



Immune cell - produced ROS and their impact on tumor growth and metastasis

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ABSTRACT

Reactive oxygen species (ROS) are derivatives of molecular oxygen (O₂) involved in various physiological and pathological processes. In immune cells, ROS are mediators of pivotal functions such as phagocytosis, antigen presentation and recognition, cytolysis as well as phenotypical differentiation. Furthermore, ROS exert immunosuppressive effects on T and natural killer (NK) cells which is of particular importance in the so-called “tumor microenvironment” (TME) of solid tumors. This term describes the heterogenous group of non-malignant cells including tumor-associated fibroblasts and immune cells, vascular cells, bacteria etc. by which cancer cells are surrounded and with whom they engage in functional crosstalk. Importantly, pharmacological targeting of the TME and, specifically, tumor-associated immune cells utilizing immune checkpoint inhibitors - monoclonal antibodies that mitigate immunosuppression - turned out to be a major breakthrough in the treatment of malignant tumors. In this review, we aim to give an overview of the role that ROS produced in tumor-associated immune cells play during initiation, progression and metastatic outgrowth of solid cancers. Finally, we summarize findings on how ROS in the TME could be targeted therapeutically to increase the efficacy of cancer immunotherapy and discuss factors determining therapeutic success of redox modulation in tumors.

1. Introduction

The availability of molecular oxygen (O₂) is a prerequisite for metazoan life. O₂ is consumed during oxidative phosphorylation in mitochondria, thereby generating adenosine triphosphate (ATP). ATP, in turn, is the universal energy carrier needed for many cellular functions. Reactive oxygen species (ROS) comprise a variety of bioactive molecules which are derived from O₂. Important representatives of ROS are hydrogen peroxide (H₂O₂) and the superoxide anion radical (O₂^{•−}) but other forms have been described as well including the hydroxyl radical (•OH), hypochlorous acid (HOCl) and organoid hydroperoxides (ROOH) [1]. In humans, ROS are produced in virtually all cells in varying amounts and while many biochemical processes can result in ROS production, it is suggested that the quantitatively most relevant generators of physiological ROS levels are the mitochondrial electron transport chain and enzymatic reactions involving nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs) [1,2]. As the intracellular presence of excessive ROS, a condition termed “oxidative distress”, leads to damaging of DNA, lipids and proteins which ultimately can result in

cell death, ROS levels need to be tightly regulated [1]. Thus, several ROS-scavenging systems have evolved encompassing the enzymes superoxide dismutases (SODs), glutathione peroxidases (GPXs), peroxiredoxins (PRDXs) and catalase (CAT) as well as glutathione (GSH) and thioredoxin (TRX) that serve as reducing agents for oxidized GPXs and PRDXs, respectively [3]. SODs dismutate O₂^{•−} to H₂O₂, whereas GPXs, PRDXs and CAT catalyze the reduction of H₂O₂ to H₂O [4]. In humans, several isoenzymes have been described for SODs (SOD1-3), GPXs (GPX1-8) and PRDXs (PRDX1-6) which differ with respect to tissue-specific expression levels and intracellular localization [5]. Under homeostatic conditions, these enzymes restrain intracellular ROS concentrations and help to maintain the structural and functional integrity of various cell types. Consequently, quantitative alterations of both oxidants and the antioxidative system participate in the development of common pathological conditions including inflammation, cardiovascular diseases and cancer [3,6].

From a cancer research perspective, ROS and oxidative stress started to attract attention when reports showed that induction of H₂O₂ and depletion of GSH are able to induce tumor cell death [7,8]. Surprisingly,

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just a few years later, another study demonstrated that human cancer cell lines generate more H_2O_2 than untransformed cells, hinting toward a pro-tumorigenic role of ROS [9]. Since then, a myriad of studies on this topic have been undertaken and revealed a role of ROS in the cancer context that is fairly ambiguous and many-faceted [3,10–12]. ROS regulate several critical aspects of tumor biology such as proliferation, resistance to cell death, epithelial-to-mesenchymal transition (EMT) and angiogenesis by interacting with key oncogenic molecular pathways including PI3K/Akt/mTOR, RAS/ERK, JNK/p38, NF- κ B, HIF and Src kinase signaling [3,13]. Depending on the experimental conditions, outcomes of these studies were heterogeneous and suggest both tumor-suppressive and -promoting effects of ROS. In line with these pre-clinical results, clinical investigations were similarly inconclusive. For example, markers of oxidative stress in the serum and in primary tumor tissue of several cancer entities have been linked to poor prognosis in large patient cohorts [14–16]. Conversely, recently performed comprehensive meta-analyses of studies evaluating the potential of the well-known antioxidant ascorbic acid (i. e. vitamin C) as a preventive measure or therapeutic option for cancer yielded no clear positive effect [17,18].

Over the last decades, cancer research has been developing toward studying not only the mutagenized cancer cells themselves but also other cell types as well as the micro- and mycobiome (i. e. the entirety of bacteria and fungi) by which cancer cells are surrounded and with whom they engage in functional crosstalk. This heterogeneous group of non-malignant cells includes cancer-associated fibroblasts (CAFs), tumor-associated immune cells (TICs) and vascular cells and creates the so-called “tumor microenvironment” (TME). On the one hand, cells of the TME are known to foster tumor growth by providing oxygen and nutrients, secreting pro-tumorigenic growth factors and cytokines and suppressing the immune response against tumors cells [19]. On the other hand, cytotoxic T cells and natural killer cells in the TME are bestowed with the ability to kill tumor cells and, thus, represent a powerful mechanism by which the TME accomplishes tumor suppression. Consequently, T cell infiltration positively correlates with prognosis in various solid cancers and the recent introduction of immune checkpoint inhibitors, monoclonal antibodies that mitigate T cell suppression in the TME, turned out to be one of the greatest successes of oncological research during the last decades. Therefore, it is now obvious that targeting the TME and, specifically, the TME-intrinsic immune response against tumors represents a promising therapeutic strategy. As they have a fundamental impact on diverse aspects of cell biology and pathobiology, it is not surprising that ROS are also intertwined with tumor-promoting and -suppressive characteristics of TME cells. The functions of tumor cell-derived ROS and ROS produced in non-immune cells in the TME have already been comprehensively summarized elsewhere [3,20,21]. Therefore, in this review, we will give an overview of the regulation and consequences of ROS generated by the various immune cell subpopulations in the TME of solid cancers.

2. Source and regulation of ROS production in tumor-associated immune cells

ROS are indispensable for proper immune cell function. For instance, congenital mutations of genes coding for the ROS-generating NADPH oxidases (NOXs) result in a primary immunodeficiency syndrome termed “Chronic granulomatous disease” (CGD) [22]. Neutrophils of CGD patients exhibit a strikingly reduced capacity to undergo respiratory burst. This cellular program is initiated in phagocytes upon contact with microbes and fungi and mediates intracellular killing of these pathogens by exposing them to high concentrations of ROS generated by NOXs ($O_2^{\bullet-}$) and myeloperoxidases (MPO generating HOCl) [23]. Consequently, CGD patients have a strong disposition toward developing recurrent and unresolving bacterial and fungal infections resulting in substantially decreased life expectancy [22].

On the molecular level, NOXs are induced by various stimuli

including growth factors, chemokines, cytokines, the complement system, phagocytosis and cell adhesion [11,24]. Remarkably, some of the aforementioned signaling molecules including growth factors PDGF, TGF- β , GM-CSF and inflammatory cytokines TNF- α and IFN- γ are known to be abundantly produced within tumor tissues and thus likely fuel NOX-mediated ROS production in tumor-associated immune cells [25–28]. Additionally, bacterial components such as lipopolysaccharide (LPS) induce NOX activity after binding to Toll-like receptors on phagocytes (TLRs; e. g. TLR4 in the case of LPS) and activating downstream kinase signaling [29]. Epithelial cancers of e. g. the gastrointestinal tract, lung and skin are in permanent contact to commensal bacteria and fungi pre-existent at the respective primary site [30]. Therefore, it is conceivable that bacteria and fungi in the TME represent triggers for ROS production by tumor cells and TME members, specifically, immune cells. In fact, tumor-infiltrating myeloid cells in tumor-bearing, bacteria-depleted mice produce significantly less ROS when treated with the cytotoxic agent oxaliplatin than tumor-associated myeloid cells of mice exposed to a physiological intestinal microbiota [31]. Moreover, the tumoricidal effect of oxaliplatin relied in part on the capacity of tumor-associated myeloid cells to generate ROS and bacteria-depleted mice demonstrated significantly reduced tumor regression upon oxaliplatin treatment [31]. Mitochondria represent a quantitatively important site of ROS production [2,32]. Factors influencing ROS generation in mitochondria and release of ROS into the cytosol are hypoxia, fatty and bile acids, TNF- α , p53, pro- and anti-apoptotic proteins such as Bax and other Bcl-2 family members, PUMA, autophagy as well as diverse chemotherapeutic agents including 5-FU, paclitaxel, cisplatin and bleomycin [32–36]. Hypoxia is a common feature of growing tumors since the tumor cells’ demand for oxygen usually exceeds the supply [37]. Under these circumstances, a transcription factor named “hypoxia-inducible transcription factor” (HIF) is stabilized intracellularly, translocates into the nucleus and promotes expression of various genes involved in glucose metabolism, angiogenesis etc. to mediate cellular adaptation to low O_2 concentrations. Furthermore, hypoxia has direct positive effects on ROS production at the mitochondrial complex III and facilitates ROS diffusion into the cytosol [38]. Conversely, ROS generated in mitochondria such as H_2O_2 are capable of stabilizing HIF and induce HIF-mediated transcription during oxygen deprivation [39]. While hypoxia and HIF critically influence innate and adaptive immune cell functions under pathological conditions including cancer, the mechanisms and consequences of hypoxia-induced ROS production in immune cells of the TME still need to be worked out [40–43]. Nitric oxide synthases (NOs) catalyze the reaction of the essential amino acid arginine and O_2 to nitric oxide (NO) and citrulline. NOs, especially the inducible NOS2 isoform, are up-regulated in classically (i. e., proinflammatory or M1-) activated macrophages to generate sufficient amounts of NO for killing infectious pathogens [44]. Importantly, NOs produce $O_2^{\bullet-}$ in macrophages when arginine availability is reduced [45,46]. It is therefore likely that NOS2 also contributes to ROS generation in tumor-associated immune cells, particularly because NOS2 is known to be strongly expressed in both tumor cells and the TME of diverse cancer entities [47]. Furthermore, xanthin oxidase, a key enzyme of purine metabolism, drives ROS production in tumor-associated macrophages, thereby influencing tumor progression [48]. Lastly, intracellular concentrations of metal ions including iron determine ROS production. During the Fenton reaction, Fe^{2+} ions react with H_2O_2 which generates $\bullet OH$. Recently, iron overload in macrophages has been associated with the tumoricidal M1 subtype via increased production of ROS [49]. Moreover, iron-loaded macrophages were associated with tumor regression *in vivo* in the same study using a xenograft model of hepatocellular carcinoma [49]. Interestingly, also in intestinal epithelial cells, induction of the Fenton reaction as a consequence of elevated levels of mitophagy enhances antigen presentation and a cytotoxic T cell response [50].

In summary, various environmental cues are known to promote ROS production in immune cells (see Fig. 1). Therefore, it is probable that

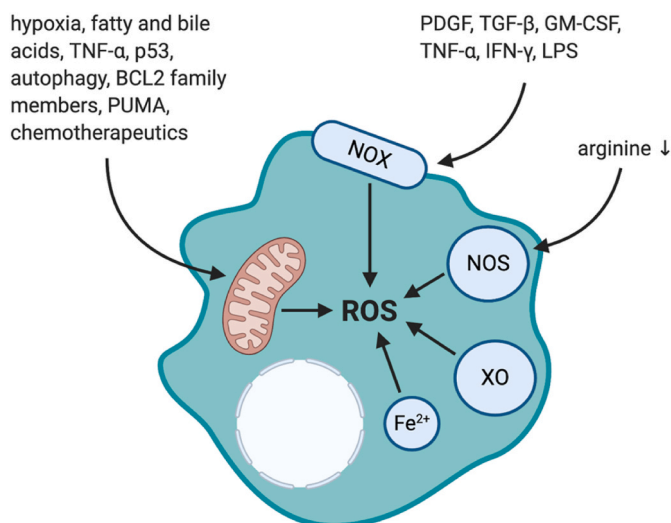


Fig. 1. Mechanisms of ROS production in immune cells. Arrows indicate positive effects on ROS production. Abbreviations: NOX = nicotinamide adenine dinucleotide phosphate oxidase, NOS = Nitric oxide synthase, XO = Xanthine oxidase, TNF- α = Tumor necrosis factor alpha, TGF- β = Transforming growth factor beta, BCL2 = B-cell lymphoma 2, PUMA = p53 upregulated modulator of apoptosis, PDGF = Platelet derived growth factor, GM-CSF = Granulocyte-macrophage colony-stimulating factor, IFN- γ = Interferon gamma, LPS = Lipopolysaccharide.

ROS sources in tumor-associated immune cells are heterogeneous and shift depending on changes in the cellular composition of the TME, the cyto-, chemokine and growth factor milieu, O₂ and ion concentrations as well as therapeutic interventions.

3. Impact of TIC-produced ROS on local tumor growth and metastasis

In both TICs of the myeloid and of the lymphoid lineage, important ROS-dependent effects on the regulation of tumor progression have been described. Intriguingly, the relevant literature suggests tumor-promoting as well as tumor-suppressive functions of immune cell-produced ROS depending on the subtype of immune cell examined, the tumor entity and the experimental model used (Fig. 2). Hence, in the following section, we provide an introduction to the ramifications of ROS produced by the various immune cell subtypes in the TME.

3.1. Myeloid cells

Neutrophil granulocytes are pivotal members of the innate immune system and are being increasingly recognized as pro- as well as anti-tumorigenic agents in the tumor microenvironment [51]. This functional heterogeneity results from a high degree of cellular plasticity and is reflected by the wide variety of neutrophil subtypes that have been described and characterized under both physiologic and pathologic conditions [52].

Early evidence of the impact of neutrophil-derived ROS on epithelial cells dates back to 1999, when Knaapen and colleagues showed that co-culture of rat alveolar epithelial cells with polymorphonuclear leukocytes (PMNs) or H₂O₂ results in increased oxidative DNA damage in the epithelial compartment [53]. Since then, several studies have confirmed ROS-mediated, cytotoxic effects of neutrophils in the TME. For example, neutrophil derived H₂O₂ mediate killing of metastatic breast cancer cells in the pre-metastatic lung, thereby substantially impeding distant outgrowth of primary tumors *in vivo* [54]. Importantly, this effect was abrogated *in vitro* upon administration of the H₂O₂ scavenger catalase, suggesting that H₂O₂ rather than any other ROS member was responsible for tumor cell apoptosis [54]. In a subsequent mechanistic study, it was further elaborated that H₂O₂-induced apoptosis in tumor cells is dependent on the influx of Ca²⁺ into tumor cells via the TRPM2 ion channel [55]. Furthermore, another study showed that the benefit of

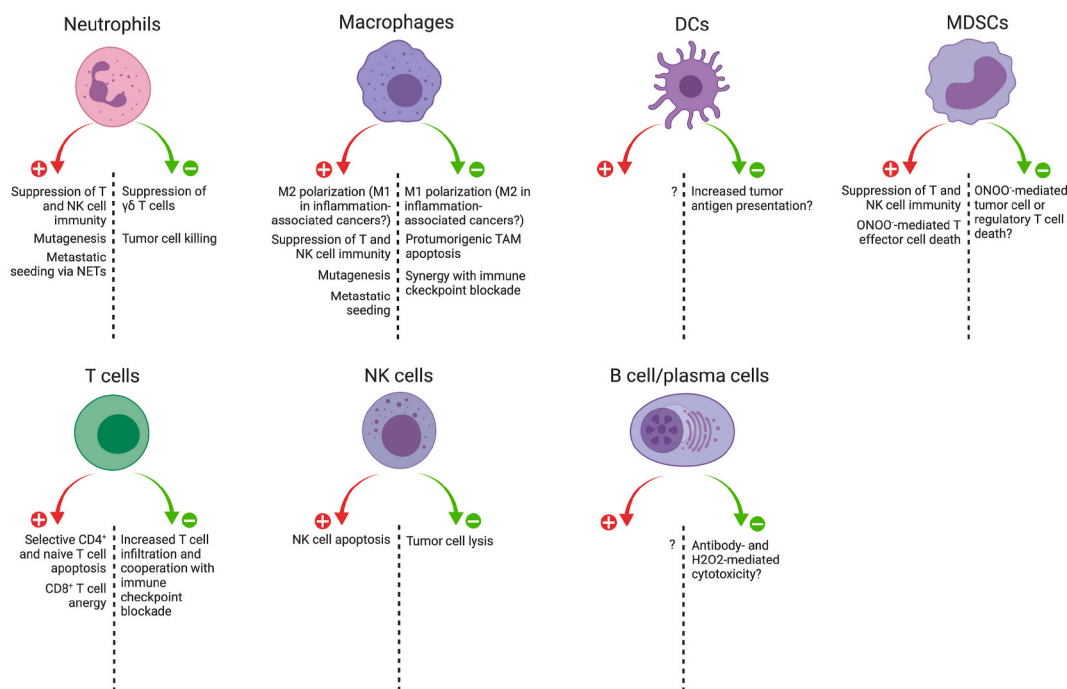


Fig. 2. Immune cell-produced ROS and its effects on tumor progression. Red arrows indicate pro-tumorigenic, green arrows tumor-suppressive effects of immune cell-derived ROS. Abbreviations: DC = Dendritic cell, MDSC = Myeloid-derived suppressor cell, NET = Neutrophil extracellular trap, NK cell = Natural killer cells, TAM = Tumor-associated macrophage. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

radiotherapy is in part mediated by the detrimental effect of neutrophil-secreted ROS on cancer cells [56].

Apart from the direct impact on tumor cells, neutrophil-derived ROS also modulate the functions of various subtypes of immune cells in the TME. For example, neutrophils exert immunosuppressive effects on T cells by producing H_2O_2 , thereby decreasing CD3 ζ chain and cytokine expression [57,58]. Additionally, neutrophil derived H_2O_2 is capable of inhibiting natural killer (NK) cell function which decreases tumor clearance as well as promotes lung colonization in a mouse model of breast cancer metastasis [28,59,60]. However, neutrophil-produced ROS can also impair the proliferation of $\gamma\delta$ -T cells, thereby decreasing the production of the pro-tumorigenic cytokine IL-17 [61].

Another aspect of neutrophil function, NETosis (i. e., neutrophil cell death resulting in release of “neutrophil extracellular traps” [NETs]), has been implicated in the regulation of tumor progression by neutrophils: Inoue and colleagues were able to demonstrate that a systemic redox imbalance generated by depletion of albumin and free thiols leads to accumulation of ROS in neutrophils which, in turn, triggers lung-predominant NETosis that promotes lung metastases in vivo [62].

Beyond their direct impact on established tumors, neutrophil ROS are also important for tumor initiation. For example, myeloid cells govern early mutagenic events during chemically induced carcinogenesis in the intestine and the lung [63,64]. Mice with a myeloid cell-specific deletion of glutathione peroxidase 4 (Gpx4), an important ROS scavenger, developed more invasive tumors in the colon after repeated injection of the carcinogenic agent azoxymethane [63] which was due to excessive ROS production by Gpx4-deficient myeloid cells that increased the mutational load of colonic epithelial cells, thus rendering arising tumors more aggressive [63]. A very recent report by the Malanchi group confirmed the role of neutrophil-derived ROS in tumor initiation by showing that lung tumors following urethane exposure were much less frequent in neutropenic (G-CSF knockout) mice [64]. Furthermore, in vitro combinatorial treatment of fibroblasts with urethane and neutrophils increased DNA damage compared to urethane treatment alone and this effect could be reverted using ROS inhibitors [64].

Macrophages belong to the best-studied immune cell subtypes in the cancer context. Like neutrophils, macrophages are of a highly plastic nature and their phenotype is subject to environmental stimuli. Depending on the quality and quantity of these cues, macrophages are thought to be polarized toward a M1 (classically activated or pro-inflammatory) or M2 (alternatively activated or anti-inflammatory) phenotype [65]. While M1 macrophages are traditionally considered to limit tumor growth, M2 macrophages are thought to promote it via secretion of various pro-tumorigenic and immunosuppressive cytokines, although this perspective is too simplistic [66]. Furthermore, these two activation states are not mutually exclusive but rather represent the two extremes on a continuous scale of polarization statuses [65]. This circumstance seems to be of particular importance in the tumor micro-environment where heterogeneous populations of macrophages expressing both M1 and M2 markers have been described [67]. The literature suggests that ROS can stimulate both activation statuses in tumor-associated macrophages. For example, two reports from 2013 to 2016 showed that $O_2^{\bullet-}$ production promotes M2 polarization through activation of ERK and JNK [68,69]. Moreover, administration of the antioxidant butylated hydroxyanisole (BHA) blocked tumor-associated macrophage (TAM) infiltration and tumor progress in vivo which insinuates a beneficial effect of ROS inhibition in macrophages for tumor therapy [68]. Indeed, another ROS scavenger, oligo-fucoidan, inhibited M2 polarization in a macrophage cell line and inhibited TAM infiltration in subcutaneous colorectal tumors [70]. In contrast, Wu et al. demonstrated a link between ROS generation and M1-polarization of macrophages. The authors showed that irradiation of macrophages increased NOX-dependent ROS production resulting in phosphorylation and activation of ataxia telangiectasia mutated (ATM) kinase and promoting a pro-inflammatory M1 phenotype that was associated with improved

response to radiotherapy in rectal cancer [71]. The induction of M1 polarization by ROS was also confirmed by others [49]. Lastly, a pro-inflammatory polarization of TAMs by NOX-produced ROS is detrimental in an inflammation-associated model of hepatocellular carcinoma through secretion of tumor-promoting cytokines such as IL-6 [72]. In cancers that arise on the ground of chronic inflammation, such as a subset of colorectal and pancreatic tumors as well as hepatocellular carcinomas, the impact of TAM-produced ROS on tumor progression might therefore substantially differ from the one on sporadic (i. e., not inflammation-associated) tumorigenesis [26]. Apart from polarization, ROS also govern TAM apoptosis. For example, inhibition of autophagy in macrophages increases ROS levels, provokes TAM apoptosis and leads to regression of the primary tumor [73]. This was confirmed in a model of ovarian cancer where a subset of TAMs deficient for a key molecular mediator of autophagy experienced increased ROS-dependent apoptosis which, in turn, was associated with increased activation of intratumoral CD4⁺ and CD8⁺ T cells [74]. In contrast, TAM-produced ROS are also known to be immunosuppressive. This was highlighted already in the mid-nineties, when two studies reported that ROS derived from macrophages could down-regulate expression of the CD3 ζ chain on T cells, resulting in immunosuppression [75,76]. Furthermore, chemotherapy-induced ROS can upregulate the expression of PD-L1, an immune checkpoint that suppresses T cell activity (see below), on the surface of TAMs [77]. Lastly, TAM-produced ROS play a role during the metastatic cascade. Kupffer cells in the liver exposed to LPS produce ROS which damages the endothelial lining, thus facilitating adherence of circulating tumor cells [78]. Taken together, it is not possible to characterize TAM-produced ROS as being clearly pro- or anti-tumorigenic. The net outcome of targeting ROS in TAMs of human cancers is context-dependent and is affected by various factors including tumor entity, stage as well as pre- and co-treatment.

Dendritic cells (DCs) are antigen-presenting cells necessary for elicitation of an antigen-specific T cell-response. While ROS in general influence DC function in manifold aspects, e. g., by regulating cytokine production, maturation, migration and antigen presentation, there is not much known about the particular functions of DC-produced ROS in the cancer context [79]. Still, it is very likely that endogenous ROS production in DCs influences the immune response against tumors. For example, ROS production is upregulated in various subtypes of DCs during cross-presentation (i. e., uptake, processing and presentation of an extracellular antigen via the MHC I protein) to cytotoxic CD8⁺ T cells [80,81]. This mechanism involves the recruitment of NOX2 to the phagosome resulting in an alkalization of its content through low-level production of ROS [80]. In turn, alkalization of the phagosome leads to structural preservation of the internalized antigen and allows its successful presentation via the MHC I complex [80]. Thus, it is well conceivable that DC-intrinsic ROS generation also affects the CD8⁺ T cell-response to tumoral antigens and influences DC-based immune therapies.

Myeloid-derived suppressor cells (MDSCs) are a heterogenous population of immature myeloid cells serving as suppressors of T and NK cell immunity under various pathologic conditions, including cancer [82]. ROS-dependent generation of peroxynitrite (ONOO⁻) represents one of the most important mediators of T cell suppression by MDSCs [83]. ONOO⁻ is formed upon the reaction of $O_2^{\bullet-}$ with nitric oxide (NO) and is capable of nitrating diverse amino acids residues, thereby affecting protein function [82]. The impact of MDSC-derived ONOO⁻ on T cell function has been reviewed elsewhere and, in brief, includes nitration of T cell receptors and CD8 which renders T cells unresponsive to MHC I and II-presented peptides and inactivation of T cell chemoattractants such as CCL2 [82,84,85]. Additionally, ONOO⁻ can also reduce the binding efficiency of peptides to MHC I on tumor cells which decreases their susceptibility to CD8⁺ T cell-mediated lysis [86]. Lastly, ONOO⁻-mediated DNA damage is known to activate the Poly (ADP-Ribose) polymerase 1 (PARP1) pathway which is capable of inducing various cell death pathways and pro-inflammatory gene expression in

multiple cell types including leukocytes [87]. However, the ramifications of the ONOO⁻-DNA damage-PARP1 pathway in the TME context haven't been studied yet. Still, it is conceivable that ONOO⁻ induces e. g. cell death of specific T cell subsets or tumor cells, thereby potentially influencing the immune response toward tumor cells.

In summary, MDSCs are an exquisite example of how ROS in the TME mediate immunosuppression and targeting of MDSCs represents a promising therapeutic strategy.

3.2. Lymphoid cells

T cells are members of the adaptive immune system responsible for mounting an immune response against antigens presented by antigen-presenting cells such as dendritic cells, macrophages etc. While CD4⁺ T cells recognize antigens associated with the MHC II complex, CD8⁺ effector T cells can directly neutralize cells presenting an antigen via a MHC I molecule which includes virus-infected and neoplastic cells [88]. Additionally, a subset of CD4⁺ T cells named "regulatory T cells" (T_{reg}^S) exerts immunosuppressive functions and is known to hamper CD8⁺ T cell-mediated killing of tumor cells in the TME. Recently, inhibitors of specific immunosuppressive molecules on the surface of both T cells (e. g. CTLA4 and PD1) and other cells (PD1's ligands PD-L1 and -2), so-called "immune checkpoints", have been developed [88]. The translation of immune checkpoint blockade into the clinical setting represented a major breakthrough in the treatment of various cancer entities and dramatically improved the prognosis of, e. g., patients with advanced melanoma ([89–91], see below). As they are known to be connected to various aspects of T cell biology including activation, differentiation, apoptosis and antigen recognition, it is not surprising that T cell-intrinsic ROS also influence tumor progression [36,92–96]. For example, in a model of non-alcoholic fatty liver disease (NAFLD)-associated hepatocellular carcinoma, increased ROS production in CD4⁺ but not CD8⁺ T lymphocytes promoted CD4⁺ T cell apoptosis and tumorigenesis and, consequently, antioxidative treatment with N-acetyl cysteine (NAC) decelerated tumor growth [34]. In clear cell-renal cell carcinoma (ccRCC), CD8⁺ tumor-infiltrating T cells were found to be inactivated and produce great amounts of ROS [97]. This inactivation could be partly rescued using ROS scavengers [97]. Furthermore, naïve T cells in the TME are prone to undergo ROS-associated apoptosis due to increased lactate uptake which is associated with impaired antitumor immunity [98]. Lastly, oxidative distress in T_{reg}^S can influence their immunosuppressive capacity. A low concentration of antioxidative factors in this particular T cell subset can induce T_{reg} apoptosis which, in turn, leads to the release of high levels of adenosine that promotes immunosuppression in neighbor immune cells and accelerates tumor growth [99].

Natural killer (NK) cells are lymphocytes capable of eliminating virus-infected and tumor cells without prior sensitization to a specific antigen [100]. In principle, NK cells therefore should be able to effectively control tumors of diverse origins. However, studies in human cancers such as non-small-cell lung cancer (NSCLC) or colorectal cancer (CRC) suggest that NK cells are either inactivated in the tumor or do not invade the malignant tissue at all [101,102]. Hence, extensive effort is currently being invested into studying mechanisms and identify therapeutical targets that increase NK cell activation and migration into the tumor [103]. Soon after the first characterization of NK cells in the 1970s it was suggested that •OH is necessary for NK cell-mediated cytotoxicity [104]. However, in the TME, ROS seems to be rather detrimental to NK viability and function. Precisely, ROS induced in NKs by the tryptophane catabolite kynurenine or by lactate promoted NK cell apoptosis [105,106]. In line with that, activation of antioxidative pathways including thioredoxin and peroxiredoxin increased the resistance of NKs to oxidative distress and NK cells in the tumor core of NSCLC patients possess a higher thiol capacity which likely protects them and other lymphocytes against ROS in the TME [107,108].

After activation by antigen-specific T_H cells, B cells can become

plasma cells and generate antibodies which mediate the humoral immune response. In contrast to the extensively studied role of T cells in solid cancer immunity, evidence pertaining to B cell function in the same context is relatively scarce. However, B cells can both promote and inhibit tumor growth by suppressing or activating other immune cells in the TME, produce antibodies against cancer-specific epitopes or secrete pro-tumorigenic cytokines such as lymphotoxins [109].

While it was demonstrated that H₂O₂ is a factor involved in the generation of functional B cells by influencing B cell activation as well as differentiation and that a deregulated redox homeostasis participates in the progression of B cell hematological malignancies, practically nothing is known about the ramifications of B cell-produced ROS in solid cancers [110–112]. One study from 2000 demonstrated that antibodies possess the capacity to destroy antigens by independent generation of H₂O₂, thereby linking antigen binding to an effective antibody-intrinsic killing mechanism [113]. It is conceivable that this also happens in solid cancers and might therefore represent one potential mechanism how B cells indirectly engage in ROS-dependent tumor control. Still, considerably more preclinical studies are needed in order to better understand the role of B cell-produced ROS in solid malignancies and estimate their potential as a therapeutic target.

4. Therapeutic manipulation of ROS and consequences for cancer immunotherapy

Although a clear benefit of antioxidative treatment in human cancer has not been established to date, ROS targeting might still yield positive effects, especially when combined with other therapeutic strategies. For example, i. v.-administered ascorbic acid (vitamin C) can reduce side effects of concomitant chemotherapy and may even increase chemosensitivity (reviewed in Ref. [114]). And given the tremendous success that cancer immunotherapy in general and immune checkpoint blockade in particular brought in the therapy of various cancer entities, it is not surprising that researchers are now increasingly investigating into potential synergistic effects of ROS blockade and immunotherapy, such as high-dose ascorbic acid that synergized with immune checkpoint inhibitors in in vivo models of breast, pancreatic and colorectal cancer [115]. In line with this notion, a ROS nano-scavenger binding to the extracellular matrix of the TME and reducing extracellular ROS levels increases immunogenic cell death and improve T cell responses in colorectal tumors [116]. Also, adoptively transferred T cells pre-treated with antioxidants achieved superior tumor control [117]. However, higher tumor microenvironmental ROS concentrations have also been described to cooperate with immunotherapy. For instance, combinatorial treatment with an anti-PD-L1 agent co-delivered with the photo-sensitizer indocyanine green (which generates ROS when activated during photodynamic therapy), effectively increased tumoral infiltration with CD8⁺ T cells and impaired primary and metastatic tumor growth [118]. Another study confirmed that administration of a ROS generator increased PD-1 blockade efficiency and even cured some mice in a xenograft model of highly immunogenic colorectal cancer [119]. Intriguingly, macrophages can also be reprogrammed via ROS to facilitate cancer immunotherapy: a study from 2018 demonstrated that an amino acid-restricted diet increases ROS production in tumor-associated macrophages and polarizes them toward a M1 phenotype that was associated with a better response to immune checkpoint blockade [120].

5. Considerations on personalized cancer treatment with redox modulators

For all interventions that aim at manipulating the tumoral redox balance, either to synergize with other therapeutic measures or to individually positively influence tumor biology, timing is likely to be critical. For example, large meta-analyses evaluating the potential of various antioxidants in the primary prevention of several cancers could

not identify any positive effect [121]. This suggests that a significant impact of antioxidative treatment on tumor initiation and early phases of tumor progression in humans is rather improbable. This may be related to the fact that in pre-malignant lesions such as e. g. colorectal adenomas, alterations in typical oncogenic signaling pathways associated with increased ROS production (e. g., loss of p53 or SMAD4, activation of RAS/ERK signaling) are less frequent than in advanced cancer stages and manipulation of ROS levels might therefore not be sufficient to significantly impede early tumor growth. In line with that, markers of oxidative stress were found to be stronger expressed in colorectal carcinomas than in adenomas and more abundant in metastatic tissue than in primary tumors [122,123]. However, it has to be noted that in multiple clinical trials involving cancer patients also at more advanced clinical stages (incl. metastatic disease), antioxidative treatment with ascorbic acid yielded similarly underwhelming results implying that other factors than just tumor stage are important in determining the effects of antioxidants in cancer therapy [17]. In fact, the tumoral redox landscape is profoundly altered by treatment with chemotherapeutics or radiation which underscores how important the timely delivery of redox modulation will be in order to achieve positive outcomes [123,124]. Beyond that, molecular markers such as specific mutations and/or transcriptomic signatures will be equally relevant in predicting potential benefits of redox-modulating therapy. For instance, in primary colorectal cancers of the stroma-rich mesenchymal transcriptomic subtype (consensus molecular subtype [CMS] 4), expression levels of the antioxidative transcription factor NRF2 are much lower than in tumors of the epithelial (CMS2) subtype, insinuating that CMS4 patients could benefit from antioxidative treatment [123,125]. Additionally, KRAS or BRAF-mutant but not KRAS/BRAF-wildtype colorectal tumors are sensitive to treatment with ascorbic acid in a pre-clinical study [126]. In contrast, inactivating mutations in the KEAP1 gene which lead to increased expression of NRF2 render cancers resistant to immune checkpoint blockade suggesting that an increased antioxidative capacity could be detrimental during treatment with immune checkpoint inhibitors [127]. Taken together, it is now evident that redox modulation in cancer patients can certainly “not be viewed as a one-size-fits-all modality” (Ngo et al. [128]). Instead, it will be crucial to further investigate into already known and new molecular markers linked to redox states in tumors and associate them with clinical parameters (e. g. primary and acquired resistance to chemo- and targeted therapy), mutational profiles, as well as transcriptomic, proteomic and metabolomic signatures. Along with innovative pre-clinical approaches such as genome-wide CRISPR/Cas- or transposase-based screenings for new therapeutic targets, these investigations will help to elucidate the role of the TME in determining sensitivity to redox manipulation and point toward the subgroups of cancer patients that might ultimately profit from redox-modulating treatment [129].

6. Concluding remarks

ROS are involved in virtually all aspects of cancer biology. Yet, the kaleidoscope of tumor-promoting and -suppressive effects of ROS described so far for tumor cells and the TME precludes any solid prediction as to whether altering ROS in cancers will result in an overall therapeutic advantage. In line with that, results from clinical studies evaluating the benefits of antioxidative therapy in cancer have been at best inconclusive. However, the advent of targeted therapies and, specifically, immunotherapy has added a new dimension to cancer medicine and impressively demonstrated that the TME can be leveraged for the treatment of tumors even in metastatic states. We believe that a multimodal therapeutic approach involving simultaneous or sequential administration of redox modulators, conventional chemotherapy and targeted therapies such as immune checkpoint blockade possesses the highest potential of demonstrating an overall therapeutic benefit for redox manipulation in cancer patients.

Declaration of competing interest

The authors declare no competing interests.

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