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Original Article

Autoamplificatory singlet oxygen generation sensitizes tumor cells for intercellular apoptosis-inducing signaling

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ABSTRACT

Tumor cells express NADPH oxidase-1 (NOX1) in their membrane and control NOX1-based intercellular reactive oxygen and nitrogen species (ROS/RNS)-dependent apoptosis-inducing signaling through membrane-associated catalase and superoxide dismutase.

Treatment: of tumor cells with high concentrations of H_2O_2, peroxnitrite, HOCl, or increasing the concentration of cell-derived NO causes initial generation of singlet oxygen and local inactivation of membrane-associated catalase. As a result, free peroxynitrite and H_2O_2 interact and generate secondary singlet oxygen. Inactivation of further catalase molecules by secondary singlet oxygen leads to auto-amplification of singlet oxygen generation and catalase inactivation. This allows reactivation of intercellular ROS/RNS-signaling and selective apoptosis induction in tumor cells.

The initial singlet oxygen generation seems to be the critical point in this complex biochemical multistep mechanism. Initial singlet oxygen generation requires the interaction between distinct tumor cell-derived ROS and RNS and may also depend on either the induction of NO synthase expression or NOX1 activation through the FAS receptor. FAS receptor activation can be achieved by singlet oxygen.

Autoamplificatory generation of singlet oxygen through the interaction between peroxynitrite and hydrogen peroxide inherits a rich potential for the establishment of synergistic effects that may be instrumental for novel approaches of tumor therapy with high selectivity towards malignant cells.

1. Introduction

1.1. ROS and multistep oncogenesis

Multistep oncogenesis is significantly controlled by the chemical biology of extracellular reactive oxygen/reactive nitrogen species. Malignant transformation is dependent on extracellular superoxide anions generated by membrane-associated NADPH oxidase-1 (NOX1) (Irani et al., 1997; Irani and Goldschmidt-Clermont, 1998; Suh et al., 1999; Bittinger et al., 1998; Yang et al., 1999; Mitsushita et al., 2004; Laurent et al., 2008; Ma et al., 2009; Kim et al., 2010; Du et al., 2011; Tominaga et al., 2007; Lambeth, 2007; Lopez-Lazaro, 2007a; Kamata, 2009; Weinberg and Chandel, 2009) (Fig. 1). Their dismutation product H_2O_2 is required for the maintenance of the transformed state and for the control of proliferation (Irani et al., 1997; Suh et al., 1999; Yang et al., 1999; Arnold et al., 2001; Chamulitrat et al., 2003; Mitsushita et al., 2004; Lopez-Lazaro, 2007b). However, extracellular superoxide anions also drive the efficiency and selectivity of intercellular ROS/RNS signalling. This eliminates transformed cells through induction of apoptosis (Jürgensmeier et al., 1994; Panse et al., 1997; Beck et al., 1997; Engelmann et al., 2000; Herdener et al., 2000; Schwieger et al., 2001; Heigold et al., 2002; Ivanovas and Bauer, 2002; Bauer et al., 2008; Bauer 2000, 2012, 2014, 2015, 2017a). The central elements of intercellular ROS/RNS signalling are summarized in Fig. 2A. Further details of intercellular ROS/RNS signaling as well as interfering mechanisms (such as oxidation of NO, peroxynitrite/carbon dioxide interaction and $HOCl/H_2O_2$ interaction) have been recently reviewed (Bauer, 2015; Bauer 2017b; Bauer and Graves, 2016).

Tumor progression requires the expression of membrane-associated catalase (Deichman and Vendrov, 1986; Deichman et al., 1989; Deichman et al., 1998; Deichman 2000, 2002; Bechtel and Bauer, 2009; Heinzelmann and Bauer, 2010; Bauer, 2012, 2014; Böhm et al., 2015). Membrane-associated catalase interferes with ROS/RNS signalling and thus protects bona fide tumor cells from elimination through intercellular ROS/RNS signaling (Bechtel and Bauer, 2009; Heinzelmann and Bauer, 2010; Böhm et al., 2015) (Fig. 1, details in Fig. 2B). As catalase is inhibited by free superoxide anions (Kono and Fridovich, 1982; Shimizu et al., 1984; Fridovich, 1986; Gebicka et al., 1989;

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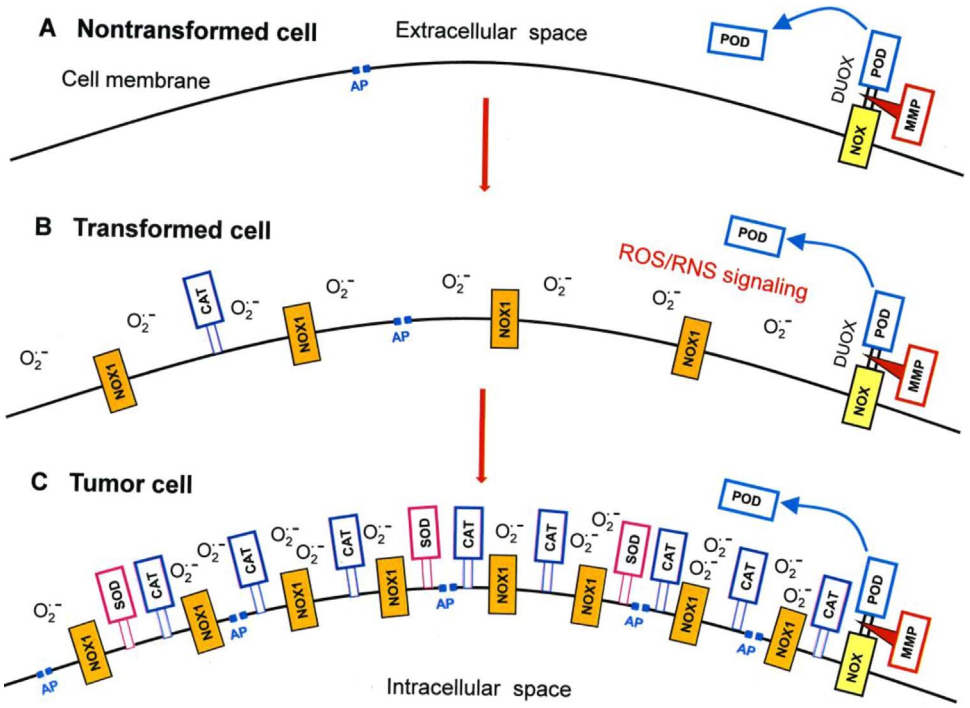

Fig. 1. ROS and tumor progression: schematic view of general principles.
Nontransformed cells (A), transformed cells (early stages of oncogenesis) (B) and bona fide tumor cells (C) release a dual oxidase (DUOX)-related peroxidase (POD) through the action of matrix metalloproteases (MMP). Oncogenic transformation depends on the expression of NADPH oxidase-1 (NOX1) that generates extracellular superoxide anions. These are essential for the proliferation of the cells, but also drive apoptosis-inducing ROS/RNS signaling. Tumor progression is characterized by an increase in NOX1 expression, and substantial expression membrane-associated catalase that allows tight control of intercellular apoptosis-inducing ROS/RNS signaling. SOD plays a comodulatory role through prevention of superoxide anion-dependent inhibition of catalase. Tumor progression also causes an increase in aquaporins (AP).

Pigeolet et al., 1990; Lardinois, 1995), catalase activity in the neighbourhood of active NOX1 requires coexpression of SOD. SOD prevents superoxide anion-dependent inhibition of catalase and thus allows tight control of intercellular ROS/RNS signalling by membrane-associated catalase. SOD also contributes to the inhibiton of ROS/RNS signaling (Bauer, 2013; Bauer and Motz, 2016). The module NOX1/catalase/SOD has been found on bona fide tumor cells derived from many different tissues, including the skin (Bauer, 2014). Tumor cells prevent HOCl signalling through catalase-mediated decomposition of H_2O_2. In this way, POD-dependent HOCl synthesis is prevented through removal of the substrate for POD (Bechtel and Bauer, 2009; Heinzelmann and Bauer, 2010) (Fig. 2B). In addition, membrane-associated catalase oxidates NO and decomposes peroxynitrite (Fig. 2B). It thus tightly controls NO/peroxynitrite signalling (Heinzelmann and Bauer, 2010: for review see Bauer, 2015, 2017d). Details of the complex enzymology of catalase and its modulation by various ROS and RNS are summarized in Supplementary Fig. 1 A, B. They demonstrate that catalase represents a central enzyme at the crossing point of ROS/RNS chemical biology during multistep oncogenesis.

Inhibition or inactivation of tumor cell protective catalase is required for the reactivation of intercellular ROS/RNS-mediated apoptosis-inducing signalling. This may be relevant for the elimination of tumor cells and for novel strategies of tumor therapy. Neutralization of catalase by specific single domain VHH fragments has been shown to cause ROS/RNS-mediated apoptosis induction in tumor cells in vitro. It also affects tumor xenotransplants in mice in vivo (Bauer and Motz, 2016). A systematic study has shown multiple ways to cause tumor cell apoptosis through targeting their protective catalase (Heinzelmann and Bauer, 2010; Bauer, 2015, 2016, 2017c,d; Scheit and Bauer, 2015; Bauer and Zarkovic, 2015; Riethmüller et al., 2015).

1.2. Significance of singlet oxygen generation

Singlet oxygen ($^1O_2$) is one of the most interesting and versatile molecules within the family of ROS (Klotz et al., 2003; Ogilby, 2010). Until now, its potential for the control of oncogenesis and for therapeutic antitumor approaches has probably been underestimated. This article solely refers to the $^1\Delta_g$ form of singlet oxygen, which will be called “singlet oxygen” throughout the manuscript. Illuminated photosyntesizers like photofrin generate singlet oxygen through the type II reaction (Ogilby, 2010; Castano et al., 2005). In biological systems, singlet oxygen can be generated through the reaction between i) HOCl and H_2O_2 (Held et al., 1978; Bauer, 2013), ii) peroxynitrite and H_2O_2 (Di Mascio et al., 1994; Alvarez et al., 1995; Riethmüller et al., 2015), iii) biological lipid hydroperoxides and HOCl (Miyamoto et al., 2006, 2007), iv) biological lipid hydroperoxides and peroxynitrite (Miyamoto et al., 2003, 2007) and v) glutathione and superoxide anions (Wefers and Sies, 1983). Direct generation of singlet oxygen from peroxynitrite ($ONOO^-$) (the reaction product of superoxide anions and NO_2) had been claimed (Khan et al., 2000), but has been substantially disproven (Merenyi et al., 1998; Martinez et al., 2000). In contrast, peroxynitrate (O_2NOO^-) (the reaction product of superoxide anions and NO_2) (Goldstein and Czapyki, 1998) decomposes spontaneously into singlet oxygen and nitrite with an efficiency of 50% (Miyamoto et al., 2009):

$$O_2NOO^- \rightarrow {}^1O_2 + NO_2^-$$

Alternatively, peroxynitrate decomposes into superoxide anions and NO_2:

$$O_2NOO^- \rightarrow O_2\cdot + \cdot NO_2$$

Generation of singlet oxygen through the interaction between two hydroperoxide radicals or one hydroperoxyl radical with a superoxide anion, i. e. the spontaneous dismutation reaction, has been claimed by many researchers (Fridovich, 1975; Aurand et al., 1977; Koppenol and Butler, 1977; Krinsky, 1977; Mayeda and Bard, 1974; Corey et al., 1987; Khan and Kasha, 1994; Kerver et al., 1997; Steinbeck et al., 1993; Tarr and Valenzeno, 2003; Devasagayam and Kamat, 2002):.

$$HO_2\cdot + HO_2\cdot \rightarrow H_2O_2 + {}^1O_2$$

$$HO_2\cdot + O_2\cdot^- + H^+ \rightarrow H_2O_2 + {}^1O_2$$

However, these reactions have also been frequently questioned and disputed (Klotz et al., 2003; Badway and Kanovsky, 1980; Kanofsky, 1989; Evans and Upton, 1985). Finally, the reaction between hydroxyl radicals and superoxide anions has been claimed to lead to the generation of singlet oxygen