and paracrine actions, having an influence on metabolic processes in cells where it is being synthesized by adjacent cells. Physiological and pathophysiological activity of NO are classified by cGMP-dependent and cGMP-independent transducing pathways. NO is enzymatically synthesized from L-arginine through the five-electron oxidation of L-arginine to citrulline and biologically active NO (32, 33). Nitric oxide synthases are a family or a group of enzymes which are able to form NO from L-arginine. Three types or three isoenzymes coded by different genes are found. Type I NO-synthase (nNOS, NOS I) is present in neurons in the brain and is often called neuronal or constitutive with the highest activity in the neurons of cerebellum and in astroglia. Type II NO-synthase (iNOS, NOS II) was first isolated from macrophages. This enzyme is mainly in the soluble form and known as an inducible isoform of NO-synthase (iNOS). Recently iNOS has been purified from interferon-g and lipopolysaccharide-activated mouse macrophages. Type III NO-synthase (eNOS, NOS III) is typical for endothelial cells. This enzyme is characterized by reversible binding with calmodulin and its activity depends on the intracellular concentration of Ca^{2+}. The 57% of the amino acid sequence of type III NO-synthase is identical to type I NO-synthase. It exists in both soluble and membrane-bound forms. Localization of this enzyme in the plasmatic membrane appears to be of substantial significance for the signal transfer mechanisms with participation of NO (34).

A significant part of biogenic NO is oxidized in hydrophobic phases and oxidation products are similar to thionitrite formation. Inspite of high chemical reactivity, NO molecules can be transported to a distance several-fold exceeding cell sizes. S-nitroso-cysteine (cys-NO) protects NO against oxidation. NO is included in formation of dinitrosyl iron complexes (DNICs), and the system including NO, thiols, and free iron provides interconversion of DNIC and RS-NO. Iron plays an important role in this system by acting as a catalyst which promotes both the conversion of NO into NO^+ important for thiol S-nitrosylation and decomposition of RS-NO. It is beleived that these complexes can have destroying effects on active sites of iron-sulfur proteins, showing in this way the cytotoxic effects of NO (35).

Beside a wide range of regulatory properties, NO may be involved in the regulation of apoptosis. The effects of apoptosis vary depending upon the dose of NO and the type of cell used, because it has been shown that NO is able to induce apoptosis or to protect from apoptosis in different cell types. The other factor can be capability of expressing iNOS, which has the capacity to synthesize large amounts of NO for prolonged period of time. The effects of NO on apoptosis are generally classified as cGMP dependent or independent (36).

Exposure of neuronal cells to NO can lead to direct induction of programmed cell death, capable of abolishing approximatelly 70% of viable cells. Furthermore, the death of heart muscle cells could be blocked by inhibitors of NOS or by the administration of transforming growth factor (TGF)-β, which potently suppressed iNOS expression. NO, produced by iNOS in cytokine-treated macrophages, was capable of triggering apoptosis of both macrophages and certain tumour cells cocultured with the macrophages. Subsequently, it has been documented to promote apoptosis in chrondrocytes, pancreatic islet cells, macrophage-like cells, certain tumours and vascular smooth muscle cells. The fact that NO is capable of triggering apoptosis is consistent with the effect of NO to induce DNA damage (37 39)). NO has been documented to

induce DNA scission by processes involving deamination of nucleotides, with cleavage occurring especially at cytosine-rich regions. Beside this, NO can induce a wide range of damage to cells, including inhibition of DNA synthesis and production of DNA strand breaks, and cell cycle arrest (40).

This proapoptotic effect of iNOS and NO has been documented to be associated with p53 accumulation. The tumor suppressor gene p53 is a transcription factor that can effect cycle arrest or apoptosis, maintaining genomic integrity, acting as a »guardian of the genome«. Normally, the half-life of p53 is short, resulting in undetectable or low level of p53 protein in normal tissue. In response to DNA damage, the protein accumulates, causing a G1 cell cycle arrest. When severe DNA damage occurs, p53 initiates apoptosis (41). The accumulation of p53 during NO-mediated cell death was established for macrophages and insulinoma cells (40). Different cytokine and LPS- induced p53 accumulation was suppressed by NO synthase inhibitors, confirming the functional role of NO in p53 pathway. Although expression of p53 may be a component of the apoptotic gene programme triggered by NO, in some cells the transcription of proapoptotic gene Bax and suppression of antiapoptotic gene Bcl-2, is also documented. Beside this, PARP cleavage was established in response to endogenously generated or exogenously supplied NO in parallel to DNA fragmentation. The enzyme poly (ADP-ribose) polymerase (PARP) was the first protein identified as a substrate for caspases. PARP is involved in repair of damaged DNA by catalyzing the synthesis of poly (ADP-ribose) and by binding to DNA strand breaks, in this way modifying nuclear proteins. The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3 (42). Possible factors implicating NO-induced apoptosis can be protein kinase C and NF-kB. NF-kB plays a role in regulating the host inflammatory and immune response and in preventing cellular apoptosis in response to cellular stress, through the activation of cellular inhibitors of apoptosis (clAP1, clAP2 IXAP, TRAF1, TRAF2 and bcl-2). NO donors have been shown to inhibit NF-kB binding activity of vascular smooth muscle cells. The physiological action of NO is also mediated through inhibition (S-nitrozylation) of protein kinase C, which, in turn, is responsible for the phosphorylation of lkBa (43). Beside this, peroxynitrite can acts as asignaling molecule in the activation of c-Jun NH2-terminal kinase (JNK) that determines cell survival in response to enviromental stress. It seems that different apoptotic signaling pathways are integrated at a point resulting in proteolytic events and caspase activation during NO exposure (43). In contrast, NO has also been reported to inhibit apoptosis in other cells including leukocytes (eosinophils), hepatocytes, trophoblasts, endothelial cells, murine and human lymphocytes, certain tumor cell lines and rat cardiac muscle cells subjected to mechanical stress (44). The antiapoptotic effects of NO can be mediated through a number of mechanisms such as nitrosylation and inactivation of many of caspases including caspase 3, caspase 1 and caspase 8. Other mechanisms include blockade of the recruitment of pro-caspase 9 to Apaf-1 apoptosome. Nitric oxide is able to activate cGMP signaling through the interaction of NO with the haem group of guanylate cyclase. The production of cGMP leads to the activation of cGMP-dependent protein kinases and probably increased expression of antiapoptotic proteins Bcl-2 and Bcl-XL (followed by inhibition of cytochrome c release from the mitochondria). The cGMP signaling leads also to activation of cGMP-dependent protein kinases as well as suppression of caspase activity (45).

Endonucleases – downstream mediators of nitric oxide and peroxynitrite effects

It is evident that overproduction of NO in cells induces their death by apoptosis, followed by the active enzyme-catalyzed DNA degradation (46). Beside the presence of an constitutive Ca^{2+}/Mg^{2+} dependend endonuclease, the endonuclease active in the pH range of 7.2 8 is probably NO-inducible form of DNase. This endonuclease exhibited the greatest activity 5 hours following the exposure to NO, dependent on the presence of Mg^{2+} ions. The biochemical properties of this enzyme are consistent with the recent observations about the mollecular weight of 95 108 kDa. The mollecular weight of constitutive (NO-independent) and inducible (NO-dependent) endonuclease are similar, as well as their optimal pH range (7.5 8). The conclusion seems to be that Mg^{2+}-dependent endonuclease is a result of proteolytic cleavage of primary, Ca^{2+}/Mg^{2+}-dependent endonuclease, following NO exposure. Some investigators documented sequential DNA cleavage by Ca^{2+}/Mg^{2+} dependent endonuclease that could yield Mg^{2+}-dependent endonuclease by proteolytic cleavage. In this way, the active form of endonuclease is a prerequisite, downstream mediator of NO-induced PCD. Another form of endonuclease that can be activated early following NO exposure is the most probably acidic (DNase II), since the exposure of cells to NO donors leads to significant and rapid change of neutral pH environment toward acidic (47). Thus, DNA degradation could begin as early as 30 min following NO exposure via the activation of acidic DNase (48). But the biphasic change of pH occurs over the next hour, maintained over a 5h period. The activity of alkaline endunuclease(s) could be responsible for the next step of DNA degradation. But under some conditions the formation of peroxynitrite could be sufficient to degrade and destruct DNA directly (48, 49).

In conclusion, the modulation of cell signalling by NO and peroxynitrite is an emerging area of research which is providing insight into the orchestration of cell function during many disease states, such as inflammation, ischemia/reperfusion and xenobiotic metabolism. They contribute to multistage process of tissue and DNA damage and progarmmed cell death.

ULOGA AZOT MONOKSIDA I PEROKSINITRITA U APOPTOZI INTERAKCIJA SA ENDONUKLAZNOM AKTIVNOŠĆU

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Kratak sadržaj: Apoptoza predstavlja formu ćelijske smrti, čiji je smisao održanje homeostaze tkiva u flzioloških uslovima, takođe i kao odgovor na delovanje različitih toksičnih i zapaljenjskih agenasa ili tokom terapije kancera. Budući da je proces apoptoze praćen fagocitozom, razgradnja DNK do niskomolekularnih fragmenata može imati protektivnu ulogu kako bi se smanjila verovatnoća prenosa gena u susedne zdrave ćelije. Različite endonukleaze su opisane kao odgovorne za internukleozomalnu razgradnju DNK u apoptozi. Najveći značaj se pridaje alkalnoj DNazi I (Mg^{2+} i kaspaza zavisnoj) i kiseloj, DNazi II. Pokazano je da i jedan i drugi enzim sadrže potencijalno mesto podložno delovanju proteaza (kaspaza), ali se mogu aktivirati i pod uticajem drugih »fragmentacionih faktora«, kao što je azot monoksid (NO). Kompleksnost bioloških efekata indukovanih delovanjem NO mogu biti posledica i promene redoks statusa ćelije, zbog potencijalne interakcije sa superoksidnim anjonom. Apoptotični efekti i azot monoksida (NO) i peroksinitrita (ONOO) su dozno-zavisni i specifični za ćelijske tipove i mogu ukazati na postojanje specifične »inducibilne« forme DNaze.

Ključne reči: apoptoza, azot monoksid (NO), peroksinitrit (ONOO), endonukleaze-DNaze

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