Reactive Oxygen Species: Central Regulators of the Tumor Microenvironment

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An Immuno-Oncology Perspective

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Abstract

Reactive oxygen species (ROS), a group of molecules generated by partial reduction of oxygen, have been postulated as central regulators of essential cellular functions through the modulation of signaling pathways activity. Excessive production of ROS, also known as oxidative stress, is a common feature of tumor microenvironment (TME). A large number of studies have provided strong evidence supporting a role of oxidative stress in the regulation of tumor development and progression through the modulation of the TME. In this chapter, we summarize the state of the field as it relates to causes and consequences of ROS elevation in the TME. In addition, we describe the molecular and biological mechanisms governing the intricate network of events driven by oxidative stress leading to an immunosuppressed TME. Finally, we discuss the translational significance of ROS induction as new therapeutic strategies with an emphasis in the role of photodynamic therapy, as a ROS-based potent antitumor agent regulating inflammation and immune system activation during tumor progression.

Keywords

Reactive oxygen species · Oxidative stress · Tumor microenvironment · Immune cells

Abbreviations

APC	Antigen presenting cells
CAFs	Cancer Associated Fibroblasts
COX	Ciclooxigenase
DCs	Dendritic cells
ECM	Extracellular matrix
ERK	Extracellular signal-regulated kinase
HGG	High-grade glioma
HIF-1	Hypoxia Inducible Factor-1
ICD	Immunogenic cell death
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MDSCs	Myeloid-derived suppressor cells
NK	Natural killer
NOX	NADPH oxidase
PDT	Photodynamic Therapy
PGE2	Prostaglandin E2
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TAMs	Tumor-Associated Macrophages
TCR	T-cell receptor
Th1	T-helper 1
Treg	T-regulatory

Introduction

In the current dogma of tumorigenesis, it is well establish the role of the tumor microenvironment (TME) in the regulation of tumor initiation, growth, and maintenance. (Kim et al. 2011). This tumor ecosystem is a complex network composed of cellular and noncellular elements. Malignant cells (parenchyma) and host cells (stroma) constitute the principal populations in this microsystem. TME's stroma is composed by fibroblasts, cells of the immune system, tumor vasculature and lymphatics, among others. Cancer-associated fibroblasts (CAFs) are one of the most abundant components of this ecosystem. CAFs provide physical support for tumor cells through synthesis and remodeling of the extracellular matrix (ECM) and production of growth factors. Through these mechanisms, they play key roles in promoting angiogenesis, metastasis, and modulation of infiltrating leukocytes. Recognition and elimination of tumors by adaptive immune cells ($CD8^+$ and $CD4^+$ T-cells) and innate immune cells including natural killer (NK) cells and dendritic cells (DCs) play a pivotal antitumor immunological activity and are often impaired in TME. In concordance, immunosuppressive populations, such as T regulatory (Treg), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs), are recruited and enriched within tumor stroma. The bidirectional interaction between stroma and tumor cells shapes their phenotype and response to the environmental conditions (Rumie Vittar et al. 2013).

Diffusible molecules present in the TME, like O_2, are essential mediators controlling tumor dynamics (Wang et al. 2017). Oxygen plays a key role in metabolism and its presence is a required condition for tumor growth. When the microenvironment senses lack of oxygen, molecular pathways are triggered to ensure provision of this molecule. At the same time, secondary products are generated as a consequence of oxygen metabolism. These compounds, named reactive oxygen species (ROS), are reactive molecules eliciting multiple biologicals effects through modifications of key redox-sensitive residues in biomolecules like DNA and proteins. ROS include radicals (molecules with unpaired electrons) that are formed by partial reduction of oxygen (O_2), such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot -}$), and hydroxyl ($OH^{\cdot}$). Even though tumor cells are damaged and genetically modified by ROS produced inside itself, diffusible ROS from surroundings are able to reach tumor cells establishing a positive loop of damage and genetic modification on these cells. For this reason, in order to distinguish these differential origins of tumor oxidative stress, in this chapter we classified the sources ROS within tumor in two major categories: (a) ROS from tumor cells, (RTC) and (b) ROS from nontumor cells (RNTC) which represent diffusible species that are impacting on tumor cells coming from their neighbors inhabiting tumor microenvironment (Fig. 1). ROS have been considered as important signaling molecules in cancer (Costa et al. 2014). While ROS are known to be enhancers of tumor development through direct effect on the tumor cells, the induction of immune cell suppression has emerged as new mechanism controlling carcinogenesis (Yang et al. 2013). In this chapter, we will focus mainly on the central role for ROS in the modulation of antitumor immunity.

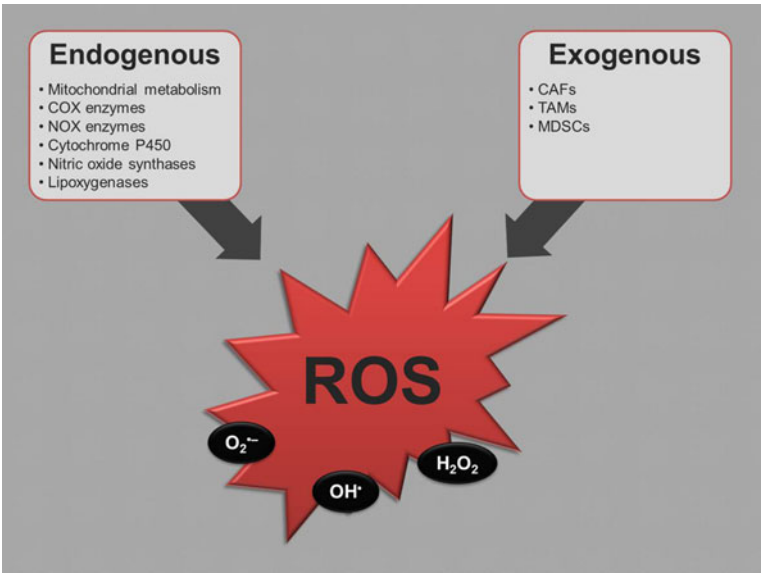

Fig. 1 Tumor and nontumor cells sources of reactive oxygen species in cancer. Intracellular ROS pool in tumor cells is generated from endogenous enzymatic complex and also from surrounding sources, in particular cellular populations inhabiting tumor microenvironment

The Origin of ROS: Mitochondrial and Nonmitochondrial Sources

Intracellular ROS sources include mitochondria electron transport chain, NADPH oxidases (NOX), cyclooxygenases (COX), cytochrome P450 (CYO), among other enzymes (Fig. 1). Cancer cells exhibit a persistent metabolic oxidative stress compared with normal cells: intrinsic mitochondrial dysfunction, NOX activation, COX, and CYP aberrant overexpression, partially explain this phenomenon (Kumari et al. 2018; Snezhkina et al. 2019). As a part of metabolic reactions, high levels of ROS are generated and unregulated levels can lead to oxidative damage such as DNA mutation–causing cancer initiation and progression.

The principal radical produced by mitochondria is superoxide, which comes from one-electron reduction of oxygen in mitochondrial electron transport chain. In particular, mitochondrial complex I, II, and III are the largest contributors of superoxide cellular pool. This radical is then converted into H_2O_2 by superoxide dismutase (SOD). The conversion mediated by SOD is important to generate a more diffusible molecule, which can get access to the cytoplasm. The amount of ROS produced by mitochondria not only depends on biochemistry of this organelle; also the number of mitochondria inside the cells is important to regulate ROS concentration (Moloney and Cotter 2018). This process is able to ensure an adequate supply of O_2 while keeping ROS under tolerable levels. Altered function of mitochondria clearance activity increases cytoplasmic ROS

accumulation (Shefa et al. 2019) leading to an enhanced DNA damage and cellular transformation.

NOX enzymes comprise a transmembrane family of proteins with important role to generate intracellular ROS. Their principal function is to transport electrons across biological membranes to reduce oxygen. The H_2O_2 produced rapidly diffuses using aquaporin channels. Although these enzymes have similar role to produce ROS, their expression changes among different tissues and cells. Currently, a total of seven members of this family have been described (NOX1-5 and DUOX1-2). NOX2 was the first member identified in phagocytes, while others members were found in colon (NOX2), kidney (NOX4), and pancreas (NOX5) among others (Bedard and Krause 2007).

COX family of enzymes comprises of three members (COX-1, COX-2, and COX-3) located mainly at the luminal side of nuclear and endoplasmic reticulum (ER) membrane. These isoenzymes are involved in prostaglandin synthesis which control process like inflammation, platelets aggregation, cell growth, calcium flow, among others. COX-1 and COX-2 are closely related to ROS production in two major ways. First, ROS can induce COX-2 expression, leading to increased PGE2 (prostaglandin E2) synthesis that in turn promotes cell proliferation, survival, and invasion in different cancer models. On the other hand, COX is able to induce ROS production by itself or by increasing NADPH oxidase activity. These properties of COX had shed light about how tumorigenesis could be increased in some tumors (Sobolewski et al. 2010).

Cytochrome P450 is an important superfamily of monooxygenase placed on the endoplasmic reticulum, in particular its activity is associated with the membrane-bound microsomal system, a complex controlling xenobiotics clearance (drugs, alcohol, polychlorinated biphenyls, synthetic organochlorides). Inefficient catalytic cycle of P450 increases ROS production and in turn induces changes promoting to cellular transformation (Veith and Moorthy 2018). Other important enzymes nitric oxide synthases and lipoxygenases can induce intracellular ROS accumulation and have been linked with tumorigenesis (Moloney and Cotter 2018). However, description of their function exceeds the purpose of this chapter.

Major Producers of ROS in the TME

Tumor cells enzymatic complex can increase intracellular ROS pool; however, there are other relevant sources of these radicals in other cellular components of the TME that are in part regulated by tumor cells. ROS produced by cancer cells can diffuse into tumor stroma and increase oxidative status of microenvironment. Under these conditions, nonmalignant cells in the microenvironment modify their phenotype in response to increment level of ROS. Moreover, these newly adaptive stroma populations contribute to enhance oxidative stress in the tumor niche by nontumor cells (Weinberg et al. 2019).

Among these cells, cancer associated fibroblasts (CAFs) are major regulators of the level of extracellular ROS in TME. In this context, ROS can stimulate

differentiation of CAFs, leading to metabolic reprograming of certain subpopulation, which impacts on tumor development and further enhancing ROS production (Arcucci et al. 2016). Similarly, immune cells constitute another important source of ROS. For example, TAMs and myeloid-derived suppressor cells (MDSCs) can produce a large amount of ROS that can impact on immune cells phenotype by impairing the normal function of T-cells leading to a immunosuppressive TME, supporting tumor growth and dissemination (Weinberg et al. 2019) (Ostrand-Rosenberg et al. 2020) (Srivastava et al. 2010). In summary, the TME is enriched in ROS sources from different cell population to orchestrate a meshwork of pathway promoting an immunosuppressive environment leading to tumor progression.

Molecular Events Triggered by ROS: Impact on Tumorigenesis

It is possible to point two opposite effects of ROS in health and disease or in other words the "Yin and Yang" of ROS (Fig. 2). At lower levels, in the "light side," ROS seem to contribute to normal physiology, regulating cellular homeostasis (Weinberg et al. 2019). In the "dark side," in terms of pro-tumoral events, ROS are crucial to regulate molecular pathways leading to tumorigenesis and inhibition of antitumor immune response. In this section, three principal molecular events triggered by ROS are discussed: DNA damage, regulation of signal transduction, and gene transcription.

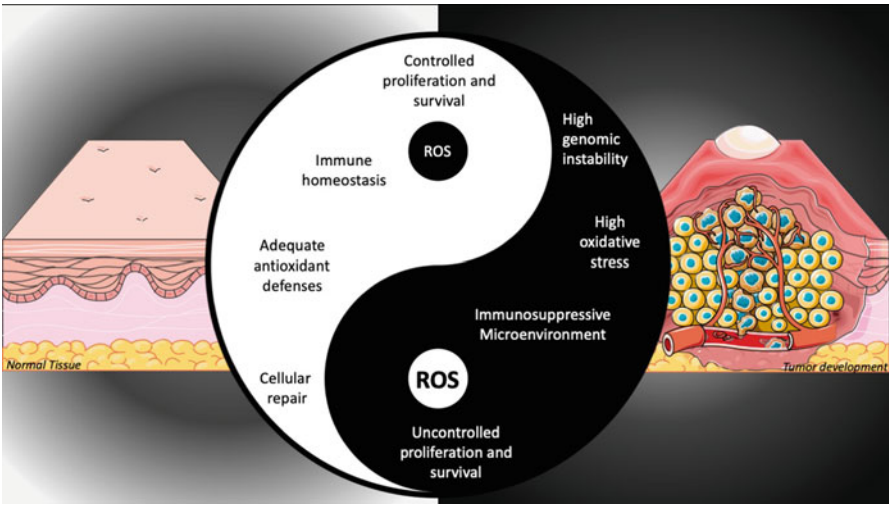

Fig. 2 Opposite role of ROS in health and disease. As described in this chapter, ROS generated by tumor and nontumor cells are able to play different roles depending on the context. In normal tissue, lower-moderate levels of ROS promote adequate homeostasis and normal physiology. When the concentration of ROS in the cells or TME is high, processes such as immune deregulation, uncontrolled proliferation, and genetic instability have place. These molecular events are important to initiate, support, and promote tumor growth and invasiveness

DNA damage: A major impact of excessive ROS in cancer cells is the induction of genomic instability through the generation of DNA damage. Under high ROS levels, DNA repair pathways are not capable to effectively fix the damage and mutations arise in tumor and nontumor cells. This instability has been proposed as an important force driving oncogenesis by promoting genetic variability essential for tumor adaptation, resistance, and evolution (Moloney and Cotter 2018). Mechanistically ROS are able to impact on DNA integrity in different ways, which include: single lesion in purine and pyrimidine bases, DNA-protein adducts, and interstrand crosslinking (Cadet and Richard Wagner 2013). In the first case, the hydroxyl radical ($OH^{\bullet}$) reacts with DNA adding double bonds and abstracting H atoms in DNA bases. These single lesions can induce conformational changes in DNA and enhance mutagenic rates. Another important impact of ROS on DNA functionality is the crosslinking that rise when lysine residue of peptides reacts with guanine radical cations. These DNA-protein structures may obstruct the normal process of transcription and replication leading to genomic instability. Finally, through oxidative mechanism (Nucleophilic Addition) •OH is responsible for crosslinking of opposite DNA strands. This lesion is highly harmful due to it prevents transcription and replication by inhibiting the correct DNA strand separation (Cooke et al. 2003).

Signal Transduction and Gene Transcription: The effect of ROS on molecular pathways could be evaluated at different levels. ROS can influence the activation of proteins involved in signal transduction axis or may affect directly the activation of transcription factors. One of the principal pathways activated by ROS is mitogen-activated protein kinases (MAPK) signaling. This pathway plays an important role in signal transduction from plasmatic membrane to the nucleus to regulate oncogenic gene expression. In mammalian cells, there are three categories of MAPKs: the c-Jun N-terminal kinases (JNKs), the extracellular signal regulated kinases (ERKs), and the p38 MAPKs. In each category it is possible to find different isoforms for MAPKs, which are activated through phosphorylation. MAPKs activated by ROS have the major activity to enhance proliferation, cellular growth, and avoid apoptosis leading to a more extended cell survival (Zhang et al. 2016). In the case of ERK axis, ROS have been reported to activate the EGF and PDGF receptors in a ligand-independent manner, which can induce Ras and subsequently trigger ERK pathway activation. Also, other reports have shown how ROS can inactivate the dual specific phosphatase 3 (DUSP3) leading to ERK activation (Wentworth et al. 2011). It has been pointed out that ROS are able to act on redox-sensitive proteins or mediate the detachment of JNK from its inhibitor protein the glutathione S-transferase pi (GSTp). High levels of ROS can also inactivate phosphatases resulting in a sustained JNK activation (Davies and Tournier 2012). The p38 pathway is activated by extracellular stresses, and similar to JNK pathways ROS can activate different intermediate proteins such as MLK3 and MEK 1/2. Finally it is important to point out that through pathway crosstalk, the activation of one axis by ROS may activate colateral signalings (Soares-Silva et al. 2016).

Another molecular impact mediated by ROS in cancer development is the activation of oncogenic transcription factors. For example, ROS stabilize the Hypoxia Inducible Factor-1 (HIF-1). This transcription factor is composed of two subunits: HIF-1α and HIF-1β. In order to regulate gene expression, HIF-1 needs to be stabilized and activated. ROS are able to mediate HIF-1α stabilization and promote its transcriptional activity (Lamberti et al. 2017). HIF-1 activity is essential for tumor cells to adapt to hypoxia and metabolic conditions in TME. HIF-1 allows cancer cells to survive in hypoxic conditions and at the same time increases the pathways that tend to enhance the nutrient flow to tumor through improving angiogenesis. For example, HIF regulates genes involved in VEGF secretion (Jun et al. 2017; Lamberti et al. 2019a). Also, the relationship between HIF-1 and ROS has been observed in tumor resistance to different treatments (Lamberti et al. 2017). HIF-1 activity is not only important in cancer cells; this transcription factor also is active in stromal cells. In particular, HIF-1 activity in infiltrating immune cells impacts on tumor evolution. For example, HIF-1 is important for TAMs recruitment and tumor growth. Also, HIF-1 regulates Treg differentiation, creating in some cases an immunotolerant microenvironment (Palazon et al. 2014). Another important molecular effect of HIF-1 is its ability to regulate autophagy. During tumor initiation, autophagy operates in order to protect the cells against oxidative stress. In this stage, autophagy is responsible for elimination of damaged mitochondria; in this way, the level of ROS decreases and some signal transduction pathway associated with ROS and transformation are prevented. The opposite effect of autophagy is observed on tumor progression. In this stage, autophagy is needed to the adaptation of cancer cells to the hypoxic microenvironment (Rodríguez et al. 2017). Particularly, ROS induce HIF-1 stabilization, which triggers the expression of BNIP3, a protein necessary to interact with Beclin-1 and Atg5 to form the autophagosome (REFs). In this way, mitochondria are recycling and cells are able to survive during hypoxia stress (Zhang et al. 2008).

GLI1-related factor 2 and NF-E2 (Nrf2) are also in the group of transcription factors that are regulated and can act as effectors of ROS. In the case of GLI1, in recent years it has been observed that ROS are able to activate this protein. For example, NOX4 enzyme promotes gastric cancer, modulating cells growth via GLI1 pathway (Tang et al. 2018). Nrf2 is a transcription factor of numerous antioxidant genes. Under physiological conditions, Nrf2 protein is repressed through its association to Keap1, which leads to Nrf2 degradation by the ubiquitin-proteasome system. Cellular oxidative stress increases Nrf2 level through posttranslation regulation, as a result of Keap1 inactivation mediated by ROS and thereby Nrf2:Keap1 complex dissociation. The multiple functions of Nrf2 have mainly been elucidated by the identification of Nrf2 target genes with a common antioxidant element response (ARE) binding motif. Particularly, those genes are involved in antioxidant defense, drug metabolism, and oxidant signaling. Overall, Nrf2 is considered to have cytoprotective role that protects both normal and tumor cells from oxidative damage. However, when cancer is developed, Nrf2 expression correlates with a bad prognostic given its connection to therapeutic resistance (Zimta et al. 2019).

Modulation of Immune Cells by Tumor Microenvironment-Associated Oxidative Stress

The effect of ROS in the immune cells present in the tumor stroma is controversial (Yang et al. 2013). Here, we discussed the state of the field on the role of ROS in the modulation of immune composition and functionality within the TME.

Natural Killer Cells

Natural killer (NK) cells are innate immune cells which show the ability to eliminate cancer cells, without previous antigen presentation. NK cells can be subdivided into two major subsets based on the relative expression of the surface marker CD56: $CD56^{dim}$ and $CD56^{bright}$. NK cells release preformed cytolytic granules, including perforin and granzymes, after forming immune synapses between germline-encoded stimulatory receptors (such as NKG2D and NKp46) and target cells (Vivier et al. 2012). Thus, their main cytotoxic strategy is the induction of cell lysis, which it has demonstrated to be highly dependent on ROS-production, in particular in the early steps after the synapses formation (Suthanthiran et al. 1984; Duwe et al. 1985). Paradoxically, NK cells exposed to ROS impaired their cytolytic activity by decreasing CD3ζ and CD16ζ (Kono et al. 1996), NKG2D and NKp46 expression, and secretion of IFN-γ (Houze et al. 1996). In the TME, this inhibition can be triggered by monocytes ROS in a NOX2-dependent fashion (Aurelius et al. 2012). NK cells are exceptionally sensitive to ROS-mediated cytotoxicity, which promoted their apoptotic cell death (Hansson et al. 1996). Epidemiological studies have demonstrated that levels of mitochondrial DNA (mtDNA) content were positively correlated with ROS-mediated immunosuppressive phenotype in cancer patients. In particular, high leukocyte mtDNA content was associated with ROS-mediated secretion of TGF-β, leading to lower NK presence in the tumor (Chen et al. 2015; He et al. 2016). Interestingly, $CD56^{dim}$ NK cells (highly cytotoxic) exhibited higher sensitivity to phagocyte-derived or exposure to ROS than $CD56^{bright}$ (less cytotoxic), associated to their antioxidant differential activities (Romero et al. 2006; Harlin et al. 2007; Thorén et al. 2007). These differential subset-dependent resistance explains, at least in part, the preferential enrichment of NK cells $CD56^{bright}$, which display less antitumor activity, in ROS high tumor microenvironment (Izawa et al. 2011), contributing to cancer immunosuppression.

Dendritic Cells

Dendritic cells (DCs) are specialized antigen presenting cells (APCs) linking innate and adaptive immunity. The central role of DCs is the capture, processing, and cross-presentation of tumor-associated antigens to adaptive immune cells, regulating their polarization into effector cells and thereby generating tumor-specific immunity. Upon exposure to "activating stimuli," iDCs turn into mature

DCs (mDCs) through a complicated series of phenotypic and functional changes (Anguille et al. 2014). ROS promote this maturation process, by the upregulation of MHC and co-stimulatory molecules (Rutault et al. 1999; Kantengwa et al. 2003). ROS increased during differentiation in monocyte-derived DCs leading to an enhanced antigen uptake and processing (Sheng et al. 2010). Antigen cross-presentation relied on NOX2-mediated ROS generation and subsequent prevention of acidification within DCs phagosomes (Savina et al. 2006; Mantegazza et al. 2008). On the other hand, tumor-associated DCs demonstrated to be defective in their ability to cross-present antigens. It has been reported a harmful accumulation of oxidized lipids in DCs, which caused defect in the traffic of peptide–MHC class I complexes to the cell surface (Ramakrishnan et al. 2014; Veglia et al. 2017). Taken together, this inhibition of antigen cross-presentation partially explains the failure of DCs within tumor microenvironment adequately stimulate T-cells responses.

T-Cells

Within $CD4^+$ and $CD8^+$ T-cell compartment (referred here as T-cells), responses to oxidative stress are multifaceted (Chen et al. 2016). Adequate T-cell response requires the generation of 3 major signals: antigen recognition by TCR-MHC engagement (signal 1), co-stimulation (signal 2), and cytokine priming (signal 3). After that, antigen-specific naïve resting T-cells proliferate and differentiate into different classes of effectors. Under physiological conditions, low-concentration of ROS showed to be necessary for those molecular events (Yang et al. 2013). Intracellular ROS generation increased immediately after TCR stimulation (Kwon et al. 2003; Jackson et al. 2004) in a NOX2-dependent manner (Jackson et al. 2004). This ROS signaling is an essential requirement for T-cell expansion and IL-2 and IL-4 activating autocrine/paracrine action (Chaudhri et al. 1988; Kaminski et al. 2010; Sena et al. 2013). In addition, ROS also regulate T-cell death during the terminal phase of immune response. By modulating FasL and Bcl-2 expression, intracellular ROS are involved in the regulation of both extrinsic and intrinsic apoptosis (Bauer et al. 1998; Hildeman et al. 2002; Hildeman 2004).

Given that the main features of T-cells are governed by a tightly regulated redox balance, it seems logical to assume that tumor-infiltrating T-cells could be dysfunctional due to the presence of TME-associated oxidative stress. It is well known that T lymphocytes isolated from patients with cancer displayed reduced proliferative responses upon TCR ligation ex vivo (Miescher et al. 1988). This observation appears to reflect an in vivo tumor-associated T-cell hypo-responsiveness by affecting TCR-signaling pathways (Cemerski et al. 2002). Further, it was demonstrated that granulocyte activation in cancer patients correlated with the inhibition of TCR expression and cytokine production by their T-cells (Schmielau and Finn 2001). Taken together, these findings point to the contrasting role of low or high levels and the closely network that redox balance could control on antitumor T-cell functions.

Treg Cells

$Foxp3^{+}CD25^{+}CD4^{+}$ regulatory T (Treg) cells are the main adaptive cellular mediators controlling self-tolerance and immune homeostasis. The underlying mechanisms to driving this suppressive activity include the secretion inhibitory cytokine (e.g., TGF-β), cytolysis of target cells and metabolic disruption of the effector T-cell target by adenosine generation and IL-2 consumption. During cancer development, it is well established that Treg promote tumor progression by obstructing effective antitumor immunity (Takeuchi and Nishikawa 2016). It has been demonstrated that Treg-mediated suppression of $CD4^{+}$ T-cells was NOX2-dependent, involved TGF-β, and could be blocked by antioxidant (Efimova et al. 2011). As mentioned in the previous sections, excessive ROS have shown to be harmful for those antitumor immune populations, such as NK cells and T-cells during cancer development. Paradoxically, tumor sites exhibited a superior number of Tregs, indicating that they could subsist in this environment of increased oxidative stress. ROS-mediated secretion of TGF-β, simultaneous with high leukocyte mtDNA, was also associated with higher levels of Treg cell and overall lower cancer patient survival (He et al. 2016). The extraordinary ability of Treg to resist oxidative stress while maintaining their immunosuppressive activities might be attributed to their stronger intracellular antioxidative machinery (Mougiakakos et al. 2009). Overall, this evidence could explain Treg cells enrichment in malignancies stroma and their contribution in the immunosuppressive phenotype of cancer.

ROS Involvement on Immunomodulation by Antitumor Therapeutics Immunotherapy

A large number of reports support the fact that most of the anticancer strategies shared the ROS generation displaying both supporting or suppressing cancer cells-intrinsic signaling programs (Gorrini et al. 2013; Raza et al. 2017; Zou et al. 2017; Lamberti et al. 2018; Perillo et al. 2020). In addition, FDA-approved agents or the ones currently in clinical trials triggered ROS generation in both direct or indirect manner (Gorrini et al. 2013; Raza et al. 2017; Perillo et al. 2020).

Considering that the immunosuppressive properties in TME are mediated by oxidative stress, the employment of antioxidant agents or supplements on antitumor regimen would contribute to immunoregulatory mechanisms improving the therapeutic intervention. Similarly, studies showed that NOX inhibition could immunoimprove cytotoxicity in human cancers (Raza et al. 2017). As mentioned in the previous sections, NOX are enzyme catalyzing reactions generating ROS (Gorrini et al. 2013). It has been demonstrated by suppressing NOX activity, and histamine protects NK cells and T-cells dysfunction and apoptosis and also maintains their activation by IL-2 among other activators (Hellstrand 2002). Several agents have been evaluated for NOX inhibition in vivo but they blocked the action of several other ROS generating enzymes (Altenhöfer et al. 2015; Raza et al. 2017). To conclude, NOX inhibition can play a vital role in cancer immunotherapy but further investigation is needed to address by pivot a therapeutic intervention.

Some evidences have shown that oxidative stress in the tumor microenvironment is able to alter phenotypically and functionally DCs blunting antitumor immunity. Accordingly, deletion or selective silencing DCs fueled oxidation byproducts by ROS restored their immunostimulatory activity evoking protective type 1 antitumor responses thus offering a cancer immunotherapy approach (Cubillos-Ruiz et al. 2015). Several therapeutics have the ability to initiate a productive immunostimulatory action whose impact depends on schedules, dose, and administration routes. Thus, many anticancer cytotoxic agents interfere often to the occurrence of an immune tolerance and prevent cancer. A prominent activation of the immune system against cancer constitutes the immunogenic cell death (ICD), which determines the long-term success therapies by promoting adaptive immunity and involves a well-defined spatiotemporal scheme of cell surface composition changes as well as release of soluble mediators. Moreover, accumulating clinical evidence demonstrates the ability of several agents to drive ICD in oncological settings including chemotherapeutics and physical therapeutic modalities (Kroemer et al. 2013; Bezu et al. 2015; Faè et al. 2016; Lamberti et al. 2019b, 2020; Galluzzi et al. 2020). Enclosed to those physical interventions and regarding ROS-based therapeutics, photodynamic therapy (PDT) constitutes a potential strategy in generation these photooxidizing reactive molecules as pivotal mechanism to kill cancer cells by three reported ways. One of them relies on the promotion of release of cytokines and acute inflammation into tumor tissue invoking immune cells to destroy the tumor (Agostinis et al. 2011; Rumie Vittar et al. 2013; Raza et al. 2017; Zou et al. 2017; Kessel and Oleinick 2018; Lamberti et al. 2020). The relevance of PDT leading effectiveness as immunotherapeutic interventions has been analyzed (Kroemer et al. 2013; Wachowska et al. 2015). In this context, PDT has been linked as ICD inducer associated with several damage-associated molecular patterns (DAMPs) involved in (Garg et al. 2011, 2012; Kroemer et al. 2013; Verfaillie et al. 2013; Lamberti et al. 2019a). As an adjuvant strategy that aimed trigger and enhance immune system activation, PDT is currently evaluated in vaccine protocols (Lamberti et al. 2020). In this context, our research group has reported for the first time the PDT relationship with the IFN-1 pathway. Tumor cells subjected to PDT showed phenotypic and functional DC maturation IFN-1 dependent outlining a novel photomodulated mechanism (Lamberti et al. 2019b). This novel danger signal released by cancer cells subjected to PDT could represent a possible ex vivo stimulated DC cultures as adoptive or personalized immunotherapy vaccines. Further research detail is required to categorize as clinical strategy in vivo.

Another approach concerning PDT-ICD inducer for DC-based vaccines was reported an orthotopic high-grade glioma (HGG) mouse model. A strong anti-HGG survival benefit was clinically relevant when vaccines were provided. Therefore, this preclinical evidence suggests that vaccination with ICD-stimulated DCs may be clinically translated for glioma treatment (Garg et al. 2016). Another promising study shows that the combination of chemo- and photo-therapeutic protocols stimulated DCs recruitment to form an in situ DC vaccine eliciting an inhibition of primary and distant tumor growth following a single intravenous injection (Yang et al. 2019).

In summary, there are increasing evidences that oxidative stress PDT-triggered should be considered as clinical strategy that permits an immunological antitumor environment.

Conclusion

In summary, depending on the context, ROS can have an antitumor or tumor promoter roles in carcinogenesis. Regulation of immune cell function plays a pivotal role for these opposed effects. Overall, it should be considered that the redox balance which include, among other factors, different ROS sources plus antioxidant intrinsic capacity of cellular and noncellular scavengers, instead of the absolute ROS level, when estimating the significances of oxidative stress in the immune stroma. Despite the fact that immune cells require a basal level of ROS for their proper functioning, TME-associated oxidative stress modulates their viability and/or activity. The impact of this "redox imbalance" relies in part on the differential cellular sensitivity to ROS. Taken together, the evidence here suggests that the basis of ROS-mediated immunosuppression in tumor microenvironment is the inhibition of those immune cells with antitumor functions (NK cells, $CD4^+$ and $CD8^+$ T-cells, immunogenic DCs) accompanied with enrichment of immunosuppressive Tregs in tumor niche (Fig. 3).

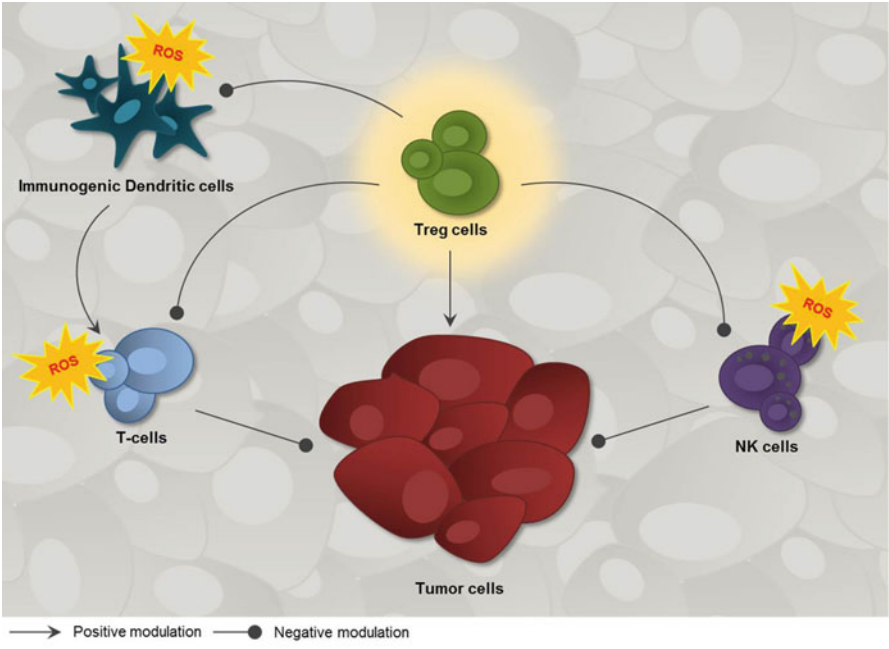

Fig. 3 ROS-mediated modulation of immune cells within tumor microenvironment. As illustrated in the review's text, ROS within tumor microenvironment acts as an immune escape mechanism by repressing the effect of antitumor effectors: NK cells, $CD4^+$ and $CD8^+$ T-cells, immunogenic DCs. On the other hand, Treg cells are recruited and enriched in tumor site and exhibit high resistance to excessive ROS. This population, by suppressing antitumor immune response, favors tumor progression

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