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Oxidative stress and apoptosis

The intrinsic balance between life and death can be influenced by several factors. Intracellular accumulation of reactive oxygen species can arise from exogenous stresses or endogenous metabolic processes. These compounds can damage antioxidant defense system and consequently various biological macromolecules, including nucleic acids, proteins, carbohydrates and lipids. Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (1, 2). It is connected with several biological and pathological processes like aging, inflammation, carcinogenesis, ischemia-reperfusion, neurodegenerative diseases, AIDS. Apoptosis has also been linked to these diseases, suggesting that both processes may be involved in these pathologies.

Cell death can go two different pathways: apoptosis or necrosis. Necrosis is rather passive process, which is an answer to acute cellular dysfunction, stress conditions or reaction to toxic compounds. It is definitely connected with depletion of ATP in the cell. Morphologically, necrosis is characterized by a significant increase in cell volume, disruption of plasma membrane resulting in penetration of cell content to extracellular environment (1). This can damage neighbouring cells and further tissues. Apoptosis occurs during several pathological situations and constitutes a common mechanism of cell replacement, tissue remodelling and removal of damaged cells. Cell shrinkage, chromatin condensation, DNA fragmentation and formation of apoptotic bodies is typical of apoptosis (3). Several proteases can be related to apoptosis, the most principal are caspases (4, 5) but also granzymes, calpains and cathepsins (5, 6, 7). Caspases are members of cysteine-containing, aspartic acid-specific proteases which form zymogens in the cytoplasm, mitochondrial intermembrane space and nucleic matrix in almost all cells. We can distinguish at least three models for caspase activation. One of the pathways which caspases regulate almost excusively is the model where apoptosis is induced by ligation of cell surface receptors like the Fas or tumor necrosis factor (TNF) called death receptors. Ligand that binds to the receptor causes the assembly of a series proteins known as DISC (death-inducing signalling complex), which then activates pro-caspase-8. Caspases are activated by cascade of the reactions, with caspase-8 causing activation of capsase-3, which can activate other caspases and ultimately cleaves various substrates. One of these substrates is endonuclease which after this acting enters the nucleus, where it cuts DNA into oligonucleosomal fragments (5, 8). The next model is involved in mitochondria and mitochondrial dysfunction during apoptosis causing cytochrome c to release into cytosol, where it binds to Apaf-1 (apoptotic protease activating factor 1). Apaf-1 contains binding sites for cytochrome c and ATP and oligomerizes with other Apaf molecules forms apoptosome. This complex binds pro-caspase-9 by using CARD (caspase recruitment domain) of Apaf-1. Caspase-9 activates the next caspases -3 and -7 (1, 9). The third pathway is initiated by cytotoxic cells (1, 5). Perforin and granzyme B collaborate to induce apoptosis in tumor cells or

those infected by pathogens. Perforin let the granzyme B come into the cytosol, where it activates caspase-3. Regardless of the pathway, caspases cleave numerous cellular proteins, e.g. poly (ADP-ribose) polymerase (PARP) and fodrin (1).

Most of this work focused on the role of caspases, which certainly play a very central role in apoptosis. However, during recent years there has been growing evidence to suggest that other enzymes, such as cathepsins, calpains and granzymes, contribute to apoptosis. In response to certain signals they are released from the lysosomes into the cytoplasm where they trigger apoptotic cell death via various pathways, including the activation of caspases or the release of proapoptotic factors from the mitochondria. Cathepsins are synthesized as inactive preproenzymes and are glycosylated post-translationally. The cathepsins B, L and D have been found to play an important role in the regulation of apoptosis. Different mechanisms have been described which can contribute, probably in a stimulus- and cell-type-dependent fashion, to lysosomal permeabilization and release the cathepsins: sphingosine accumulates within the lysosomes where it can permeabilize the membrane via a detergent-like mechanism, the endolysosomal acid sphingomyelinase can bind to cathepsin D, which in turn is responsible for the proteolytic activation of other lysosomal proteins. Another possible mechanism of lysosomal permeabilization involves the generation of reactive oxygen species, (ROS). Lysosomal destabilization has been recognized as a feature of oxidative-stress induced cell damage (6).

Granzyme B might kill a cell in diverse ways, such as by activating caspases directly, inducing DNA fragmentation through derepressing CAD (caspase-activated deoxyribonuclease), or cleaving key structural proteins in the nuclear membrane or cytoskeleton. However, an unexpected finding has been reported and recently confirmed that granzyme B preferentially mediates apoptosis by activating the mitochondrial pathway rather than by direct caspase cleavage and activation. It now seems clear that, although purified granzyme B is capable of directly activating several procaspases *in vitro*, its actions may be more constrained when considering the death of many intact cells. Caspase inhibition did not prevent the loss of mitochondrial depolarisation. This placed mitochondrial disruption upstream of caspase activation in response to granzyme B, and suggested that the mitochondrial pathway can frequently be dominant over direct caspase activation (7).

Calpains are a group of cytoplasmic neutral cysteine proteases expressed in either a ubiquitous or a tissue-specific manner. They require Ca^{2+} in their activation and optimal activity. Their activity is also regulated by a highly specific endogenous inhibitor called calpastatin. Calpains are activated during apoptosis and can be abolished by various calpain inhibitors. They cleave numerous proteins like fodrin, gelsolin, p53 and Bax (5).

Wide research relating to the role of oxidative stress in apoptosis has shown that lower doses of oxidants like hydrogen peroxide can trigger apoptosis (1). Other examinations bring up that intracellular ROS (reactive oxygen species) generation may constitute a protective apoptotic activity (1). Sometimes depletion of glutathione (GSH) pool is involved in ROS production (10).

Generation of ROS leads to the activation of protein tyrosine kinases (PTK) followed by the stimulation of downstream signalling systems including MAP kinase and phospholipase C (PLC). The activation of PLC elevates the cellular Ca^{2+} levels. Such reactions in the upstream signalling cascade activate the transcription factors (4, 11). Oxidants activate PTKs the same way as growth factors. They bind like an insulin to receptor tyrosine kinase (RTK), enhance the enzymatic activity and this results in the phosphorylation of both the receptor by itself and insulin receptor substrate -1 (IRS-1). They also activate other PTKs, e.g. c-Src, Lck, Fyn and other. Activated RTKs stimulate the MAP kinase cascade through the activation of Ras. Ras as a small G protein transduces signal from certain RTKs to the MAP kinase cascade. Hydrogen peroxide stimulates other kinases of the MAP

kinase family such as c-Jun N-terminal kinase (JNK), p38 MAP kinase (p38) and Big MAP kinase 1 (BMK1). Activated RTKs also stimulate PI 3-kinase, which in turn activates several protein kinases such as Akt, PKC. Oxidants also activate phospholipase A_2 and D (8). Oxidative radical stress induces the expression of many genes such as c-fos, c-jun, c-myc and gene for haeme oxygenase (1). Such expression may be modified by transcription factors in response to the activation of upstream cellular signalling pathways. Treatment of cells by hydrogen peroxide induce the activation of the activator protein -1 (AP-1) and nuclear factor κB (NF κB) in some types of cells. The oncogene products Jun and Fos form AP-1 by heterodimerisation (Jun/Fos) or homodimerisation (Jun/Jun) through their leucine-zipper structure located at the C-terminal region. The activity of AP-1 is regulated by redox through the conserved cysteine residues that are located in the DNA-binding domain of each protein. NF κB induces the gene expression that mediates the immune response, stress response, cell growth and cell survival. It is composed of two subunits p50 and p65, which have a significant homology to the protooncogene Rel in a region called Rel-homology domain (RHD). Myb is a protooncogene product that activates the transcription of several genes involved in cell cycle progression. Myb posseses a conserved cysteine residue of which the reduced state is essential for its DNA binding and transformation activity (4). However, many transcription factors are directly regulated by redox in the opposite manner. It is connected with structure of active groups in proteins and redox activities of oxidants (1,11).

Furthermore, cellular signalling pathways are subjected to dual redox regulation in which redox has opposite effects on upstream signalling systems and downstream transcription factors. Signalling systems regulate the cellular redox state. There is a cross talk between the cellular signalling system and the cellular redox state because ROS activating by extracellular signals stimulate other cellular signalling pathways as second messengers (4–6). Oxidative radical stress also induces the activation of caspases, whereas the oxidation of them inactivates these enzymes and switches the mode of cell death to necrosis. It is done through oxidation of their active site thiol group or by S-nitrosylation (1, 3).

Reactive oxygen species can be eliminated by a number of enzymatic and nonenzymatic antioxidant mechanisms. Superoxide dysmutase (SOD) immediately converts peroxide to hydrogen peroxide (H_2O_2), which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondria (12). Another enzyme that is important is glutathione reductase, which regenerates glutathione that is used as a hydrogen donor by glutathione peroxidase during the elimination of H_2O_2 (12, 13).

While ROS are generated under physiological conditions and are involved to some extent as signalling molecules and defense mechanisms, excess generation in oxidative stress has pathological consequences including damage of proteins, lipids and DNA. There are various systems of antioxidants in the cell which give evidence about the central role of oxidative stress in apoptosis. Nonenzymatic antioxidants include vitamins A, C and E; glutathione; α-lipoic acid; carotenoids; trace elements like copper, zinc and selenium; coenzyme Q_{10} (CoQ_{10}); and cofactors like folic acid, uric acid, albumin, and vitamins B_1, B_2, B_6 and B_{12}. Glutathione (GSH) acts as a direct scavenger as well as a co-substrate for GSH peroxidase. Vitamin E is a fat-soluble vitamin that prevents lipid peroxidation. Hydroxyl radical reacts with tocopherol forming a stabilized phenolic radical which is reduced back to the phenol by ascorbate and NAD(P)H dependent reductase enzymes. Glutathione plays an important role in metabolism, transport, redox signalling, and cellular protection. Reduced GSH is the major non-protein thiol present in virtually all cells. The glutathione (GSH) redox system is important for reducing oxidative stress. GSH, a radical scavenger, is converted to oxidized glutathione (GSSG) through glutathione peroxidase (GPx), and converted back to GSH by glutathione reductase (GR) (2,

10, 13). Another important role for glutathione relates to its impact over signal transduction of gene expression inside cells. Briefly, glutathione status has been favourably linked to two well-established redox sensitive transcription factors, nuclear factor κB (NF-κB) and AP-1(4, 10). Activation of NF-κB appears from these studies to be critically regulated by intracellular thiol redox status. Extremely high or extremely low levels of oxidized glutathione results in less than optimal activation of these transcription factors making it important for optimal levels of intracellular oxidized glutathione to be maintained throughout the cell. Glutathione status has been investigated for its role in tumor necrosis factor-α induced activation of NF-κB, which demonstrated that NF-κB activation is related to cellular glutathione levels. Consequently, these studies provide continued support that maintaining optimal cellular levels of glutathione is important for effective cellular function (8).

It is also worth paying attention to several groups of antiapoptotic proteins like Bcl-2 and p53 having antioxidant functions (5, 14, 15). Another group of protective proteins are heat shock proteins (HSPs). When cells are exposed to stress the cells induce a state of acquired thermotolerance connected with accumulation of HSPs. The major HSPs include proteins of 110, 90, 70, 60, 40, 32 and 27 kDa. The Bcl-2 family includes both pro-as well as anti-apoptotic molecules. Their members include Bcl-2, Bcl-x_L and Bax subfamily with Bax, Bak, Bik, Bad and others. The ratio between these two subsets helps determine the susceptibility of cells to death signals. This family is built of four conserved domains BH (BH1, 2 ,3 and 4). Many of antiapoptotic members display sequence conservation in all four domains. Proapoptotic proteins display sequence homology only within the BH3 domain. Bcl-2 overexpressing cells characterize higher levels of total glutathione. These findings are basis of further examinations into the regulation of GSH during apoptosis (14, 15). Another antiapoptotic protein being a broad-spectrum caspase inhibitor is p53. p53 also attenuates the generation of hydroxyl radicals acting as a sink for free radicals (4, 9).

Paradigms for oxidative stress and apoptosis are constantly being redefined. The newest research in the field of oxidative stress indicates that oxidants may act as triggers of cell death but also have much broader effects. The role of ROS as second messengers is now established from various lines of evidence (4, 5). The redox regulation of cellular signalling has multiple functions in cell physiology. Cell protect themselves from oxidative radical damage through the oxidant-induced activation of cellular signalling pathways. Moreover, cells determine their fate, such as cell proliferation, by the cross talk between the cellular signalling systems and the cellular redox state. It is obviously necessary to study further to reveal the physiological role of redox regulation and its molecular mechanism in cells.

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SUMMARY

Cell survival requires various factors, including appropriate proportions of molecular oxygen and antioxidants. Although most oxidative insults can be overcome by natural cell defense, prolonged stress may disturb the intrinsic equilibrium and cause apoptotic or necrotic cell death. The mode of the cell death depends on multiple agents. Oxidants and antioxidants determine cell fate but also can modulate it. This review shows the basic pathway of apoptosis and its connection to oxidative stress as activating and modulating factor. It brings up the problem concerning redox regulation of cellular signalling because there is evidence that the cell's fate is determined by the cross talk between the cellular signalling pathways and the cellular redox state through a complicated regulation mechanism.

Stres oksydacyjny i apoptoza

Przeżycie komórki wymaga różnorodnych czynników, włączając właściwe proporcje tlenu i antyoksydantów. Chociaż większość oksydacyjnych ataków jest przezwyciężana przez naturalne czynniki obronne komórki, przedłużający się stres może zakłócić wewnętrzną równowagę i spowodować śmierć komórki poprzez apoptozę lub nekrozę. Rodzaj śmierci komórki zależy od wielu czynników. Poziom oksydantów i antyoksydantów określa los komórki, ale także modyfikuje go. Praca przedstawia podstawowe szlaki apoptotyczne i ich połączenie z zagadnieniem stresu oksydacyjnego jako czynnika aktywującego i modulującego. Porusza także problem dotyczący regulacji redox w komórkowym przekazywaniu sygnału, ponieważ istnieją dowody, że los komórki jest określany przez związek miedzy szlakiem przekazywania sygnału a stanem redox w komórce.