Review article

Redox control of cancer cell destruction

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ABSTRACT

Redox regulation has been proposed to control various aspects of carcinogenesis, cancer cell growth, metabolism, migration, invasion, metastasis and cancer vascularization. As cancer has many faces, the role of redox control in different cancers and in the numerous cancer-related processes often point in different directions. In this review, we focus on the redox control mechanisms of tumor cell destruction. The review covers the tumor-intrinsic role of oxidants derived from the reduction of oxygen and nitrogen in the control of tumor cell proliferation as well as the roles of oxidants and antioxidant systems in cancer cell death caused by traditional anticancer weapons (chemotherapeutic agents, radiotherapy, photodynamic therapy). Emphasis is also put on the role of oxidants and redox status in the outcome following interactions between cancer cells, cytotoxic lymphocytes and tumor infiltrating macrophages.

1. Introduction

Cancer represents the toughest challenge for modern medicine and is responsible for approximately 9 million deaths worldwide with more than 14 million new cases reported each year [1,2]. Therefore, understanding formation and spreading of cancer as well as mechanisms for developing therapy resistance are of crucial importance for the development of new effective treatments.

Most aspects of cancer biology display some degree of redox regulation. Carcinogenesis, cancer cell proliferation, migration, invasion, metastasis and vascularization all appear to be under redox control. Moreover, inflammatory cells in the tumor microenvironment may

Abbreviations: ABC transporters, ATP-binding cassette transporters; ADC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cancer cell phagocytosis; AML, acute myeloid leukemia; APE1, apurinic/apyrimidinic endonuclease 1; ASK-1, apoptosis signal-regulated kinase 1; CAF, cancer-associated fibroblast; CAR-T cells, Chimeric Antigen Receptor T-Cell; CLL, chronic lymphoid leukemia; CLA, cytotoxic lymphocytes; cPLA2, cytosolic phospholipase A2; CTL, cytotoxic T lymphocyte; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DARP, damage-associated molecular pattern; DC8F 2, 7-dichlorofluorescein; DCs, dendritic cells; DDB, Diphenyl Dimethyl Dicarboxylate; DDR, DNA damage response; Dox, doxorubicin; DUOX, nicotinamide adenine dinucleotide phosphate (NAPDH) dual oxidase; EGG, Epidermal growth factor; EGR, Epidermal growth factor receptor; GM-CSF, Granulocyte-macrophage colony-stimulating factor; GPx, glutathione peroxidase; GSH, glutathione; GST, Glutathione transferase; HDC, histamine decarboxylase; HMGB1, high mobility group box 1; HR, human estrogen receptor; IFN-γ, interferon-γ; IL-2, Interleukin-2; ILT, immunoglobulin like transcripts; IMC, immature myeloid cell; HMGB1, high mobility group box 1; iKB, inhibitor of nuclear factor kappa B; IOOH, lipid hydroperoxide; LPO, lipid peroxidation products; LPS, Lipopolysaccharide; MAP, mitogen-activated protein; MAPKK, mitogen-activated protein kinase; M-CSF, macrophage colony-stimulating factor; MDR, multidrug resistance; MDSC, myeloid derived suppressor cell; MHC-I, Major histocompatibility complex type I; MnTBAP, Mn(III)tetrakis (4-benzoic acid) porphyrin; NAC, N-acetylcysteine; NBD, macrophage; NAPDH, nicotinamide adenine dinucleotide phosphate; NCR, natural cytotoxicity receptor; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NK, Natural Killer cells; nNOS, Neuronal NOS; NO, nitric oxide; NOX, nitric oxide synthase; NOX1, nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase; NOO1, NADPH:quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; NSCLC, Non-Small Cell Lung Cancer; ONOO−, peroxynitrite; PARP1, Poly (ADP-ribose) polymerase 1; PBMC, Peripheral blood mononuclear cell; PDI, Programmed cell death protein 1; PDGF, Platelet-derived growth factor; PD-L1, Programmed death-1 ligand; PDC, Photodynamic therapy; PDE, pigment epithelium derived factor; PEG2, Prostaglandin E2; PEGF, phospholipid hydroxyperoxide glutathione peroxidase; P38K, Phosphatidylinositol 3-kinase; PEDF, pigment epithelium derived factor; PGE2, Prostaglandin E2; PGF, phospholipid hydroxyperoxide glutathione peroxidase; PI3K, Phosphatidylinositol 3-kinase; PK, pyruvate kinase; SO3, superoxide dismutase; TAMS, tumor-associated macrophages; TCR, T cell receptor; TGF-β, Transforming growth factor beta; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TRxR1, thioredoxin reductase 1; VEGF, Vascular endothelial growth factor

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produce superoxide, hydrogen peroxide and nitric oxide which impacts on both the cancer cells and the neighboring regulatory or effector immune cells. Many of these aspects of cancer biology have been extensively reviewed and therefore this paper focuses on the redox control of cancer cell destruction. Killing the cancer cells is the ultimate goal of both traditional therapies such as chemotherapy and ionizing radiation and of biological therapies such as checkpoint inhibitors (e.g. anti-PD1 and anti-CTLA-4 antibodies) [3], anticaner antibodies (e.g. against EGFR or Her antigens) [4,5] and adoptive cell therapies (e.g. with NK cells, cytotoxic T lymphocytes, T cells expressing chimeric antigen receptors; CAR-T cells) [6,7]. Other targeted treatment modalities; e.g., inhibitors of tumor vascularization (VEGF pathway inhibitors), tyrosine kinase inhibitors, hormone therapy indirectly also result in tumor cell death [8].

Redox signaling is known to affect the biology of tumors at multiple levels:

a) Redox signaling has a great impact on tumor cell proliferation. Signals through growth factor receptors (GFR) as well as integrins stimulate production of superoxide (O$_2^-$), which dismutates to hydrogen peroxide (H$_2$O$_2$) or production of H$_2$O$_2$, directly. These oxidants are produced by NADPH oxidases (NOXs) that are activated via largely overlapping pathways [9]. Stimulation of GFRs (e.g. epidermal GFR, insulin-like GFR, transforming GFR beta, platelet-derived GFR) by their specific growth hormones or ligation of integrins by extracellular matrix components trigger the Ras-Raf-Erk and the PI3K-Akt signaling pathways required for proliferation [10,11]. These signaling pathways also converge on NOXs that produce O$_2^-$ and H$_2$O$_2$ (mainly by growth factor receptors). Lipoxynegases, which produce lipid hydroperoxides among their products, are stimulated through integrins. Hydroperoxides produced by these sources stimulate receptor tyrosine kinases and inhibit protein tyrosine phosphatases, thus sensitizing cells to proliferation signals [12]. It should be noted that hydroperoxides and not superoxide, hydroxyl or other oxygen centered species function as a second messenger [13].

b) Even in non-transformed cells a small amount of oxygen is partially reduced by the mitochondrial electron transport chain resulting in superoxide production. Cancer cells may utilize a Warburg type metabolism; i.e., they rely on glycolysis for energy production even if oxygen is abundant (aerobic glycolysis). It has been proposed that the Warburg phenomenon aims to spare oxygen for the production of H$_2$O$_2$, that is used for redox signaling to promote tumor cell proliferation [14]. It has also been documented that defects in mitochondrial oxidative metabolism, as observed in cancer cells, may give rise to superoxide, hydrogen peroxide, hydroperoxide production and increased glucose utilization aims to provide reducing equivalents through NADPH and pyruvate necessary for metabolizing hydroperoxides [15,16].

c) Many cells in a tumor mass undergo cell death due to various factors including insufficient supply of oxygen and nutrients, attack by infiltrating cytotoxic immune cells and anti-cancer treatments (chemotherapy, irradiation and immunotherapy). Cells undergoing apoptotic or necrotic cell death overproduce H$_2$O$_2$ due to disruption of the mitochondrial electron transport system [17].

d) In relation to the previous point, tumor cells undergo repetitive ischemia-reperfusion cycles due to their irregular blood supply not always on a par with their ever increasing demand for oxygen and nutrients as required for rapid growth. e) The relationship between cancer and inflammation is complex. On the one hand, many (but not all) chronic inflammatory conditions predispose to cancer and, on the other hand, the tumor microenvironment is also characterized by varying degree of inflammation [18]. Infiltrating immune cells produce a plethora of cytokines and chemokines in the tumor fueling inflammation accompanied by the production of O$_2^-$ and hydroperoxides, and species derived from nitric oxide (NO), including peroxynitrite. NO is produced by nitric oxide synthases [19], one of which (eNOS/NOS3) is constitutive in cells and regulated through calcium and kinase signaling, a second (iNOS/NOS2) is regulated at the level of transcription, and a third (nNOS/NOS1) is both inducible and signaling regulated. NO is a well characterized second messenger that activates guanylate cyclase [20]. Signaling by peroxynitrite is controversial [21]. While H$_2$O$_2$, lipid hydroperoxides and NO are involved in signaling, other species, particularly in the presence of iron freed from proteins, can cause oxidative damage to macromolecules and disrupt cell integrity. The inflammatory tumor environment promotes tumor progression by increasing genetic instability, it stimulates metastasis and may also be involved in therapy resistance [22]. However, extensive cell death in the inflamed tumor environment (especially cell death involving oxidant-based ER stress, which occurs in response to certain therapies) may lead to release of tumor antigens. Uptake of tumor antigens by antigen presenting cells (dendritic cells and macrophages) results in antitumor adaptive immune responses (immunogenic cell death).

2. The cancer redox environment

2.1. Sources and types of oxidants in tumors

Oxygen and nitrogen centered oxidants, often called by the vague terms reactive oxygen and nitrogen species are formed by many cell types in the tumor microenvironment, including cancer cells, stroma cells, endothelial cells, innate and adaptive immune cells. As described above, the main species produced in tumors, as well as normal tissues, are superoxide, hydrogen peroxide, lipid hydroperoxides, NO and peroxynitrite. In tumors, production of these may be greater [23]. All cells generate O$_2^-$ and H$_2$O$_2$ as by-products of mitochondrial ATP generation in the electron transport chain. In addition, O$_2^-$ and H$_2$O$_2$ are produced in a regulated fashion by the nicotinamide adenine dinucleotide phosphate (DUOX). These families of transmembrane proteins comprise NOX1-5 and DUOX1-2 and their only known function is to produce O$_2^-$ and H$_2$O$_2$, some by one-electron and some by two-electron reduction of oxygen [8,24–26]. Membrane bound NOXs have been identified as major sources of oxidants in cancer. While NOX1 has been implicated mostly in the regulation of colon cancer cell proliferation, DUOX enzymes have been linked to the control of epithelial mesenchymal transition (EMT), invasiveness in cancer cell lines (DUOX1) and induction of VEGF and HIF-1α expression in pancreatic cell lines (DUOX2) [27,28]. NOX2 is, as will be discussed in a later section, highly expressed in myeloid cells but may also be expressed at lower levels by other cell types. For example EBV-infected gastric cancer cells have been shown to express enhanced NOX2 levels, which contributes to tumor progression [29] and NOX2-derived radicals have been suggested to contribute to Bim-induced apoptosis in small-cell lung cancer cell lines [30]. Recently NOX4 attracted increasing attention. NOX4 was found to localize to the perinuclear region, nucleus, endoplasmic reticulum, plasma membrane and also to the mitochondria [31–33]. NOX4 primarily generates H$_2$O$_2$ but mutation of a conserved histidine in its third extracyslosic loop (E-loop) can switch the protein to a superoxide generating enzyme [34]. The role of NOX4-derived oxidants in cancer is tumor context and therapeutic modality specific. For example, in renal cancer cells NOX4 has been found to localize to the mitochondrial inner membrane in an ATP bound inactive form [35]. NOX4 was activated upon ATP redistribution from mitochondria which led to metabolic reprogramming (a hallmark of cancer) and resistance to the anticancer drug etoposide. The underlying mechanism involved NOX4 derived oxidants which caused inhibition of acetylation-mediated lysosomal degradation of the “oncogenic” M2 splice variant of pyruvate kinase (PKM2) in etoposide-treated cells. These data identify NOX4 as a novel energetic sensor...
within the mitochondria, which serves as a checkpoint to couple mitochondrial energy metabolism to drug resistance in cancer cells [35]. Similar tumor promoting role of NOX4 has been reported in breast and ovarian cancer. These tumors overexpress NOX4 resulting in H2O2-dependent senescence, increased tumorigenicity and etoposide resistance [33]. The tumor promoting, prosurvival and antiapoptotic role of NOX 4 which is mediated by Akt kinase activation as demonstrated in pancreas adenocarcinoma cells [36,37] may also contribute to therapy resistance. In contrast, in head and neck cancer cells the EGFR tyrosine kinase inhibitor Erlotinib caused upregulation of NOX4 and down-regulation of NOX1,2,5. H2O2 produced by NOX4 was essential for Erlotinib-induced cancer cell death [38].

Another well examined enzyme that generates O2•- and H2O2 is xanthine oxidase, which catalyzes the oxidation of hypoxanthine to xanthine and ultimately to uric acid [39]. There are many other oxidoreductases in cells that can produce O2•- and H2O2, but many are confined to peroxisomes where the catalase concentration is very high. As mentioned above, nitric oxide is produced by nitric oxide synthase (NOS) enzymes with NOS2 (inducible NOS) and NOS3 (endothelial NOS) being the most important sources in tumors. No can react with superoxide to form peroxynitrite. The presence of this short-lived and highly reactive oxidant is evidenced by detection of its footprints in nitrotyrosine and 8-nitroguanine in proteins and DNA/RNA of tumors, respectively [40]. The tumor infiltrating myeloid cells, such as myeloid derived suppressor cells (MDSCs) and macrophages often constitute the major source of oxidizing species generated via the enzymatic systems. However, in different tumors the dominant source, the amount of oxidants produced and the role of these radicals for cancer progression or destruction may vary. Also, the applied anti-cancer treatments and antioxidant enzyme expression in the tumor microenvironment may affect redox status and outcome.

### 2.2. Redox sensors

While oxidants have been viewed for decades as cell damaging agents, hydroperoxides and nitric oxide are now widely recognized as unique signaling molecules. Their signaling role is mediated mostly by enzymatic oxidation of certain cysteine residues by H2O2 to either disulfides or mixed disulfides with glutathione resulting in altered protein structure and functional state [41]. These modifications are reversed by specialized reducing proteins glutaredoxins and protein disulfide reductases. For example in non-oxidatively stressed cells, one of the MAPKKK enzymes named apoptosis signal-regulated kinase 1 (ASK-1) is kept inactive by association with reduced thioredoxin. Upon formation of a disulfide bond in thioredoxin via peroxiredoxin-catalyzed oxidation of two critical cysteines by H2O2, the oxidized thioredoxin dissociates from ASK-1 permitting its oligomerization and activation [42]. H2O2 may also prolong MAP kinase signals by directly inactivating MAP kinase phosphatases via cysteine oxidation, again most likely through an enzyme catalyzed process [43]. Similarly, in growth factor signaling, oxidation of the phosphatase PTEN permits peptide growth factor (e.g. PDGF or EGF)-induced H2O2 production to mediate sustained proliferation signals [44].

Induction of certain cellular antioxidant enzyme systems also occurs via redox signaling mechanisms. The key regulator in this pathway is Nrf2 (nuclear factor erythroid 2-related factor 2) which is targeted for degradation via association with KEAP-1 (kelch-like ECH-associated protein 1). Upon oxidation of key cysteines in KEAP-1, it no longer can facilitate the ubiquitination of Nrf2 which in turn allows newly synthesized Nrf2 to translocate to the nucleus and bind to EpRE (also known by the misnomer antioxidant response element, ARE) in the promoters of antioxidant genes such as glutathione S-transferase, glutathione peroxidase subunits of glutamate-cysteine ligase and NADPH-quinone oxidoreductase 1 to induce their expression [45].

### 2.3. Cellular antioxidant systems

Tissue damage caused by excessive production of oxidants is prevented by antioxidant enzymes. In mammals, the former class include superoxide dismutases (SOD1 and SOD2), catalase, the glutathione peroxidases (GPX 1–8) and the peroxiredoxins (Prdx 1–6) [46]. SODs converts superoxide to H2O2 (and O2) while catalase, the glutathione peroxidases and peroxiredoxins reduce H2O2 to H2O. GPxs and Prdx6 use glutathione as the reducing substrate, Prdxs 1–5 use reduced thioredoxin, and catalase dismutates H2O2 to H2O and O2.

While all antioxidant enzymes have been linked to various aspects of cancer biology, SOD2 deserves special attention. On the one hand, many cancers downregulate SOD2 expression while, on the other hand, SOD2 overexpression has been shown to suppress tumorigenesis and cancer cell proliferation with hydrogen peroxide and nitric oxide being likely effectors [47]. In mouse embryonic fibroblasts the tumor suppressive effect of SIRT3 has been shown to be mediated by SOD2 overexpression pointing towards a role of mitochondrial superoxide in cell transformation in a single oncogene expression setting [48]. Mitochondrial superoxide also plays a central role in the anticancer effect of pharmacological ascorbate [49]. Ascorbate selectively induces mobilization of iron from Fe-S clusters and thus increases labile iron pool. Increased steady state levels of superoxide and H2O2 were commonly observed in many types of cancer and have also been implicated in the disruption of homeostatic iron metabolism; a finding also observed in tumor cell killing by pharmacological ascorbate. Under this condition SOD2 increased resistance of tumor cells to ascorbate-mediated cell killing. In different therapeutic settings, where the effects of the DNA alkylating agent and glutathione reductase inhibitor BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) was investigated, higher SOD2 activity was accompanied by higher toxicity. Combination of SOD2-mediated H2O2 production and impaired H2O2 removal due to glutathione peroxidase inhibition appeared to be a key factor in this toxicity [47].

In MCF7 cells superoxide was found to stabilize hypoxia inducible factor-1α (HIF1α), a positive regulator of transcription of genes responsible for angiogenesis, proliferation, survival and metastasis [50] and this pathway was inhibited by SOD2 overexpression.

There are thousands of compounds that have antioxidant chemistry in vitro and appear to have some antioxidant effect in vivo. Scavenging of oxidants is actually a competition between organic molecules, including both small metabolites, vitamins and macromolecules, because the rate constants for these reactions are usually very close to each other. For a molecule to be effective as oxidant scavenger in vivo, it would need to outcompete all other molecules [51]. This simply does not happen inside of cells except for vitamin E, which is concentrated in membranes where it reacts with hydroperoxyl radicals. Scavenging of superoxide, hydrogen peroxide and other hydroperoxides is carried out efficiently by enzyme catalyzed reactions with rate constants that are 100,000 times faster than for the non-enzymatic reactions. Thus, among the naturally occurring metabolites and vitamins, with the exception of vitamin E, there is no physiologically meaningful scavenging by non-enzymatic reactions. The only other significant antioxidant effect of small molecules are chelators that tie up iron so that it cannot catalyze the production of hydroxyl radical, and inhibitors of NOX, NOS and other oxidoreductases, as inhibitors of production rather than scavengers of oxidants; e.g., NOX2-inhibitors [52], NOS inhibitors, xanthine oxidase inhibitors) or induce antioxidant defenses (e.g. Nrf-2 activators) [53]. In cells, the dominant cellular “antioxidant” molecule is glutathione, which is used by GPxs and Prdx6 rather than as a direct scavenger of any oxidant.

### 3. Oxidative stress induced by conventional anticancer therapy (ionizing radiation, chemotherapeutic agents, PDT)

Conventional anticancer therapy includes the use of chemotherapeutic agents, radiotherapy or photodynamic therapy. Hydroxyl
radical and singlet oxygen production are common mechanisms of many therapeutic approaches for cancers (photodynamic therapy, radiotherapy, doxorubicin) and is also responsible for many of their side effects. These approaches involve the production of species that are associated with damage rather than redox signaling. Of course, damage itself is recognized by cells and stimulates signaling that may include redox signaling.

3.1. Radiotherapy

Radiotherapy is given for eliminating tumor cells (curative treatment) or for relieving the symptoms of patients (palliative treatment). It can be applied internally (with a radioactive material) or externally (high-energy x-rays at the affected area). Radiotherapy utilizes high-energy rays and its main cellular target is DNA [54]. Single and double strand breaks, crosslinks between DNA strands and chromosomal proteins are induced. While electromagnetic radiation ionizes indirectly through inducing the formation of hydroxyl radicals, heavy particles such as alpha particles [55] and carbon ions [56] can cause DNA damage directly, hence with higher biological effectiveness. Alpha particles have relatively short range (≤100 μm) resulting in partial tumor irradiation and limited killing. **222**Radium and many other newly developed alpha emitters may offer the potential for targeted therapy via conjugation with specific antibodies or targeted nanoparticles [57]. Carbon ion has quite favorable physical and biological properties for cancer therapy [56]. It provides a sufficient radiation dose to the tumor, while causing acceptable damage in the surrounding normal tissues. Adenocarcinoma, adenoid cystic carcinoma, malignant melanoma, hepatoma, and bone/soft tissue sarcoma respond favorably to carbon ion radiotherapy. Radiation induces tumor cell death or permanent cell cycle arrest [58]. It destroys cancer cells, but may also damage normal cells [59]. However, compared to non-transformed cells, DNA repair in cancer cells is often defective making them more vulnerable to radiotherapy.

3.2. Chemotherapy

Classical chemotherapeutics can be classified as alkylating agents, antimitabolites, topoisomerase inhibitors and anti-microtubule agents. **3.2.1. Alkylating agents**

By definition, alkylating agents are compounds capable of replacing a hydrogen atom in another molecule by an alkyl radical. Alkylating agents (cyclophosphamide, ifosfamide, chlorambucil) can be toxic, mutagenic, carcinogenic or teratogenic at different doses. They decrease the rate of cell division, and may cause breakage and other abnormalities of chromosomes [60]. Alkylating agents interfere with DNA replication by crosslinking DNA strands, causing DNA strand breaks and abnormal base pairing. They tend to be more effective against rapidly dividing cells. In particular, impairment of replicative fidelity of DNA during the S-phase could contribute to some of the mitotic and chromosomal effects, as well as to their carcinogenic and teratogenic potencies [60]. The nitrosoureas are a subgroup of the alkylating agents interfering with DNA replication and repair. Platinum-containing compounds include agents such as Cisplatin, Carboplatin and Oxaliplatin and their cytotoxic properties also extend to alteration of the cell membrane transport systems and suppression of mitochondrial function [61]. Cisplatin is used to treat various types of cancers such as testicular, lung, and ovarian cancers. This drug exhibits multiorgan toxicity with redox imbalance as a possible mechanism [62,63].

**3.2.2. Topoisomerase inhibitors**

Topoisomerases I and II can relax DNA supercoiling (e.g. during replication or transcription) by breaking and rejoining the backbone of DNA strands. They also play a significant role in fixing DNA damage that occurs as a result of exposure to harmful chemicals or UV rays. Inhibitors of topoisomerase I (e.g. topotecan, camptothecin) and topoisomerase II (e.g. etoposide, doxorubicin) work by binding to the topoisomerase enzymes and blocking DNA religation after strand cleavage [64,65].

3.2.3. Anti-microtubule agents

Tubulins proteins form spindle fibers (also called microtubules) which is essential for cell division. Vinca alkaloids (Vinblastine, Vincristine) bind to tubulin, inhibiting cytoskeletal dynamics [66].

3.2.4. Antimetabolites

Antimetabolites (methotrexate, 5-fluorouracil and cytosine arabinoside) act as analogs of nucleotides interfering with DNA and RNA synthesis. Most of these agents are specific for S phase, therefore they are mostly effective against fast-growing tumors.

3.2.5. Epigenetic reprogrammers

In the pathogenesis of various hematological malignancies (e.g. myelodysplastic syndrome and different forms of leukemias) a critical event is the silencing of tumor suppressor genes via hypermethylation of their promoters. The cytosine analogs 5-azacytidine and 5-aza-2’déoxycytidine (also known as decitabine) can inactivate DNA methyltransferase [67] and can thus restore the activity of suppressed genes.

3.3. Photodynamic therapy (PDT)

Photodynamic therapy (PDT) utilizes the combination of light and photosensitizing drugs [68]. When exposed to light with a specific wavelength, photosensitizers (e.g. porphyrin sodium) produce singlet oxygen. Singlet oxygen is a dienophile that attacks histidine in proteins and bases in DNA among other targets. The wavelength determines how far the light can travel into the body. Generally, the light needed to activate most photosensitizers cannot pass through more than 1 cm. The photosensitizing agent (given systemically) accumulate in the tumor cells. The tumor is exposed to the light 24–72 h after the injection, when most of the drug is eliminated from the normal cells. PDT can act in two additional ways as well: it can activate the immune system to kill tumors or it can inhibit the metabolism of the tumors by damaging their blood vessels. PDT is a local treatment (typically on or just under the skin) and generally cannot be used to treat metastasized cancer, but can be combined with other therapies. As laser light can be directed through optical fibers, it can deliver light to the inside of the body, so that esophagus or lung cancers can also be treated. Singlet oxygen reacts with the polyunsaturated fatty acids of the membranes, producing lipid hydroperoxides (LOOHs) [69]. The effectiveness of PDT depends on various factors. The addition of ascorbate and ferrous iron increase the cytotoxic effect of PDT by further augmenting lipid peroxidation and the conversion of LOOHs to more cytotoxic species [69]. It was reported [70] that singlet oxygen generated by photodynamic treatment can inactivate cellular antioxidant enzymes (catalase, SOD1 and SOD2) in nucleated mammalian cells, which can contribute to cytotoxicity. Elevated phospholipid hydroperoxide glutathione peroxidase (PhGPx) enzyme activity, on the other hand, could contribute to the resistance of tumor cells to PDT [71].

3.4. Oxidative stress induced by conventional cancer therapy

In radiotherapy and PDT singlet oxygen production is in the very heart of their mechanism of action (see above). The anthracycline doxorubicin (Dox) may generate oxidants by more than one mechanism [72,73]. Dox itself can be converted by mitochondrial reductions to anthracycline semiquinone free radicals. Under aerobic conditions, they are able to reduce molecular oxygen to O2· and H2O2 [74]. Reactions between iron and Dox can also generate hydroxyl radical through the Fenton reaction. A dose-dependent cardiotoxicity is a
well-known side effect of Dox therapy and is a major limitation to its use. Cardiotoxicity is partly caused by doxorubicin-dependent free radical formation, lipid peroxidation and mitochondrial dysfunction [75]. The alkylating agent cyclophosphamide is widely used to treat ovarian, breast, and hematological cancers. An adverse effect of cyclophosphamide chemotherapy is a reductive production of free radicals, which can be inhibited by curcumin [92]. Curcumin protected against the toxicity of cisplatin, which causes mitochondrial caspase 3 activation and apoptosis. Downregulation of HIF1α, PKB (Akt), ERK and activation of NFκB was detected in MDR cells [93]. Huang et al. [94] could selectively target cancer cells with estrogen derivatives, which caused apoptosis in human leukemia cells but not normal lymphocytes. The selectivity was found to be based on the inhibition of SOD1 and SOD2. Overproduction of catalase (or application of its analogs) combined with chemotherapeutic drugs was also found to suppress the proliferation and aggressiveness of lung cancer [95].

Human AP endonuclease is an essential enzyme of base excision repair, but also acts as a redox signaling factor. Silencing of APE1/Ref1 in A2780 and CP70 cell lines resulted in increased apoptosis [96]. Illyasova et al. [97] found at least two different types of redox homeostatic mechanisms balancing oxidative stress in humans, which predispose to drug resistance and toxicities during doxorubicin and cyclophosphamide chemotherapy.

4. Redox regulation of cancer cell killing by cytotoxic T lymphocytes and natural killer cells

Tumors that show a dense infiltration of immune cells are regarded as “hot” while tumors containing few immune cells are regarded as “cold”. In many cancers, the hotter the tumor the better are the patient’s chances [100–102]. However, the relation between types of infiltrating immune cells are of relevance for the outcome. In the fight against cancer, cytotoxic lymphocytes (CTLs) represent the most powerful soldiers in the army of the cellular immune system [103,104]. On the contrary, tumor infiltrating macrophages and myeloid derived suppressor cells (MDSCs) often represent poor prognostic markers [104]. Hence, in several cancers, including lung cancer [105], bladder cancer [106], glioblastoma [107] and renal cell carcinoma [108], a high ratio between tumor infiltrating T cells and myeloid cells prognosticates a favorable outcome [104]. The reason for the negative impact of tumor infiltrating myeloid cells is assumed to be the suppressive factors, including oxidants that are produced by myeloid cells and inhibit the cytotoxic functions of CTLs. However, all myeloid cell infiltrates are not disadvantageous. As discussed in more detail below certain types of macrophages may inhibit tumor growth and dendritic cells that also represent a myeloid cell type are necessary for proper tumor-specific T cell responses to evolve.

In the following section, we will summarize how the tumor redox environment affect CL-mediated killing of cancer cells. Though cytotoxic lymphocytes are sensitive to excessive levels of oxidants that trigger inactivation and apoptosis, low levels of oxidants are needed for the lymphocytes to exert their cytotoxic functions.

4.1. CL-induced cytotoxicity is accompanied by oxidant production

CD8 positive cytotoxic T lymphocytes (CTLs) express T cell receptors (TCRs) and recognize tumor antigen peptides associated with MHC-I (major histocompatibility type I) cell surface proteins (Fig. 1). Thus CTL-mediated tumor cell killing is antigen-specific and requires expression of MHC-I proteins by the tumor cells. Natural killer (NK) cells, on the other hand, are more effective against cancer cells with defective expression of MHC-I molecules. Hence, recognition of tumor cells by NK cells does not require the presentation of antigens on MHC-I, but instead the NK cells interact with a wide range of activating and inhibitory receptors such as the natural cytotoxicity receptors (NCRs), killer immunoglobulin-like receptors (KIR), C-type lectin receptors and immunoglobulin like transcripts (ILT) (Fig. 1) [109]. Since NK cells express Fc receptors (recognizing the variable Fc region of immunoglobulins) they can also bind tumor cells via tumor-bound antibodies (e.g. therapeutic antibodies such as anti-EGFR or anti-Her Ab)
Chemotherapeutic agents induce oxidants, alter antioxidant systems
cervical cancer, 5-fluorouracil, followed by cisplatin and bleomycin
in vivo, human
The alterations in the circulating pro/antioxidants in advanced cervical
cancer patients were investigated, before and after neoadjuvant chemotherapy. The
pretreatment levels of “antioxidants” and oxidants and also the extent of their
change during treatment can predict the therapeutic response to neoadjuvant
chemoradiation in advanced cervix cancer [86].
lung cancer, cisplatin
in vivo, human
Oxidative stress was detected after cisplatin based combination chemotherapy
induced in NSCLC patients. The pretreatment levels of LPO and NO in NSCLC
patients were significantly higher while GSH and SOD were significantly lower,
compared to control. A higher elevation of oxidative stress was detected after the
chemotherapy and was more evident in higher stage than lower stage
patients [87].
non-small cell lung cancer patients, cisplatin + etoposide
in vivo, human
Oxidative stress markers (LPO and NO) and antioxidant levels (GSH and SOD)
were investigated in control and in NSCLC patients, before and after cisplatin +
etoposide combination chemotherapy. In responders LPO and NO were low
while GSH and SOD were high [88].
keratinocyte apoptosis, Doxorubicin Mitochondrial superoxide
in vitro (HaCaT)
Doxorubicin induces keratinocyte apoptosis. Mitochondrial superoxide can
mediate the apoptotic process through the oxidative modification of ERK and
Bcl2 ubiquitination [91].
human NSCLC (non-small cell lung cancer) cell lines, MCF-7 cells, A549
cells, MDA-MB triple negative breast cancer cells, MiaPaCa2
orthotopic xenografts
in vitro and in vivo (mice)
β-lapachone undergo redox cycling-dependent bioactivation by NAD(P)
H-equinone oxioreductase 1 (NOQ1) which is accompanied by H2O2 production.
Subsequent DNA breakage and PARP1 activation depletes NAD+ /ATP pools
culminating in necrotic cell death. Combination of β-lapachone with the PARP
inhibitor rucaparib cause synergistic cell death by apoptosis [85].

Non-traditional treatments for the elimination of cancer cells that utilize oxidative stress
prostate cancer, Sodium selenite
in vitro
Human prostate cancer cells were treated with sodium selenite. Upon treatment,
mitochondrial-dependent superoxide production was detected, that was at least
partly responsible for the induction of apoptosis [89].
MDR cancer cells, NO
in vitro
Bifendate (DDB) nitric oxide, a synthetic nitric oxide releasing compound,
effectively decreased viability of both sensitive and MDR tumor cells. The
proposed mechanism includes mitochondrial tyrosine nitration and apoptosis on
the one hand, and HIF1α downregulation and the phosphorylation (activation)
of PKB (AKT), ERK, and NFκB in MDR cells on the other hand [90].

Intervention with endogenous antioxidant systems enhance tumor killing by chemotherapeutic agents
doxorubicin, selenium compounds, and D-pantethine
in vitro and in vivo study
Protective effect of specific agents (sodium selenite, selenomethionine, D-
pantethine) during cytotoxic action of doxorubicin was demonstrated in vitro in
drug-sensitive human tumor cells and in adult male Wistar rats. In contrast, was
no protective effect could be detected in drug-resistant sublines [90].
Glutathione transferase overexpressing cancer cells, doxorubicin
derivatives
in vitro
GSTs are often overexpressed and TrxR1 is often upregulated in tumors and
frequently correlated to bad prognosis and resistance against a number of
different anticancer drugs. These cells could be selectively targeted with drug
derivatives, incorporating a sulfonamide moiety (ANS-etoxydox, ANS-DOX)
[92].
head and neck squamous cell carcinoma, rat model of cisplatin-induced
ototoxicity, cisplatin, curcumin
in vitro, in vivo
Cisplatin has an ototoxic side effect. The modulating effect of curcumin was
investigated in the rat model of cisplatin-induced ototoxicity, and in head and
neck squamous cell carcinoma cells. Curcumin attenuated all stages of tumor
progression (survival, proliferation) and, by targeting pSTAT3 and Nrf2
signaling pathways, curcumin sensitized cells to cisplatin in vitro and protected
from its ototoxic adverse effects in vivo [91].
human leukemia cells, SOD, selective tumor killing
in vitro
Certain estrogen derivatives selectively kill human leukemia cells but not
normal lymphocytes. Superoxide dismutase (SOD) was identified as a target of
this drug action and show that chemical modifications at the second carbon (2-
OH, 2-OCH3) of the derivatives are essential for SOD inhibition and for apoptosis
induction [94].
lung cancer cells, catalase, cisplatin chemotherapy
in vitro
In lung cancer cells, combining Catalase (or CAT analogs) with traditional
chemotherapeutic drugs, especially cisplatin, was found to be a promising
therapeutic strategy. The overexpression of the antioxidant enzyme catalase
(CAT) might control tumor proliferation and aggressiveness [95].
A2780 and CP70 cell lines, platinum based chemotherapy APE1/Ref1
inhibitor
in vitro
In patients not responding to platinum based chemotherapy, altered levels and
subcellular distribution of APE1/Ref1 expression was found comparing with those
who responded to platinum based chemotherapy. In A2780 and CP70 cell
lines APE1/Ref1 silencing resulted in increased apoptosis after platinum based
chemotherapy [96].

breast cancer patients, urine samples, Doxorubicin, Cyclophosphamide
chemotherapy
in vivo, human
Urine samples of breast cancer patients show, that there are differences in the
redox homeostatic control between cancer patients. These differences may
underlie predisposition to drug resistance and toxicities. There may be at least
two distinct redox phenotypes with different homeostatic mechanisms balancing
oxidative stress in humans [97].

human head and neck cancer cells (FaDu cells)
in vitro
If combined with cisplatin, 2-deoxy-glucose increases the steady-state levels of
H2O2 and enhances the disruption in thiol metabolism, leading to increased
oxidative stress and increased cell killing [99].
oxidants in cancer cell killing or treating breast cancer cells with the combination of the membrane function. Evans et al. [115] modelled CL-mediated cytotoxicity by protease granzymes unleash apoptotic and non-apoptotic cell death include FAS-ligand, TRAIL (TNF-related apoptosis-inducing ligand), through mannose-6-phosphate receptors or via electrostatic linkage to these pores. Alternatively, granzymes can also enter target cells according to the widely held but experimentally not fully proven view, the other powerful weapons of CLs, granzymes can enter cancer cells resulting in ADCC (antibody-dependent cell-mediated cytotoxicity) [110]. Thus, ADCC is of special importance for antibody-based cancer immunotherapies.

Although NK cells and CD8+ CTL cells recognize target cells through different receptors and operate different activation pathways, their effector functions are basically identical. One of the most potent cytotoxic mechanisms of CLs (cytotoxic lymphocytes: CTLs and NK cells) is mediated by perforins [111]. In resting CLs perforin is localized to the cytotoxic granules and upon activation it is released by exocytosis. Secreted perforin undergoes calcium dependent polymerization to form cylindrical pores of 5–20 nm in the target cell membrane. According to the widely held but experimentally not fully proven view, the other powerful weapons of CLs, granzymes can enter cancer cells through these pores. Alternatively, granzymes can also enter target cells through mannose-6-phosphate receptors or via electrostatic linkage to the target cell membrane [112]. Once inside the tumor cells, the serine protease granzymes unleash apoptotic and non-apoptotic cell death pathways [113].

Both CTLs and NK cells utilize cell surface death ligands to induce programmed cell death in the target cells [114]. These death ligands include FAS-ligand, TRAIL (TNF-related apoptosis-inducing ligand), TNFs and they act through death receptors belonging to the TNF receptor superfamily.

Oxidants have a dual role in the regulation of CTL and NK cell function. Evans et al. [115] modelled CL-mediated cytotoxicity by treating breast cancer cells with the combination of the membrane damaging, pore forming protein streptolysine O (to mimic perforin) and granzyme B and reported oxidant production accompanying cancer cell death (Table 2). The crucial role of oxidants in cancer cell killing or sensitization was indicated by the observation that expression of the antiapoptotic XIAP protein (which rendered tumor cells resistant to PBMC-induced ADCC) suppressed oxidation of the dye, 2',7-di-chlorodihydrofluorescein (DCFH). Oxidation of DCFH is often, although falsely, thought to be a measure of H2O2 production [116]. Nevertheless, DCFH oxidation often correlates with oxidative stress.

The inhibitory effect of MnTBAp, which scavenges both O2− and H2O2, on ADCC reaction provided further support for the active role of oxidants in CL-mediated cancer cell destruction [115]. A possible mechanism underlying the sensitizing effect of oxidants to CL-mediated killing of cancer cell may be the upregulation of NK cell activating molecules on the surface of cancer cells. In multiple myeloma cells, subtoxic concentrations of melphalan or doxorubicin induced the DNA damage response (DDR) and caused cell senescence [117]. Senescent myeloma cells upregulated ligands (MICA, MICB and PVR) for NK cell activating receptors NKG2D and DNAM1 in an oxidant-dependent manner resulting in enhanced NK cell activation [118].

4.2. Effects of tumor-associated inflammatory stress and therapy-induced oxidant production on CLs

The relationship between cancer and inflammation is complex [119]. It has been well established that inflammation is a common feature of most solid tumors. Chronic inflammation has a predominant role in tumor survival and proliferation, angiogenesis and immunosuppression [120]. As detailed above, cancer cells also produce H2O2 and use it for proliferation signaling. Moreover, conventional cancer therapy with chemotherapeutic agents, ionizing radiation, photodynamic treatments or therapeutic antibodies are all known to induce oxidant production [62,64,68,72]. But, it is important to remember that low level production of H2O2 is involved in enzyme-cat-
alyzed redox signaling, while production of high levels of H2O2 or production of hydroxyl radicals by radiation or singlet oxidation in photodynamic therapy causes indiscriminant damage. Also therapeutic antibodies, such as the anti-CD20 antibody rituximab that is used in the treatment of chronic lymphoid leukemia (CLL), triggers oxidant production by interacting with the Fe-receptor of myeloid cells [121]. The myeloid derived oxidants decrease the capacity of NK cells to exert ADCC against antibody bound tumor-cells. Treatment with NOX2-inhibitors or H2O2 scavengers restored the NK cell-mediated ADCC activity in cocultures between CLL cells, NK cells and monocytes [121].

On a similar note, CTLs equipped with engineered T cell receptors (CAR-T cells = chimeric antigen receptor-redirected T cells) that co-expressed catalase were protected from hydrogen peroxide stress and maintained high tumor killing activity indicating that hydrogen peroxide contributes to T cell anergy [122].

While performing their tasks, CLs are constantly exposed to oxidants and strategies to reduce the oxidative stress have been proposed to enhance the ability of CLs to kill tumor cells. However, activated CLs may partly adapt to the oxidatively stressed tumor environment by upregulating antioxidant proteins (e.g. peroxiredoxin 1 and thioredoxin 1) as demonstrated with IL-2-activated NK cells [123]. Also, DCs have been shown to provide antigen-specific T cells with antioxidative thiols during antigen presentation, which made them more resistant to oxidi-
anted-induced apoptosis [124].

The inflammatory and redox environment may differ significantly between individual tumors and between different parts of the same tumor mass. In general, macrophages and MDSCs play a central role in creating an inflammatory environment in the tumors (see below), that other tumor-infiltrating lymphocytes such as CTLs and NK cells are also exposed to and have to cope with. One of the suppressive features of macrophages and MDSCs is the production of O2− and H2O2 via the NADPH oxidase NOX2. The NOX2 enzyme is highly expressed in cells of the myeloid lineage, such as monocytes/macrophages and neutrophils [125]. While NOX2-derived H2O2 is critical for these cells to eliminate microbes during infections, the high localized concentration of H2O2
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that may be achieved via NOX2 and has also been linked to immunosuppression in cancer [126–128]. Hence, in a confined inflammatory site, NOX2-derived H₂O₂ triggers dysfunction and apoptosis of adjacent cytotoxic lymphocytes, including cytotoxic T cells and NK cells [126–129]. Genetic disruption of Nox2 has in mouse models been shown to reduce melanoma metastasis formation by protecting tumor killing NK cells from oxidant-induced inactivation [52]. Also Nox2⁻/⁻ mice showed a reduced growth rate of subcutaneous melanoma and lung carcinoma, but sarcoma growth and prostate cancer growth were not affected [130,131]. Pharmacological inhibition of NOX2 by histamine dihydrochloride (HDC) is, together with low doses of IL-2, utilized as a relapse-preventive strategy for acute myeloid leukemia (AML) patients in complete remission [132,133]. The proposed mechanism of action for HDC is to protect anti-leukemic lymphocytes from drug-induced ligand upregulation on senescent MM cells [117].

Table 2
Redox regulation of the antitumor functions of natural killer cells, cytotoxic T lymphocytes and lymphokine-activated killer cells. Oxidants may be produced and may even contribute to perforin and granzyme B-induced cancer cell killing. On the other hand, tumor-associated inflammatory stress and therapy-induced oxidant production may compromise the tumor killing effect of CLs.

| NK/CTL/LAK | Tumor model/context | In vitro/vivo | Findings |
|------------|---------------------|---------------|----------|
| human NK   | triple combination therapy with bortezomib, oHSV, and NK cells | in vitro human and in vivo mouse xenograft | • Combination treatment with bortezomib and oHSV, induced necroptotic cell death and increased the mitochondrial H₂O₂ and JNK phosphorylation production. |
| human NK   | myelogenous leukemia/general cell mechanism/oxidative stress | in vitro human | • IL-2 NK and expanded NK are more resistant to H₂O₂ than resting NK. |
| human NK   | human melanoma/NK cells | in vitro human | • During NK-mediated tumor cell killing (in vivo mouse xenograft) | • PRDX1 and TXN1 are upregulated in activated NK cells |
| primary NK | MCF7 (breast cancer), A549 (lung carcinoma), MDA-MB-231 (breast adenocarcinoma), U937 (monocytic leukemia) | in vitro human | • IL-2 confers protection on NK cells against oxidative stress mainly by up-regulation of TXN1 [123] |
| transduced T cells/NK cells | Her2 + Shv3 cells and Her2-specific CAR-transduced T cells K562 (Human myeloid leukemia cell line) were used as targets for NK cells | in vitro human | • CAR-T cells (Chimeric Antigen Receptor coexpressing catalase) protect in trans both T and NK cells from oxidative stress-mediated repression. |
| Human CD3+/CD56 + NK cells | chronic lymphocytic leukemia (CLL) CD14+ monocytes | in vitro human | • CAR-T cells display an increased inhibition of intrinsic oxidant production upon T cell activation |
| CD8+ Cytotoxic T lymphocytes (CTL) | nanogels for cancer vaccine delivery to dendritic cells | in vivo human | • CAR-T cells maintain their activity under H₂O₂ stress |
| Cytotoxic T lymphocytes (CTL) | HLA-A2+ human melanoma CTL homeostasis | in vitro human | • CAR-T cells mediate a protective bystander effect [122] |
| PBMC | human | in vitro human | • Inhibitors of oxidant formation preserved NK cell viability and restored NK cell-mediated ADCC [121]. |
| Activated PBMC | parental IBC cell lines Inflammatory breast cancer (IBC) HER2 resistance | in vitro human | • Superoxide production increases upon TCR (T-cell receptor) stimulation with the cognate epitope |
| mature plasma cells (PCs) in BM | chemotherapeutic stress on cancer cells promote antitumor immune responses in MM (multiple myeloma) cells | in vitro human | • Inhibition of oxidant production rescues CTL from AICD (Activation-induced cell death) without impairing their effector functions |
|                          |                                  |               | • Antigen-reactive primary CTL and TIL (tumor infiltrating lymphocytes) escaped AICD when treated with MnTBAP (SOD and catalase mimic) [143] |
|                          |                                  |               | • Treatment with ox-LDL induced a significant down-regulation of proliferative response to mitogens, antigens and interleukin-2 in PBMC. |
|                          |                                  |               | • NK cell-mediated cytotoxic activity was significantly down-regulated by ox-LDL while treatment with N-acetylcycteine (NAC), a precursor for cysteine used in glutathione biosynthesis, induced a significant up-regulation of NK-cell activity. |
|                          |                                  |               | • Ox-LDL and NAC exerted opposite effects on the cytokine network [144]. |

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mechanism to cause T cell unresponsiveness or T cell apoptosis, and are reportedly more suppressive compared to granulocytes and monocytes from healthy subjects [134]. Granulocytic MDSC produce peroxynitrite (via combination of NOX2-derived superoxide and eNOS-derived NO) to induce T cell unresponsiveness (due to nitration-mediated T cell receptor inactivation) and T cell apoptosis [135-137]. Monocyctic MDSC express iNOS, generate NO but their T cell suppressing effect doesn’t appear to require peroxynitrite formation and may be due to a direct effect of NO [137,138].

5. Redox regulation of interactions between cancer cells and macrophages

5.1. Tumor associated macrophages: enemies within or potential anticancer weapons?

Macrophages (Mφ-s) are part of the innate immune system and together with monocytes and dendritic cells they comprise the mononuclear phagocyte system. While in most organs, tissue resident macrophages populate the organs prenatally, in the gut and skin circulating blood monocytes significantly contribute to the macrophage pool [145,146]. Under inflammatory conditions, however, circulating monocytes can also enter most tissue niches and differentiate to macrophages upon exposure to CSF1 (M-CSF) and GM-CSF [147].

Macrophages are also among the first host cells infiltrating the tumor mass [119]. But their role in the tumor environment is a classic case of a double-edged sword situation. On the one hand, Mφ-s have the potential to kill cancer cells. However, the presence and high number of Mφ-s in the tumor tissue is widely recognized as a negative prognostic marker. This is especially true in breast, head and neck, mesotheliom, thyroid, liver, pancreas, kidney, bladder, ovarian, uterus, and cervix cancer as well as in glioma, melanoma and non-Hodgkin lymphoma while in colorectal cancer, high macrophage density was correlated with increased patient survival [148]. Thus the question arises whether macrophage-induced tumor killing is only an in vitro phenomenon which is not relevant in tumors or the cytotoxic activity of Mφ-s is “real” but suppressed in tumors.

Traditionally Mφ-s have been viewed as cells capable of destroying cancer cells. Macrophages activated in vitro with interferon gamma, LPS, glycans etc. display tumor cell killing activity. Although unlike T cells, Mφ-s are not equipped with specific antigen recognition receptors, they are still capable of binding to tumor cells (Fig. 2). To recognize cancer cells Mφ-s utilize – among other molecules - calreticulin binding receptors [149]. In damaged tumor cells (e.g. after chemotherapy), the ER protein calreticulin translocates to the plasma membrane [150] where it can bind to the Mφ-s cell surface protein CD91. Other tumor-derived DAMPs (damage-associated molecular patterns) such as ATP, HMGB1, nucleic acids also activate Mφ-s via different TLRs (HMGB1 and nucleic acids) [151] and purinoceptor P2X7R (ATP) [152]. Mφ-s can phagocytose bound tumor cells. In addition to calreticulin-CD91 interactions, cancer cell phagocytosis by macrophages is also facilitated by opsonization of cancer cells by antibodies (e.g. therapeutic antibodies such as Herceptin). ADCP (antibody-dependent cancer cell phagocytosis) leads to processing and presentation of tumor-derived antigens triggering antitumor T cell responses and thus lays the foundation for the adaptive immune response [153].

Despite possessing a wide array of potentially cytotoxic mechanisms, Mφ-s appear to be one of tumors’ best friends. Recruitment of monocytes to cancer is mediated by tumor-derived chemokines (e.g. CCL2) and cytokines (CSF-1) [154]. Depending on the composition of the Mφ-s environment, Mφ-s may exist in many functional states. Although these polarization pathways most likely represent a continuous spectrum, they are often characterized by the extremes of these continuums: i.e. M1 and M2 Mφ-s [155]. IFNγ and TNFα stimulate polarization towards M1 (inflammatory) Mφ-s, while IL4 and IL13 (and tumor-derived CCL2, CSF1 and IL10) initiate M2 polarization (Fig. 3) [156]. (Markers of M1 and M2 Mφ-s differ greatly between human and mouse and are summarized in [157].) M1 macrophages produce O2−, H2O2, and NO and the cytotoxic cytokine TNFα and can thus keep tumor cells under control. However, due to exposure to IL4, IL13 and IL10, the typical phenotype of TAMs (tumor-associated macrophages) is M2, often referred to as anti-inflammatory, remodeling or “wound healing” Mφ-s. M2 cells express T cell inhibitory PD-L1 and produce T cell suppressing mediators (TGFβ, and PGE2) [158]. Thus, the role of Mφ-s in the tumor microenvironment is to mediate immunosuppression and thus protect cancer cells from attack by cytotoxic effector cells [159]. In certain cancer types (e.g. melanoma, colorectal and gastric cancer), however, Mφ polarization is skewed towards the M1 state and infiltration of these tumors with Mφ-s is recognized as a positive prognostic marker [160-162].

Fig. 2. Recognition of tumor-associated molecular patterns by macrophages. Surface bound antibodies, externalized calreticulin or released nucleic acids or ATP can modify macrophage phenotype via interactions with specific cell surface receptors.

Fig. 3. Macrophage polarization. Exposure of M0 macrophages to IL-4, IL-13 and IL-10 induces differentiation towards the M2 phenotype. Stimulation by LPS and IFNγ induces M1 polarization. While M2 macrophages promote cancer cell growth, M1 macrophages are potentially cytotoxic to cancer cells.
5.2. Redox control mechanisms of MΦ-tumor cell interactions

The encounter of MΦ-s and cancer cells may involve antibody-dependent cellular phagocytosis/trogocytosis and consequent cytotoxicity (ADCP and ADCC, respectively). MΦs utilize their cell surface Fc receptors to bind tumor associated antibodies (e.g. produced by the humoral anti-tumor immune response or administered to the patient as a therapeutic antibody). This step can be followed by phagocytosis of the tumor cells (ADCP) [163] or MΦs biting out pieces of the tumor cells’ plasma membrane (trogocytosis) [164]. The outcome of antibody-mediated MΦ responses against tumor cells can lead to tumor cell destruction (ADCC) via production of cytolytic proteases, oxidants or TNFα [165].

MΦs are prototypical O2−, H2O2, and NO producing cells and oxidants represent one of the most potent weapons of activated MΦ-s in the combat against cancer [166,167]. While monocytes preferentially produce O2− and H2O2, MΦ-s predominantly use peroxynitrite [168] for in vitro tumor killing. Monocytes and MΦ-s operate NADPH oxidase (NOX) enzymes for superoxide production. NOX2, the dominant NOX in MΦ-s is a multisubunit protein and its components are localized in the cell membrane as well as in the cytosol. Various signals [167] can lead to NOX2 activation which requires translocation of the cytosolic subunits (p47phox, p67phox and Rac1) to the cell membrane where they associate with the membrane localized NOX2 components (gp91, p21) to form the functional holoenzyme [169]. Critical steps in the signaling cascade leading to NOX2 activation include elevated cytosolic calcium concentrations, activation of PKCα and PLA and production of arachidonic acid with the latter inducing membrane translocation of cytosolic subunits [170].

Activated MΦs express iNOS via an NFκB-mediated pathway [171]. iNOS derived NO can combine with superoxide to form the cytotoxic product peroxynitrite (ONOO−). While NO is not considered as a cytotoxic mediator against cancer cells, it still stimulates tumor cell proliferation, extravasation and metastasis formation, peroxynitrite on the other hand is a potent cytotoxic molecule with the potential to destroy any cells.

The question arises whether any of the MΦs antimtumor effector functions are under redox control (Table 3). The early phases of MΦ-tumor cell interactions (i.e. ADCP and trogocytosis) appear to lack any significant redox control mechanism. It has been shown for example that intrasplenically injected melanoma and colon carcinoma cells are taken up rapidly by MΦs (Kuffer cells) via high affinity FcgRII and low affinity FcgRIV without any involvement of oxidants [172]. Opsonization of tumor cells by antibodies (e.g. anti-CD47) not only stimulates ADCP but it also induces a shift from M2 to M1 and results in slower tumor progression and prolonged survival of the animals [173]. Although the role of oxidants was not directly assessed in this study, the M2 to M1 shift was likely accompanied by increased oxidant producing potential of the reprogrammed MΦs. This hypothesis is supported by other studies documenting MΦ reprogramming (e.g. by gold or silver nanoparticles [174] or heme oxygenase-derived carbon monoxide and these studies demonstrated a requirement for oxidants for the shift to M1 state.

Numerous studies have addressed the role of oxidants in the tumor-killing effect of MΦs. For example pigment epithelium derived factor (PEDF) stimulated not only migration of macrophages into tumor spheroids, but phagocytosis and oxidant-mediated apoptotic killing of cancer cells [175]. Moreover, a synthetic analogue of 15-epi-lipotoxin A4 reprogrammed macrophages from M2 to M1 like phenotype and reprogrammed cells expressed iNOS, produced O2− via NADPH oxidase, and thus inhibited cancer growth [176]. Furthermore, GSH depletion caused an increased H2O2 half-life and inhibition of tumor progression [177]. These data collectively indicate that modulations of macrophage phenotype that tip the balance between oxidants and the capacity to remove them in favor of the oxidants efficiently suppress cancer progression both in vitro and in vivo.

On the other hand, a substantial body of findings questions the role of oxidants in tumor control. Several studies suggested that NOX1/2-derived superoxide or non-specified oxidants promote M2 polarization and cancer progression [178,179]. H2O2 production by macrophages or myeloid cells has also been shown to fuel tumor progression via driving angiogenesis, promoting cancer cell proliferation, inhibition of mir328 and blocking differentiation of DCs and MΦs [180]. Radiotherapy has also been shown to stimulate invasion and metastasis formation via oxidants (and H2O2-induced CXCR4 expression) [181].

6. Therapeutic considerations

Oxidants play a multifaceted and highly complex role in tumor biology. Tremendous efforts have been made to take advantage of intratumoral redox imbalance and turn it against cancer. The controversy surrounding the role of oxidants in cancer is exemplified by the often opposite therapeutic approaches put forward by scientists. While some of these approaches stimulate oxidant production, others were based on the use of antioxidant or NOX2 inhibitors [121]. Despite some limited success, a common feature of these opposite approaches is that they all failed to provide an answer to the questions:

a) Why accelerating oxidant production or using antioxidant therapies work in some instances but fail in others?

b) How can we predict which type of redox-based therapy might provide therapeutic benefit?

In order to resolve these controversies surrounding redox therapies of cancer, one must first appreciate the complexity of the problem with special recognition to be given to the facts that

a) Tumors cannot be viewed as a single disease as different cancer types differ greatly in the way they form, what drives their growth, invasion and metastasis and what type of redox dysregulation they display.

b) Tumors cannot be viewed as a mass of highly proliferating transformed cells as the tumor stroma (including regulatory and effector immune cells) plays a fundamental role in the behavior of tumor cells and their responses to chemotherapy, immunotherapy and redox therapy.

c) Oxidants are not equal and the term reactive oxygen species is inaccurate and misleading. The chemical identity of the produced oxidants, as well as compartmentalization and kinetics of oxidant production need to be given careful consideration.

d) The role of oxidants should be determined individually in every tumor to decide if it is a oxidant-driven cancer or a non oxidant driven cancer.

e) Immunosurveillance need to be taken into account [118]. Oxidant-induced immunosuppression of CLs promote tumor growth. The relative importance of this phenomenon relates to the content of tumor-infiltrating leukocytes and the sensitivity of the tumor cells to immune-mediated clearance.

f) Molecules referred to as antioxidants do not function efficiently as scavengers of free radicals or other oxidants, but may induce anti-oxidant enzymes, or be used as a precursor of cysteine used for glutathione biosynthesis (N-acetylcysteine in particular), or have other effects on signaling that counters the effects of oxidants.

A detailed redox characterization and classification of tumors including antioxidant enzymes expression, cell signaling and transcription factor activation profiles need to be established to identify whether and how a specific type of cancer can be targeted by redox-based therapies [191]. Attempts to compile such a combinational set of predictive cellular parameters have already been made [97] and we should continue and broaden such efforts.
Table 3: Role of oxidants and antioxidants in the regulation of MΦ-mediated cancer cell killing. Depending on the model systems used, studies either report A) lack of effects of certain oxidants in MΦs effector function; B) demonstrate the active role of oxidants in mediating MΦ-induced cancer cell killing or C) regulate MΦ function in a way favoring cancer cell survival.

| Macrophage | Tumor model/context | In vitro or in vivo | Finding |
|------------|---------------------|---------------------|---------|
| **Lack of effect of oxidants in some MΦ effector functions** | | | |
| Kupffer cells | Mouse B16F10 melanoma cells and C26 colon carcinoma cells injected intrasplenically in mice | in vitro/in vivo | ● Kupffer cells effectively arrest and phagocytose intact tumor cells after antitumor mAb treatment  
● Phagocytosis is dependent on FeR1 and FeR1B  
● Antibody-dependent phagocytosis is not affected by reactive oxygen or nitrogen species production [172]. |
| Human PBMC-derived MΦ and murine BM-derived MΦ | Glioblastoma (GBM) cells, mouse xenograft model | in vitro/in vivo | ● Following anti-CD47 treatment, both M1 and M2 macrophages displayed increased tumor cell phagocytosis rates in vitro (higher rates by M1).  
● Anti-CD47 treatment In vivo changed the macrophage polarization profile toward anti-tumorigenic microenvironment (increased the ratio of M1 macrophages) [173]. |
| Kupffer cells | Metastases model in Wag/Rij rats (CC531s tumor cell line injected to mesenteric vein) | in vivo | ● Tumor specific monoclonal antibody prevents liver metastases from colorectal cancer.  
● Antibody-dependent phagocytosis is the main mechanism.  
● Kupffer cells are the main effector cells in eliminating tumor cells [182]. |
| **Oxidants promote MΦ-mediated cancer cell killing** | | | |
| RAW264.7, BMDM | Lung adenocarcinoma mice (Kras model), A549 xenograft models | in vivo | ● Exogenous CO at low doses blocks progression of lung cancer.  
● The effects of CO are mediated by H2O2-dependent activation of MAPK/Erk1/2 - c-myc pathway as well as Notch 1-dependent negative feedback on the metabolic enzyme heme oxygenase-1 (HO-1).  
● CO treatment modulates macrophage phenotype and alters macrophage function to anti-tumoral [183].  
● Gold and silver nanoparticles increase the production of O2-•, H2O2, and NO in tumor-associated macrophages.  
● High oxidant production is associated with a suppressed antioxidant enzyme system, resulting in a shift of TAMs from M2 (pro-tumorigenic) to M1 (anti-tumorigenic) nature [174].  
● The model and experimental data predict that tumor-associated macrophages, specifically through HIF-1α activity, can augment tumor intracellular GSH to help tumor cells develop resistance to therapy.  
● Tumors with HIF-1α deficient macrophages grow slower and have reduced levels of intracellular GSH.  
● GSH depletion can raise the rate of production of oxidants above a toxic threshold and result in inhibition of tumor growth [177].  
● Pigment Epithelium-Derived Factor (PEDF) stimulates the migration of macrophages towards tumor 3D spheroids and 2D cultures.  
● PEDF induces the phagocytosis of tumor cells through an indirect apoptosis-dependent mechanism.  
● PEDF increases superoxide production by macrophages.  
● Conditioned media from PEDF-treated macrophages induces apoptosis, suggesting that oxidants may be involved in tumor cells apoptosis.  
● PEDF-mediated signaling involves PNPLA2 up-regulation on macrophages to induce M1 polarization and CD47 down-regulation on tumor cells which in collaboration with ATP5B elevation on macrophages leads to phagocytosis [175].  
● ATL-1, a synthetic analogue of 15-epi-lipoxin A4, could modulate TAM activity profile.  
● ATL-1 selectively decreased M2 surface markers in TAM, induces NO production by increasing the iNOS/arginase ratio and activated NADPH oxidase, triggering H2O2 production.  
● ATL-1 inhibits tumor progression in a murine model in vivo [176]. |
| WT, HIF-1α KO, and HIF-2α KO mouse MΦ | Mouse model of breast cancer | in vivo model, mathematical model | ● The model and experimental data predict that tumor-associated macrophages, specifically through HIF-1α activity, can augment tumor intracellular GSH to help tumor cells develop resistance to therapy.  
● Tumors with HIF-1α deficient macrophages grow slower and have reduced levels of intracellular GSH.  
● GSH depletion can raise the rate of production of oxidants above a toxic threshold and result in inhibition of tumor growth [177].  
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● ATL-1 inhibits tumor progression in a murine model in vivo [176]. |
| RAW264.7, THP-1, BMDM | Mouse and human tumor cell lines | in vitro | ● Pigment Epithelium-Derived Factor (PEDF) stimulates the migration of macrophages towards tumor 3D spheroids and 2D cultures.  
● PEDF induces the phagocytosis of tumor cells through an indirect apoptosis-dependent mechanism.  
● PEDF increases superoxide production by macrophages.  
● Conditioned media from PEDF-treated macrophages induces apoptosis, suggesting that oxidants may be involved in tumor cells apoptosis.  
● PEDF-mediated signaling involves PNPLA2 up-regulation on macrophages to induce M1 polarization and CD47 down-regulation on tumor cells which in collaboration with ATP5B elevation on macrophages leads to phagocytosis [175].  
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● ATL-1 selectively decreased M2 surface markers in TAM, induces NO production by increasing the iNOS/arginase ratio and activated NADPH oxidase, triggering H2O2 production.  
● ATL-1 inhibits tumor progression in a murine model in vivo [176]. |
| Human peripheral blood mononuclear cells (PBMC) | MV3 human melanoma cells, murine melanoma model (subcutaneous injection with B16F10 melanoma cells) | in vitro/in vivo | ● ATL-1, a synthetic analogue of 15-epi-lipoxin A4, could modulate TAM activity profile.  
● ATL-1 selectively decreased M2 surface markers in TAM, induces NO production by increasing the iNOS/arginase ratio and activated NADPH oxidase, triggering H2O2 production.  
● ATL-1 inhibits tumor progression in a murine model in vivo [176]. |
| Tumor-associated macrophages | MCF-10A and A549 cell lines, mouse xenograft model | in vitro/in vivo | ● Oncogenic MCT-1 (multiple copies in T-cell malignancy 1) activity promotes oxidant generation.  
● Overexpression of MCT-1 elevates MnSOD level via the YY1-EGFR signaling cascade, which protects cells against oxidative damage [184]. | (continued on next page)
Table 3 (continued)

| Macrophage                        | Tumor model/context                                                                 | In vitro or in vivo | Finding                                                                                                                                                                                                 |
|-----------------------------------|--------------------------------------------------------------------------------------|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Oxidants inhibit MΦ-mediated cancer cell killing |                                                                                       |                   | • Pancreatic cancer-associated fibroblasts (CAFs) induce a tumor-promoting TAM phenotype in monocytes                                                                                                 |
|                                   |                                                                                       |                   | • Secreted M-CSF from CAFs led to enhanced H$_2$O$_2$ production and M2 polarization in monocytes [185].                                                                                                 |
|                                   |                                                                                       |                   | • NOX1 and NOX2 are critical for the differentiation of monocytes to macrophages, the polarization of M2-type but not M1-type macrophages, and the occurrence of tumor-associated macrophages (TAMs). |
|                                   |                                                                                       |                   | • Decrease in M2 macrophages and TAMs contributes to the delay in wound healing and the inhibition of tumor growth and metastasis in NOX1/2 double knockout mice [178]. |
|                                   |                                                                                       |                   | • Radiotherapy can promote the invasion and metastasis of several types of cancer.                                                                                                                          |
|                                   |                                                                                       |                   | • After irradiation, hypoxia-inducible factor 1α (HIF-1α) was increased and translocated into the nucleus and promoted the transcription of CXCR4.                                                              |
|                                   |                                                                                       |                   | • Oxidants also play a role in the radiation-induced expression of CXCR4.                                                                                                                                       |
|                                   |                                                                                       |                   | • NAC reduce the transcriptional activation of CXCR4 promoter by 2 Gy irradiation [181].                                                                                                                      |
| MΦs (myeloid-derived suppressor cells) |                                                                                       |                   | • Oxidant production is up-regulated in myeloid-derived suppressor cells (MDSC) in seven different tumor models and in cancer patients.                                                                       |
| Mouse peritoneal macrophages       |                                                                                       |                   | • Increased production of O$_2^·$ and H$_2$O$_2$ in MDSCs is regulated by NADPH oxidase-2 (NOX2).                                                                                                          |
|                                   |                                                                                       |                   | • MDSCs from NOX2 deficient mice lost the ability to suppress T cell responses and quickly differentiated into mature macrophages and dendritic cells [126].                                                    |
| Subcutaneous tumor models in mice: DA3 mammary carcinoma, CT26 colon carcinoma, MethA sarcoma, EL4 thymoma, Lewis lung carcinoma, MC38 colon carcinoma, C3 sarcoma. |                                                                                       |                   | • Differentiation of ImC (immature myeloid cells from tumor-bearing mice was significantly delayed.                                                                                                             |
| Blood samples of head and neck cancer patients |                                                                                       |                   | • Rates of oxidant production were significantly higher in ImC from tumor-bearing mice. Hydrogen peroxide but not superoxide was found to be the major part of increased oxidant production. |
|                                   |                                                                                       |                   | • ImC transferred into tumor-bearing recipients failed to differentiate into DC or macrophages [186].                                                                                                          |
| Macrophages (and DCs, granulocytes) |                                                                                       |                   | • The increase in oxidant production was mediated by integrins [187].                                                                                                                                     |
| Mouse tumor models (CT-26 colon carcinoma and C3 sarcoma) |                                                                                       |                   | • TAMs have anti-inflammatory and pro-angiogenic effects on C26 tumor cells.                                                                                                                               |
| ImC (immature myeloid cells)       |                                                                                       |                   | • Inhibition of NADPH oxidase in macrophages reduced the production of angiogenic proteins.                                                                                                                    |
| C3 fibrosarcoma                    |                                                                                       | in vitro (ex vivo)| • The stimulatory effects of TAMs on C26 cell proliferation may be related mainly to their pro-oxidant actions exerted by NADPH oxidase activity, which maintains the redox status and the angiogenic capacity of the tumor microenvironment [188]. |
| murine TAMs (tumor-associated macrophages) |                                                                                       |                   | • O$_2^·$ and H$_2$O$_2$ production is critical for macrophage differentiation and inhibition of superoxide production specifically blocks the differentiation of M2 macrophages [179]. |
| C26 murine colon carcinoma cells   |                                                                                       | in vitro          | • Oxidative stress by M1 and M2 macrophages induced downregulation of mirt-328 and upregulation of CD44                                                                                                      |
| Human and mouse monocytes and macrophages |                                                                                       | in vitro/in vivo  | • CD44 is a direct target of mir-328.                                                                                                                                                                    |
| Mouse tumor models: Urethane model, Kras model, Breast tumor model |                                                                                       |                   | • Increased CD44 expression results in tumor progression by enhancing antioxidant defense [180].                                                                                                          |
| Tumor-associated macrophages       |                                                                                       |                   | • Oncogenic MCT-1 (multiple copies in T-cell malignancy 1) activity promotes oxidative generation.                                                                                                          |
| MCF-10A and A549 cell lines, mouse xenograft model |                                                                                       | in vitro/in vivo  | • Overexpression of MCT-1 elevates MnSOD level via the YY1-EGFR signaling cascade, which protects cells against oxidative damage [184].                                                              |
|                                   |                                                                                       |                   | (continued on next page)                                                                                                                                                                                  |
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