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Research Paper

Singlet oxygen treatment of tumor cells triggers extracellular singlet oxygen generation, catalase inactivation and reactivation of intercellular apoptosis-inducing signaling ☆

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ABSTRACT

Intracellular singlet oxygen generation in photofrin-loaded cells caused cell death without discrimination between nonmalignant and malignant cells. In contrast, extracellular singlet oxygen generation caused apoptosis induction selectively in tumor cells through singlet oxygen-mediated inactivation of tumor cell protective catalase and subsequent reactivation of intercellular ROS-mediated apoptosis signaling through the HOCl and the NO/peroxynitrite signaling pathway. Singlet oxygen generation by extracellular photofrin alone was, however, not sufficient for optimal direct inactivation of catalase, but needed to trigger the generation of cell-derived extracellular singlet oxygen through the interaction between H_2O_2 and peroxynitrite. Thereby, formation of peroxynitrous acid, generation of hydroxyl radicals and formation of perhydroxyl radicals ($HO_2^{\cdot}$) through hydroxyl radical/H_2O_2 interaction seemed to be required as intermediate steps. This amplificatory mechanism led to the formation of singlet oxygen at a sufficiently high concentration for optimal inactivation of membrane-associated catalase. At low initial concentrations of singlet oxygen, an additional amplification step needed to be activated. It depended on singlet oxygen-dependent activation of the FAS receptor and caspase-8, followed by caspase-8-mediated enhancement of NOX activity. The biochemical mechanisms described here might be considered as promising principle for the development of novel approaches in tumor therapy that specifically direct membrane-associated catalase of tumor cells and thus utilize tumor cell-specific apoptosis-inducing ROS signaling.

1. Introduction

Reactive oxygen and nitrogen species (ROS) can cause destructive effects through their mutagenic potential and through their ability to react with proteins and lipids, but they can also establish specific and fine-tuned signaling pathways [1,2]. During multistep oncogenesis, reactive oxygen and nitrogen species establish remarkable signaling pathways that induce oncogenic- and antioncogenic effects. Extracellular superoxide anions generated by membrane-associated NADPH oxidase (NOX) and their dismutation product H_2O_2 control the proliferation of malignant cells and are involved in the maintenance of the transformed state in vitro [3–8] and in vivo [8–14]. These oncogenic effects of superoxide anions are counteracted by ROS-dependent intercellular induction of apoptosis, a process that selectively eliminates transformed cells [1,15–30]. ROS-dependent intercellular apoptosis induction is mainly based on the HOCl [1,23,24,29,30] and the NO/peroxynitrite signaling pathway [1,24,26,29,30]. In both pathways, extracellular superoxide anions derived from NOX1 determine the efficiency and the selectivity of apoptosis-inducing signaling. Please find details in Fig. 1A. Tumor progression in vivo requires the acquisition of the "H_2O_2-catabolizing phenotype", i.e. resistance against ROS-mediated apoptosis induction [49–53]. This resistance is established through expression of membrane-associated catalase, which interferes with HOCl signaling through decomposition of H_2O_2 [54–56], and with NO/peroxynitrite signaling through oxidation of NO [57] and decomposition of peroxynitrite [56,58] (Fig. 1B). The combination of the two phenotypic features "NOX-dependent generation of extracellular superoxide anions" and "protection by membrane-associated catalase" has been found

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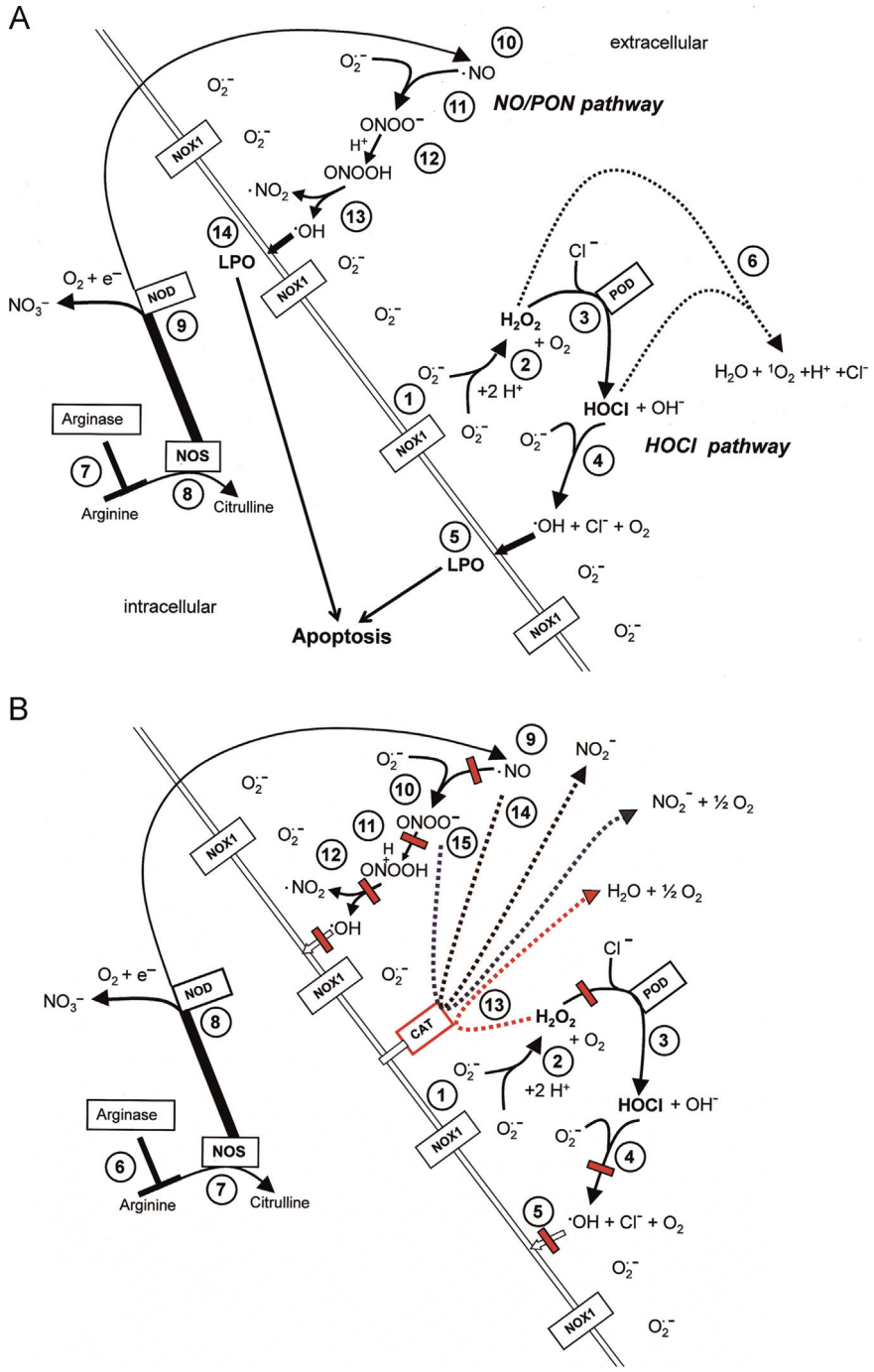

Fig. 1. Intercellular apoptosis-inducing ROS signaling. (A) Transformed cells. The figure shows the membrane of a transformed cell with the intracellular space on the left side, the extracellular space on the right side. Transformed cells are defined as malignant cells that have the potential to form tumors but have not yet been confronted with the natural antitumor mechanisms of an organism. Transformed cells are characterized by expression of NOX1 that generates extracellular superoxide anions (#1). These dismutate and form H_2O_2 ($2O_2^{\cdot-}+2H^+ \rightarrow H_2O_2+O_2$) (#2) which is used by the peroxidase domain of DUOX (POD) as substrate for the generation of HOCl ($H_2O_2+PODFe^{III} \rightarrow POD\ Fe^{IV}=O^{\cdot+}+H_2O$; $POD\ Fe^{IV}=O^{\cdot+}+Cl^-+H^+ \rightarrow PODFe^{III}+HOCl$) (#3). HOCl interacts with superoxide anions, leading to the generation of hydroxyl radicals ($HOCl+O_2^{\cdot-} \rightarrow .OH+O_2+Cl^-$) (#4) [24,31,32] that induce lipid peroxidation (#5) and subsequent apoptosis induction through the mitochondrial pathway of apoptosis. In the presence of an high excess of H_2O_2 compared to POD, a consumption reaction between H_2O_2 and HOCl (#6) blunts HOCl signaling. The level of arginine is controlled by arginase (#7). NO synthase (NOS) utilizes arginine as substrate for the synthesis of NO (#8) [33-35]. A substantial part of NO may be converted into nitrate by NO dioxygenase (#9), which is connected to the activity of cytochrome P 450 oxidoreductase (POR). NO passes the cell membrane (#10) and reacts with superoxide anions, resulting in the formation of peroxynitrite ($.NO+O_2^{\cdot-} \rightarrow ONOO^-$) (#11) [36–40]. Protonation of peroxynitrite leads to the formation of peroxynitrous acid ($ONOO^-+H^+ \rightarrow ONOOH \rightarrow .NO_2+.OH$) (#12) [37,41–43]. As malignant cells have efficient proton pumps that establish a high local concentration of protons on the outside of their cell membrane [44], the formation of ONOOH seems to be locally favored over the competing reaction between $ONOO^-$ and CO_2 ($ONOO^-$ and $CO_2 \rightarrow ONOOCOO^- \rightarrow NO_2+CO_3^{\cdot-}$) [45–48]. Peroxynitrous acid spontaneously decomposes into NO_2 and hydroxyl radicals (#13), which induce lipid peroxidation and the mitochondrial pathway of apoptosis (# 14). (B) Tumor cells (defined as malignant cells derived from a *bona fide* tumor) are protected against intercellular apoptosis-inducing ROS signaling through expression of membrane-associated catalase. Tumor progression causes the selection of a phenotype that is characterized by the expression of membrane-associated catalase [54,56]. Membrane-associated catalase protects the tumor cells against ROS signaling by the HOCl pathway (#1–#5) and the NO/peroxynitrite pathway (#6–#12) through decomposition of H_2O_2 (#13), oxidation of NO (#14) and decomposition of peroxynitrite (#15). Decomposition of H_2O_2 and peroxynitrite by catalase are two step reactions with compound I ($CATFe^{IV}=O^{\cdot+}$) as intermediate. NO is oxidated to NO_2^- by compound I.