Reactive oxygen species, apoptosis and cancer

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Introduction

Reactive oxygen species (ROS) comprise oxygen free radicals, including superoxide anion (O$_2^-$), hydroxyl (HO•), peroxy (RO$_2^-$) and alkoxy (RO•) radicals, and oxygen-derived non-radical species like hydrogen peroxide (H$_2$O$_2$) and singlet oxygen (’O$_2$)\(^1,2\). As a consequence of aerobic metabolism, ROS are continuously generated in biological systems and simultaneously detoxified by complex antioxidative mechanisms\(^3\). Oxidative stress develops due to imbalance between the systems that generate and scavenge free radicals\(^4\). Persisting oxidative stress leads to the accumulation of oxidative damage of the crucial biomolecules: genomic DNA, lipids and proteins.

Reactive oxygen species in carcinogenesis

Aside from DNA-damaging function, ROS can act as second messengers and control various signaling cascades which induce and maintain the oncogenic phenotype of cancer cells\(^5\). The effects of ROS include anticancer activities (cell cycle arrest, senescence, apoptosis or necrosis, inhibition of angiogenesis) and pro-cancer activities (promotion of cell proliferation, invasiveness, angiogenesis, metastases and apoptosis suppression)\(^6\). The incidence of malignant neoplasms increases with age. Lifelong constant attacks of free radicals are considered one of the main culprits for this. The average rate of DNA oxidative products generation is about 1 in 10^5 DNA bases, but is this enough to cause the spontaneous malignancy related to age? It was found that free radicals primarily damage specific genomic sequences that are important for carcinogenesis\(^6,7\). For example, oxidative DNA damage in gastric mucosa in patients with Helicobacter pylori infection is unevenly distributed among the genes, with a tropism for TP53 gene\(^5\).

Significant number of enzymatic and non-enzymatic systems protects the cell from ROS toxicity. Their impairment could also lead to deteriorating effects of ROS. Glutathione (GSH), an important guardian against the oxidative damage, accumulates abundantly within the mitochondria of cancer cells\(^8\). In vivo studies demonstrated that GSH depletion sensitizes tumor cells to oxidative cytolysis\(^3,9\). The study on knockout mice with the deficiency of copper-zinc superoxide dismutase (SOD), the enzyme which is main intracellular scavenger of superoxide radicals, revealed the increased liver cancer incidence in such animals\(^10\). Deficiency of manganese SOD, which is main O$_2^-$ cleaner in mitochondrial matrix, leads to mice mortality quickly after birth\(^11\). The animals that are heterozygotes for manganese SOD survive, but carry increased risk to develop lymphoma, adenocarcinoma and pituitary adenoma\(^12\).

Radiation induced carcinogenesis is largely based on generation of very reactive HO• radical, which interacts with DNA causing the formation of mutagenic purines, pyrimidines and oxidative deoxyribose products, such as 8-hydroxy-2-deoxyguanosine (8OHdG)\(^6,13\). The defects in enzymes responsible for reparation of oxidative DNA damage increase the level of 8OHdG and other mutagenic bases, leading to higher incidence of age-related cancer in animals\(^14\). The DNA itself or its precursors can be altered by ROS. In mice with MTH1 enzyme deficiency, which hydrolyses DNA precursors damaged by oxidative mechanisms, the incorporation of these defective nucleotides in DNA is enabled. This increases the rate of spontaneous tumorigenesis primarily in lungs, stomach and liver\(^14,15\).

The role of ROS in apoptosis

It is well-established that mediators of apoptosis can induce intracellular production of ROS. Moreover, various reactive species can either initiate apoptosis or modify its course\(^4\).
Apoptosis can be induced by extracellular or intracellular signals and is conducted through two major pathways: mitochondrial (intrinsic) or death receptor pathway (extrinsic). Extrinsic pathway of apoptosis is triggered by interaction of death receptor and its ligand in cellular plasma membrane, which activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and generation of ROS. This further leads to activation of acid sphingomyelinase, generation of ceramide and clustering of the receptors. The described processes form signal platform that induces apoptotic cascade. Activation of NF-κB survival path increases the transcription of anti-apoptotic proteins (FLIP, MnSOD, Bcl-X, IAP) and blocks the apoptosis. In the presence of high ROS concentrations, the impossibility of NF-κB induction triggers the activation of ASK1/JNK kinases, which initiates apoptosis.

Intrinsic apoptotic cascade is associated with the changes in permeability of outer mitochondrial membrane, which allows the release of proapoptotic proteins. ROS are well known triggers of intrinsic apoptotic pathway through interaction with outer mitochondrial membrane proteins. Proteins of the Bcl-2 family determine the susceptibility to apoptosis, shifting the balance between pro-apoptotic (Bax, Bak, Bad, Bim and Bid) and anti-apoptotic (Bcl-2, Bcl-XL and Bcl-w) members of the family in favour of apoptosis or against it. In the presence of the apoptotic stimuli like ROS, truncated form of BID protein causes Bax/Bak oligomerization which leads to creation of megapores in mitochondrial membrane. Subsequently, apoptosome complex is formed in the cytosol, activating initiator Caspase 9 and than Caspase 3, which executes the final steps of apoptosis. Caspase activation is further enhanced due to neutralization of caspase inhibitors by proteins released from mitochondria, like Smac/Diablo and Omi/HtrA2. In addition, mitochondrial proteins like apoptosis-inducing factor and Endo G stimulate caspase-independent apoptosis via translocation into nucleus, where they mediate genomic DNA fragmentation.

Programmed cell death is often characterized by generation of large quantities of ROS or transient oxidative burst. Catalase prevents H2O2 mediated apoptosis, indicating the important role of H2O2 in apoptosis. Recently it has been found that H2O2 initiates caspase activation, which is dependent on mitochondrial cytochrome c release. However, very high doses of H2O2 or other ROS induce cell necrosis. This implies that the modus of cell death induced by oxidative stress is dose dependant. Since apoptosis represents active, energy dependant process, it seems that the intracellular ATP level is crucial determinant weather the apoptotic cascade would eventuate.

The interplay of ROS and apoptosis in cancer

Although apoptosis and cancer represent opposite entities, ROS play an important role in both of the processes. Moderate levels of ROS, where “moderate” depends on the cell type, promote apoptosis in tumor cells. Nevertheless, in some malignant cells ROS can exert the opposite effect. In melanoma cell line M14, overexpression of zinc-copper SOD causes the decrease in O2 concentration and thus promotes apoptosis. Generation of ROS by NADPH oxidase enzymatic system acts as an anti-apoptotic, pro-proliferative stimulus in pancreatic cancer cell lines. The ability of mitochondrial apoptosis-inducing factor to oxidase NADH and generate superoxide anion contributes to viability of some cancer cell lines.

The mechanisms by which ROS exert their anti-apoptotic effect have not been completely elucidated so far. It has been hypothesized that caspase inactivation could be responsible. In some cells elevated O2 causes the increase in cytosolic pH value, which halts the caspase activation. In opposite, H2O2 lowers pH in cytosol and thus acts in favor of apoptosis initiation. In addition, H2O2 promotes apoptosis through conversion in HO that directly attacks DNA or damages mitochondria. An alternative mechanism includes inactivation of PTEN protein (product of tumor suppressor gene PTEN), which increases the activation of Akt signaling pathway, thus promoting cell survival. However, Akt signaling is often attributed ambiguous roles, since some studies have indicated that the activation of this path supports cancer cell survival, but suppresses invasiveness and metastases.

The phenomenon of apoptosis has been investigated in many types of cancer and its significance has been well-established for urothelial carcinoma. Recent findings have suggested that oxidative stress sensitizes tumor cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis by downregulation of anti-apoptotic proteins. Moreover, TRAIL protein has gained a lot of attention as a new therapeutic target because of its property to induce apoptosis only in cancer cells, but not in normal cells. However, some urothelial carcinomas develop resistance to TRAIL, therefore the reestablishment of this sensitivity is of great importance. Induction of oxidative stress with low concentration of H2O2 can reverse the resistance to TRAIL by significantly decreasing the expression of short living anti-apoptotic proteins such as FLIP, XIAP and Survivin. Further studies are to determine the relevancy of H2O2 application as adjuvant intravesical agent in urothelial cancer treatment, in order to lower the threshold for initiating apoptosis in malignant cells.

Apoptosis induced by ROS in anticancer treatment

Numerous studies confirmed that generation of free radicals capable of inducing apoptosis in malignant cells is the underlying mechanism of action for numerous chemotherapeutics and radiotherapy. Antineoplastic agents that induce generation of ROS in large quantities are anthra-cyclines (doxorubicin, bleomycin), platinum complexes (cisplatin), alkylating agents (cyclophosphamide), epipodophylotoxins, camptothecin derivatives. In the course of radiotherapy of malignant disease, apoptotic signal can be generated in plasma membrane through lipid peroxidation induced by radiation. Antioxidants reduce ROS generation and thus contribute to preserve the integrity of healthy tissue, but also decrease the damage on tumor cells.
ROS generating anticancer drugs lead to depletion of intracellular antioxidant capacity and when the ROS concentration reaches a certain threshold, apoptosis is initiated. In the absence of adequate antioxidative defense, damage caused by ROS induces activation of apoptosis-related genes. In addition, intracellular ROS increase can also activate redox sensitive JNK/SAPK signaling pathway, often implicated in genes transactivation and posttranslational modifications of proteins required for apoptosis.

**Future implications**

Pharmacological manipulations are able to shift the intracellular redox equilibrium toward the increase in ROS and/or the depletion of protective agents, inducing the apoptotic cascade in cancer cell. The studies investigating application of antioxidant inhibitors and/or ROS generating agents in order to induce apoptosis or to overcome the resistance to chemotherapy have provided significant advances, but further validation of the results is necessary.

Genomics and proteomics represent powerful and promising tools in unraveling complex molecular networks. Investigators have attempted to understand the subset of proteins whose expression levels are directly altered by oxidants, or those proteins that are posttranslationally modified in a redox-dependent fashion. Recent proteomics based study identified gold (III) porphyrin 1a as a new, potent anticancer drug that induces apoptosis through both caspase-dependent and caspase-independent mitochondrial pathways, and demonstrated that intracellular oxidation affected gold (III) porphyrin 1a-induced apoptosis. The difficulty with advanced “omics” methodologies is how to translate the provided data into a meaningful clinical context, into clinical trial design and subsequently into routine clinical use.

One of the most important goals in cancer studies is directed to the possibility of treating cancer by inducing apoptosis via caspase activation. ROS could play a major role in this process, acting as potent mediators. The greatest challenge remains the issue of inducing apoptosis selectively within the neoplastic cells, with maximal sparing of normal tissue. The therapeutic strategy based on triggering apoptosis by modulation of ROS levels, selectively within the neoplastic cells, carries great expectations and holds promise of a significant advance in clinical oncology.

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