Review Article

NOX2-Derived Reactive Oxygen Species in Cancer

Hanna Grauers Wiktorin,1 Ebru Aydin,1,2 Kristoffer Hellstrand,1 and Anna Martner1

1TIMM Laboratory, Salgernska Center for Cancer Research, Department of Infectious Diseases, Institute of Biomedicin, Sahlgrenska Academy, University of Gothenburg, Sweden
2Molecular Genetics, German Cancer Research Center (DKFZ), Heidelberg, Germany

Correspondence should be addressed to Anna Martner; anna.martner@gu.se

Received 2 August 2019; Accepted 21 October 2019; Published 30 November 2020

Academic Editor: Jayeeta Ghose

Copyright © 2020 Hanna Grauers Wiktorin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The formation of reactive oxygen species (ROS) by the myeloid cell NADPH oxidase NOX2 is critical for the destruction of engulfed microorganisms. However, recent studies imply that ROS, formed by NOX2 + myeloid cells in the malignant microenvironment, exert multiple actions of relevance to the growth and spread of neoplastic cells. By generating ROS, tumor-infiltrating myeloid cells and NOX2+ leukemic myeloid cells may thus (i) compromise the function and viability of adjacent cytotoxic lymphocytes, including natural killer (NK) cells and T cells, (ii) oxidize DNA to trigger cancer-promoting somatic mutations, and (iii) affect the redox balance in cancer cells to control their proliferation and survival. Here, we discuss the impact of NOX2-derived ROS for tumorigenesis, tumor progression, regulation of antitumor immunity, and metastasis. We propose that NOX2 may be a targetable immune checkpoint in cancer.

1. Introduction

1.1. Distribution and Function of NOX Enzymes. The NOX family of enzymes comprises seven structurally conserved isoforms, i.e., NOX1-5 and DUOX1-2. The only known function of these transmembrane multicomponent enzymes is to catalyze the reduction of molecular oxygen to generate superoxide (O2−) or hydrogen peroxide (H2O2) [1, 2]. Superoxide is spontaneously or enzymatically converted to H2O2 that may be further converted to additional reactive oxygen species (ROS), including myeloperoxidase- (MPO-) derived hypochlorous acid and tyrosyl radical [3].

NOX enzymes differ in distribution between cell types in their subcellular localization and composition of subunits. NOX1 is mainly expressed in the colon, NOX2 on the lysosomal and plasma membranes of myeloid cells where it contributes to phagocyte killing of microbes, NOX3 in the inner ear and fetal tissues, NOX4 in the kidney, NOX5 in lymphoid tissue and testis, and DUOX1-2 in thyroid and gastrointestinal tissues [4, 5]. Low expression levels of NOX1 and NOX4 are also detected in myeloid cells [4, 6, 7], and NOX2 is minimally expressed by hematopoietic stem cells [8]. NOX2 is further expressed at low levels by B cells that may take up and, similar to myeloid cells, degrade microbial pathogens by generating NOX2-derived ROS [9]. Additionally, within dendritic cell (DC) phagolysosomes, NOX2 generates ROS in a process that consumes protons leading to alkalization of this compartment. This protects engulfed peptides from complete degradation by lysosomal proteases, which facilitates their presentation to cytotoxic T cells [10–12].

1.2. NOX Enzymes in Cancer. ROS formed from NOX enzymes have been implicated in carcinogenesis [13]. In addition, several NOX enzymes are expressed in malignant tissue and may contribute not only to cancer progression and spread but also to apoptosis of malignant cells. NOX1 is implicated in colon cancer where its ROS-producing activity may enhance tumor cell proliferation and metastasis [14, 15]. Myeloid leukemic cells express high levels of NOX2 that compromises destruction of malignant cells by triggering ROS-induced apoptosis of adjacent antileukemic lymphocytes [16–19]. Stem cell expression of NOX2 has been implicated in leukemogenesis by maintaining survival of leukemic stem cells [8]. NOX2 is further expressed by EBV-
infected gastric cancer cells to promote tumor progression [20] and by non-small-cell lung cancer cell lines, where it mediates tumor cell apoptosis [21]. NOX4 is overexpressed in several forms of cancer, including breast cancer, where it may enhance tumorgenesis [22], and prostate cancer, where it promotes apoptosis [23]. Table 1 summarizes the proposed physiological and pathophysiological functions of NOX enzymes.

Additionally, ROS from all cellular sources, including NOX-derived ROS, participate in redox signaling by oxidizing thiol groups on proteins, thus modifying cellular functions and activation status. For example, ROS may oxidize protein tyrosine phosphatases (PTPs) and protein kinase C (PKC) with ensuing effects on differentiation, proliferation, and survival of malignant cells [69–73].

1.3. The Myeloid NADPH Oxidase: NOX2. The first discovered and by far most extensively studied member of the NOX enzyme family, NOX2, is densely expressed by myeloid cells such as monocytes, macrophages, and granulocytes [2]. NOX2 is a complex of membrane-bound and cytosolic subunits that are spatially separated in resting cells. The membrane-bound subunits, gp91phox (also referred to as CYBB or NOX2) and p22phox (CYBA), constitute the catalytic core of the oxidase. The subunits p47phox (NCF1), p67phox (NCF2), and p40phox (NCF4) remain in the cytosol as a complex. Activation of NOX2 may be induced by pathogen-associated molecular patterns, danger-associated molecular patterns, bacterial peptides, growth factors, and cytokines, which trigger the cytosolic subunits p47phox (NCF1), p67phox (NCF2), and p40phox (NCF4) to translocate and assemble at the membrane [5, 74]. Two GTPases, Rac and Rap, are also critical for NOX2 activation [75, 76]. In its GTP-bound form, the cytosolic Rac interacts with p67phox and translocates to the membrane. Rap1 is a membrane protein with a partly unknown function that is required for optimal activation of NOX2 components [77] (Figure 1).

Phagocytes are stimulated to generate NOX2-derived ROS upon encountering microbes in a process referred to as a “respiratory burst.” When the components of NOX2 assemble at the phagolysosome membrane, NOX2 generates intracellular ROS, while assembly at the plasma membrane leads to the formation of extracellular ROS [5, 78]. The respiratory burst is critical for phagocyte-mediated killing of microorganisms as highlighted by the susceptibility to bacterial and fungal infections in patients with chronic granulomatous disease, a rare genetic disorder caused by dysfunction of NOX2 [79–81], and by studies in mice that are genetically deprived of NOX2 [82]. NOX2 deficiency is also associated with hyperactive lymphocytes and autoimmunity in mice and humans, indicating that NOX2-derived ROS also participate in controlling lymphocyte reactivity [83–85]. Additionally, monocyte-derived DCs express NOX2, and the formation of NOX2-derived ROS by pathogen-activated DCs is proposed to reduce the potential transmission of pathogens to secondary lymphoid organs [86].

2. Redox Homeostasis

In addition to NOX-mediated formation of ROS, all cells generate ROS during mitochondrial ATP generation. In the process of oxidative phosphorylation, electrons pass through the electron transport chain where the final electron acceptor is oxygen, most of which is converted to water. Superoxide is produced as a byproduct in this process due to incomplete reduction of oxygen to water or premature electron leakage to oxygen [87, 88]. Intracellular levels of ROS affect cellular redox signaling and homeostasis, while ROS released into the surrounding, in particular H$_2$O$_2$, that is relatively stable and readily crosses cell membranes, may also affect adjacent cells [19, 89–91]. Under resting conditions, when there is a balance between ROS and antioxidants, redox signaling is reversible and regulates physiological processes due to the ability of ROS to reversibly oxidize cysteine residues to thus alter protein function [92, 93]. During environmental stress, infection, and inflammation, including cancer-related inflammation, the cell and tissue concentrations of ROS may increase beyond the capacity of the antioxidant defense systems. Such “oxidative stress” may result in irreversible oxidation and damage to proteins, lipids, and DNA [92]. Details

| Enzyme | Tissue expression (high to low) | Function | Cancer relevance |
|--------|-------------------------------|----------|-----------------|
| NOX1   | Colon, uterus, prostate [24–28]| Repair of colon mucosa | Colon [14, 15, 29, 30] and prostate [31] cancers |
| NOX2   | Myeloid cells [8, 32–34]      | Host defense against pathogens, lymphocyte homeostasis, stem cell maintenance, myeloid cell differentiation | Myeloid leukemia [35, 36], melanoma [37, 38], lymphoma [32] |
| NOX3   | Inner ear, fetal tissue [39–41]| Otoconia synthesis, organogenesis | Hepatocellular carcinoma [42] |
| NOX4   | Kidney [43, 44]               | Oxygen sensing* | Renal [45, 46] and ovarian [47] cancers, glioma [48], melanoma [49] |
| NOX5   | Lymphoid tissue, testis [50, 51]| Lymphocyte differentiation, spermatozoa motility | Prostate cancer [52, 53], Barrett’s esophageal adenocarcinoma [54] |
| DUOX1  | Thyroid, respiratory tract [55–57]| Hormone synthesis, innate airway host defense | Thyroid [58, 59] and lung cancer [60, 61] |
| DUOX2  | Thyroid, gastrointestinal tract [55, 62–65]| Hormone synthesis, regulation of gut microbiota/mucosa interactions | Thyroid [58, 66] and pancreatic cancer [67, 68] |
regarding redox homeostasis and its impact on cancer have recently and comprehensively been reviewed [94, 95] and is beyond the major scope of this overview.

To avoid ROS-inflicted cell damage, several cellular systems that neutralize ROS are induced in an oxidative environment. The transcription factor Nrf2 is a key regulator of production of antioxidative enzymes within cells. In resting conditions, Nrf2 is bound to Keap1 in the cytoplasm, which prohibits Nrf2 from inducing gene transcription. Upon oxidation of cysteine residues in Keap1, Nrf2 is released and translocates to the nucleus where it binds to antioxidant response elements [96]. This process stimulates the transcription of Nrf2 target genes with cytoprotective functions. These include NAD(P)H quinone oxidoreductase 1, which catalyzes the reduction of reactive quinones that otherwise cause oxidative stress [97], heme oxygenase-1 (HO-1) that catalyzes the breakdown of heme [98], glutamate-cysteine ligase catalytic and modifier that catalyzes the rate-limiting step in synthesis of the endogenous antioxidant glutathione (GSH) [99], and thioredoxin reductase 1 that reduces peroxiredoxins of relevance to the detoxification of reactive peroxides, including H₂O₂ and peroxynitrite [100].

Other cellular antioxidant enzymes include superoxide dismutase, catalase, glutathione peroxidase-1, peroxiredoxins, and thioredoxin. Together with the nonenzymatic antioxidant GSH, these antioxidant enzymes are assumed to provide the most efficient protection from oxidative damage (Figure 2). Additional nonenzymatic scavengers of ROS include naturally occurring metabolites, vitamins (such as vitamins C and E) and iron chelators that prevent formation of hydroxyl radicals in the Fenton reaction [101, 102].

3. ROS and Cancer

3.1. Cancer-Related Oxidative Stress. Cancer may be associated with oxidative stress, i.e., an imbalance between the...
production and detoxification of ROS. Rapidly proliferating cancer cells have a high energy demand and therefore exhibit enhanced cellular respiration. Consequently, cancer cells generate enhanced levels of mitochondrial-derived ROS [89]. Growth factors and integrins, which are often produced at enhanced levels in cancer tissues, also contribute to enhanced NOX-derived ROS production [103] and, as reviewed above, several cancer histiotypes exhibit dysregulated expression of NOX enzymes [8, 14–17, 20–23]. Furthermore, solid and metastatic tumors are often infiltrated by NOX2+ myeloid cells that may release ROS leading to an oxidized tumor microenvironment [104–108]. The extracellularly released ROS from myeloid cells affect redox regulation in adjacent tumor cells and may inactivate T cells and NK cells, thus compromising immune-mediated killing of malignant cells [19, 90, 91, 109–111]. Hypoxia is a common feature of the microenvironment of tumors that activates the hypoxia-inducible factor (HIF) family of transcription factors. HIFs mediate cellular adaptation to low oxygen levels and may influence several aspects of cancer such as promoting neovascularization [112], increasing cell survival [113], stimulating metastasis [114, 115], and conferring resistance to chemotherapeutics [116]. ROS may induce the activation of HIF-1α, a member of the HIF family of transcription factors, and thereby stimulate HIF-related cancer events [117].

The arguably most established role of ROS in cancer is its capacity to damage DNA with ensuing mutations and risk of cancer initiation and progression. Typically, deoxyguanosine is oxidized to 8-oxo-2-deoxyguanosine that may pair with adenine instead of cytosine, which promotes mutations in oxidatively stressed cells [118–121]. Overexpression of NOX enzymes, including NOX4, DUOX1, and DUOX2, has been shown to generate excessive H$_2$O$_2$ that may cause local tissue injury and DNA damage, thus resulting in the formation of a premalignant niche. NOX-derived ROS may thus contribute to tumor initiation and to tumor progression by inducing further DNA damage [122, 123].

Moreover, many cancer-related events, such as cell cycle proliferation, invasion, epithelial-to-mesenchymal transition, and metastasis are subject to redox regulation [47, 69–71, 73, 124–130]. For example, growth factors such as PDGF and EGF stimulate the PI3-K-AKT and RAS-MEK-ERK pathways, which are key regulators of cell proliferation and survival [131, 132]. These growth factors also stimulate NOX enzymes to produce ROS. The kinases in the PI3-K and RAS pathways phosphorylate target proteins, while PTPs serve to remove phosphate groups from proteins. This phosphorylation/dephosphorylation circuit alters protein function and controls cellular functions [133–135]. ROS may oxidize thiol groups in PTPs resulting in their inactivation. As a consequence, signaling along these pathways is boosted in an oxidative environment where PTPs are inactivated, and cancer cells may thus respond more vigorously to stimulation by growth factors [134, 135].

An additional example of the effects of ROS on PTPs is the inactivation of PTPs in pancreatic cancer cells that results in sustained activation of Janus kinase 2, which in turn activates signal transducer and activator of transcription (STAT) and antiapoptotic proteins to enhance tumor cell survival [72]. ROS may also oxidize and thus activate PKC; thereby, ROS modulate several PKC-dependent activities within cells [126, 136]. ROS have been proposed to enhance the tissue-invasive properties of cancer cells by modulating the function of mitogen-activated protein kinases via oxidation of PTPs and PKC [124–126]. However, as overproduction of ROS by cancer cells may trigger their apoptosis, the clinical efficacy of many therapies relies on induced ROS production in cancer cells, as further discussed below.

Tumor cells often show enhanced levels of antioxidative enzymes, presumably to resist the toxicity from the generation of NOX- and mitochondria-derived ROS [89]. In addition, tumor cells may acquire mutations that further boost antioxidative responses, thereby contributing to tumor cell resistance to oxidative stress. Approximately 30% of human lung cancers thus carry mutations in either Keap1 or Nrf2, resulting in Nrf2 stabilization and enhanced production of endogenous antioxidants [137]. One of the antioxidants controlled by Nrf2 is HO-1 that reduces intracellular levels of free heme; thus, in turn, stabilizes the transcription factor BACH1 to activate transcription of genes that promote glucose uptake, glycolysis, and lactate secretion in the Warburg reaction [138]. Accordingly, BACH1 activation was shown to stimulate glycolysis-dependent metastasis of lung cancer cells [137, 138]. Thus, an antioxidative response by tumor cells, or antioxidative treatment strategies such as scavengers of ROS, may enhance tumorigenesis and metastasis by modulating tumor metabolism in favour of glycolysis.

3.2. Targeting NOX2 in Experimental Cancer Models. The development of knockout mice with NOX2 deficiency has been instrumental in studies on the role of ROS in cancer from sources other than mitochondria. Mice with deficiency in the NOX2 subunit Ncf1 show reduced growth or incidence of melanomas and the Lewis lung carcinoma tumors, whereas the growth of spontaneously arising prostate carcinoma or methylcholanthrene-induced sarcoma is not affected [38, 139].

Studies in knockout mice imply a role for NOX2 in metastasis. Mice deficient in the NOX2 subunit Cybb thus show reduced lung metastasis after intravenous inoculation of melanoma cells and a lower incidence of spontaneously formed metastases from surgically removed melanomas [37, 140, 141]. The targeting of NOX2 by systemic treatment with the NOX2 transduction inhibitor histamine dihydrochloride (HDC) reduced the formation of lung melanoma metastases in wild-type but not in Nox2-deficient mice. Effects of NOX2 repression on hematogenous metastasis were absent after the depletion of NK cells in vivo and absent also in interferon-γ- (IFN-γ-) deficient mice. These results thus imply that NOX2-derived ROS trigger the formation of melanoma metastasis by downmodulating NK cell functions, and that genetic or pharmacological inhibition of NOX2 restores tumor cell clearance exerted by IFN-γ- NK cells [37]. These results were confirmed and extended by Van der Weyden et al. showing that hematogenous metastasis was markedly reduced in mice genetically depleted of any of the major NOX2 subunits (Cyba, Cybb, Ncf1, Ncf2, and Ncf4) and that tumor tissues of NOX2-deficient mice showed...
a marked increase of antineoplastic lymphocytes [141]. In accordance with the latter finding, treatment with the NOX2 inhibitor HDC resulted in enhanced NK cell counts in the lungs of wild-type mice with pulmonary melanoma metastases, but not in corresponding lungs from Nox2-deficient mice [37].

HDC suppresses ROS formation by exerting agonist activity at histamine type 2 receptors (H\textsubscript{2}Rs) [18] and thus inhibits NOX2 signal transduction rather than directly inhibiting, e.g., oxidase function or assembly. The detailed mechanisms of NOX2 inhibition and the ensuing protection of antineoplastic lymphocytes are incompletely understood. Myeloid cells deficient of MPO still exerted immunosuppression towards NK cells, which was reversible by HDC-treatment, thus suggesting that O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2} are more likely mediators of NOX2-induced immunosuppression than MPO-derived ROS such as, e.g., hypochlorous acid or tyrosyl radicals [142]. Additionally, circumstantial evidence links the NOX2-inhibitory properties of HDC to the PI3-K pathway. Activation of PI3-K thus activates Akt and PKC that triggers the assembly and ROS formation of NOX2 [143]. HDC suppresses NOX2-mediated ROS formation induced by fMLF and other bacterial peptides, but does not affect PMA-induced respiratory burst [144]. As fMLF activates the PI3-K pathway [145] whereas PMA directly induces the activation of PKC, these findings thus suggest that HDC, by activating H\textsubscript{2}Rs, targets the PI3-K pathway upstream of PKC in myeloid cells. In support for this hypothesis, PI3-K inhibitors share the NOX2 inhibition exerted by HDC and equally efficiently protect antineoplastic lymphocytes from apoptosis and dysfunction induced by adjacent, ROS-producing myeloid cells [146].

Systemic treatment with HDC in vivo suppresses tumor growth in several models of experimental cancer [147]. While these antitumor effects of HDC are likely pletotropic, it is noteworthy that beneficial effects of treatment with HDC in murine melanoma, lymphoma, and mammary cancer were only observed in NOX2-sufficient mice [32, 35, 37, 148] and that HDC only inhibited growth of NOX2\textsuperscript{+} and not NOX2 leukemic cells in a xenograft setting [35]. Additionally, the efficacy of HDC in reducing murine tumor growth and metastasis relied on the presence of NOX2-expressing Gr1\textsuperscript{+} myeloid cells since the effect was lost upon Gr1\textsuperscript{+} cell depletion [37, 148]. Furthermore, experiments using single-cell suspensions from tumors, spleens, and lungs suggested that ROS formation was confined to the Gr1\textsuperscript{+} cell fraction [37, 148]. These findings, along with results showing that HDC does not reduce metastasis after the depletion of NK cells, support the hypothesis that HDC provides a less immunosuppressive malignant microenvironment that favors NK cell-mediated clearance of tumor cells [37, 83]. Additionally, treatment with HDC was shown to increase the number of tumor-infiltrating effector CD8\textsuperscript{+} T cells in murine lymphoma and to improve the antitumor efficacy of immune checkpoint inhibitors (anti-PD-1 and anti-PD-L1) [148], thus implying that HDC may facilitate also T cell-dependent elimination of tumor cells.

Monocytic leukemic cells recovered from patients with acute myeloid leukemia (AML) frequently express functional NOX2, and studies in xenografted mice support that NOX2 is relevant to the survival and expansion of monocytic AML cells [35, 149]. NOX2-derived ROS have been proposed to stimulate the transfer of prosurvival mitochondria from stromal cells to AML cells [149]. Furthermore, NOX2 inhibition by HDC reduced the expansion of xenografted NOX2\textsuperscript{+} but not of NOX2 human AML cells, presumably by hindering S-phase entry of leukemic cells [35]. These results illustrate that the targeting of NOX2 may reduce malignant expansion independently of functional cellular immunity.

In addition, results obtained in a mouse model of Kras-induced myeloid leukemia showed that Kras\textsuperscript{+} NOX2-deficient myeloid cells (Nox2\textsuperscript{−/−} M-Kras\textsuperscript{G12D}) expanded slower than their NOX2-sufficient counterparts. In this model, treatment of mice with N-methyl-histamine (an H\textsubscript{2}R-selective analogue of HDC that shares the NOX2-inhibitory properties of HDC) reduced leukemic expansion and prolonged the survival of NOX2-sufficient but not of NOX2-deficient mice. N-Methyl-histamine-treated mice harbored leukemic cells with reduced intracellular ROS levels, reduced DNA oxidation, and reduced double-stranded DNA breaks [150]. These results thus imply that NOX2-derived ROS may promote genomic instability and malignant expansion in Kras-induced leukemia. NOX2 may also support myeloid expansion of murine Bcr-Abl1\textsuperscript{+} cells as transplantation of NOX2\textsuperscript{+} Bcr-Abl1\textsuperscript{+} cells into irradiated mice causes a more rapidly expanding and severe leukemia than the transfer of NOX2-deficient Bcr-Abl1\textsuperscript{+} cells [8, 151].

4. Myeloid-Derived Suppressor Cells and NOX2

4.1. Myeloid Cells within the Tumor Microenvironment. The presence of cytotoxic lymphocytes, including CD8\textsuperscript{+} T cells and/or NK cells, in the microenvironment of human cancer tumors is typically prognostically favorable, while the presence of infiltrating myeloid cells often, although not invariably, predicts poor survival [104–107, 152–159]. Hence, a high ratio of tumor-infiltrating T cells to myeloid cells entails favorable prognosis in several cancer forms including lung cancer, bladder cancer, glioblastoma, prostate cancer, and renal cell carcinoma [160–166]. In recent years, the neutrophil to lymphocyte ratio and the monocyte to lymphocyte ratio in peripheral blood have emerged as readily available and independent predictors of poor survival in several forms of solid cancer [167], thus underscoring that myeloid cell-induced immunosuppression may impact adversely on cancer prognosis.

Myeloid-derived suppressor cells (MDSCs) are immature and immunosuppressive myeloid cells that accumulate in the tumor microenvironment and in the periphery in patients with cancer. MDSCs comprise pathologically induced myeloid cells of the monocytic (M-MDSCs) and granulocytic (G-MDSC) lineages that suppress T cells and NK cells by several mechanisms, including enhanced production of immunosuppressive NOX2-derived ROS, arginase, nitric oxide (NO), TGF-beta, and IL-10 [168]. MDSCs are thus assumed to favor immune escape in cancer [169, 170]. MDSCs and other myeloid cells are attracted to tumors in response to...
cytokines such as CCL2 and CSF1 for M-MDSCs and CXCL1 and CXCL8 for G-MDSCs [171]. Once in the tumor microenvironment, M-MDSCs may differentiate into tumor-associated macrophages (TAMs) or DCs. TAMs may also originate from infiltrating monocytes and tissue-resident macrophages [172]. MDSCs and TAMs may release soluble molecules such as cytokines, prostaglandins, chemokines, interleukins, and growth factors into the tumor microenvironment that may contribute to the formation of premetastatic niches, promote angiogenesis, promote tumor cell survival, and enhance tumor cell invasion [173, 174]. These properties of MDSCs and TAMs may, in part, account for the unfavorable association between myeloid cell tumor infiltration and prognosis.

TAMs exhibit either M1 or M2 polarization. The M1-polarized TAMs express iNOS and TNF and are denoted proinflammatory, whereas the M2-polarized TAMs produce the L-arginine-depleting enzyme arginase and secrete IL-10 to compromise immune activation [171, 175]. M1 and M2 macrophages both express NOX2, although the expression level is higher in M1 macrophages [176]. Mice lacking NOX1 and NOX2 showed reduced M2 macrophage polarization, while single knockout of NOX1 or NOX2 did not [6]. Hence, in the Lewis lung carcinoma model, wild-type and NOX1/NOX2 double-knockout mice showed a similar degree of TAM infiltration, while the content of M2-TAMs was reduced in the double-knockout mice along with reduced tumor growth [6]. These results imply that inhibition of NOX enzymes may favor M1 polarization in cancer; however, studies of nonmalignant inflammation (spinal cord inflammation in mice) suggest that inhibition of NOX2 instead reduces M1 polarization [177], and further studies are required to define the impact of NOX enzymes on macrophage polarization.

In contrast to MDSCs and M2-TAMs, the intratumoral accumulation of other myeloid cells, such as DCs and M1-polarized TAMs, may indicate favorable cancer prognosis [178–181]. Tumor-infiltrating DCs initiate the induction of tumor-specific T cell responses and are thus critical to evoke antitumor immunity, and M1 polarized macrophages may contribute in the killing of tumor cells [182]. While the favorable impact of the presence of M1-polarized macrophages in cancer tumors is well established, the subdivision of macrophages into distinct populations is challenged by reports showing that TAMs often display features of both M1 and M2 subsets [183, 184].

4.2. Immunosuppression by MDSC-Derived ROS. Early studies showed that MDSCs displayed enhanced expression of NOX2 as a result of the activation of the transcription factor STAT3 [185, 186]. The formation of NOX2-derived ROS is considered a major immunosuppressive action mediated by MDSCs, in particular by G-MDSCs [148, 186, 187], and ROS-producing MDSCs or other immunosuppressive myeloid cells thus induce apoptosis or dysfunction in adjacent lymphocytes such as NK cells and T cells [19, 91, 188–190]. ROS induce activation of ERK1/2 in lymphocytes, which results in PARP-1-dependent accumulation of poly-ADP-ribose (PAR) and parthanatos (a form of apoptosis) [191]. In addition, MDSC-derived ROS inhibit antigen-specific CD8+ T cell responses and may thus selectively eradicate antitumor T cell clones [188]. The immunosuppression exerted by ROS towards T cells has been linked to nitration of the T cell receptor (TCR) and occurs when ROS react with NO to form peroxynitrite during MDSC–T cell interactions. Nitration was proposed to induce a conformational change of the TCR, and T cells thus display reduced affinity for MHC-peptide complexes [192]. This effect was linked to ROS as MDSCs with dysfunctional NOX2 did not suppress antigen-specific T cell responses [186]. On a similar note, MDSCs isolated from mice systemically treated with the NOX2 inhibitor HDC produced lower levels of ROS and were less prone to suppress T cells ex vivo [148].

4.3. ROS as Inhibitors of Myeloid Cell Differentiation. MDSCs isolated from mice with myeloid cells that cannot generate NOX2-derived ROS, i.e., Stat3 or NOx2 knockout mice, are prone to differentiate towards mature macrophages and DCs [186, 193] suggesting that NOX2-derived ROS inhibit myeloid cell maturation and thus promote the accumulation of immature MDSCs. Furthermore, the antioxidant N-acetyl cysteine (NAC) was found to trigger differentiation of MDSCs [194]. Similarly, all-trans-retinoic acid (ATRA), which upregulates the antioxidant glutathione synthase and thus reduces intracellular ROS, stimulates the differentiation of MDSCs in murine tumor models and of MDSCs isolated from cancer patients [195–198]. In agreement with these reports, treatment with the NOX2 inhibitor HDC reduces the accumulation of tumor-infiltrating MDSCs in EL-4 thymoma-bearing mice. The reduction of tumor-infiltrating MDSCs was accompanied by augmented levels of intratumoral DCs and by improved maturation of human DCs from monocytes [32, 148]. Figure 3 summarizes aspects of NOX2-mediated regulation of myeloid cell differentiation in cancer.

5. Targeting ROS in Human Cancer

While low ROS levels in cells are reportedly mitogenic due to the activation of the PI3-K-AKT and RAS-MEK-ERK pathways [131, 132], high ROS levels are toxic to numerous cell types including cancer cells [92, 118–121]. Several chemotherapies, as well as radiotherapy and photodynamic therapy, trigger excessive ROS production within cells. Oxidants may thus contribute to the elimination of tumor cells and to the toxicity of chemotherapeutics [199]. In addition, several antitumor agents, including erlotinib and silibinin, trigger overproduction of ROS via NOX enzymes, which contributes to killing tumor cells [21, 23].

Despite that increased intracellular ROS levels may induce killing of malignant cells, ROS have also been ascribed protumorigenic properties. Antioxidative strategies have thus been evaluated for human cancer therapy and prevention. Such strategies include ROS scavengers such as NAC, vitamin E, and beta-carotene that are aimed at reducing oxidative stress [200–202]. These studies, as well as animal experiments comprising the administration of ROS scavengers in cancer treatment, have shown partly divergent results. Whereas some studies support that antioxidants reduce the
risk of cancer [200–202], other studies, in particular those involving the administration of antioxidants to smokers to prevent lung cancer, imply enhanced cancer risk by the administration of antioxidants [203].

The mechanisms explaining the partly opposing results in studies of broad antioxidants in cancer remain to be elucidated. Recent studies imply that antioxidants trigger the activation of the transcription factor BACH1 that stimulates a metabolic reprogramming of cancer cells in favor of glycolysis, which enhances their capacity to metastasize [137, 138]. These findings may appear counterintuitive in light of the abovementioned reduction of metastasis induced by HDC and other NOX2 inhibitors that act by reducing ROS levels. However, a noticeable difference between global antioxidants and HDC is that HDC targets NOX2-derived ROS formation only in myeloid cells that coexpress H2R and NOX2. HDC or other NOX2-inhibitory strategies are hence unlikely to alter metabolically generated ROS.

ATRA is used in the treatment of acute promyelocytic leukemia where the leukemic cells carry a PML-RARA translocation giving rise to a block in myeloid cell differentiation and development of leukemia. ATRA releases this block and allows the differentiation of immature leukemic promyelocytes into mature granulocytes [204]. ATRA may also promote the differentiation of MDSCs by neutralizing intracellular ROS [195–198]. ATRA exerts antitumoral effects in several murine models [205, 206] and has been investigated in combination with immunotherapies such as IL-2 and DC vaccines in renal cell carcinoma and non-small-cell lung cancer [205–207]. The efficacy of ATRA combined with ipilimumab is currently assessed in stage IV melanoma (ClinicalTrials.gov identifier: NCT02403778).

The NOX2-inhibitor HDC is used in conjunction with low-dose IL-2 within the EU to prevent relapse of AML in the postchemotherapy phase [208]. HDC acts on H2Rs expressed on the surface of normal and leukemic myeloid cells to inhibit production of NOX2-derived ROS [208, 209]. In vitro studies support that HDC promotes cellular immunity by protecting subsets of cytotoxic lymphocytes against ROS-induced inactivation [19, 91] and that these effects of HDC are markedly enhanced by the coadministration of NK and T cell activators such as IL-2 [111]; however, complementary or alternative mechanisms are conceivable, including HDC-induced differentiation of AML cells [19, 35, 208]. While the side-effects of HDC/IL-2 were typically mild and transient with minimal impact on global health [208, 210], the incidence of grade 1/2 arthralgia and myalgia was slightly but significantly higher in treated patients. It may thus be speculated that HDC/IL-2 induces autoimmunity similar to that observed in NOX2-deficient CGD patients and in experimental animals that are devoid of functional NOX2 [83].
6. Conclusion

While details regarding the contribution by NOX2-derived ROS for the induction and progression of cancer remain to be elucidated, it seems likely that the impact of NOX2 is confined mainly to primary and metastatic tumors that are infiltrated by immunosuppressive NOX2+ myeloid cells and to myeloid leukemias, where the malignant clone comprises NOX2+ cells. In cancer, NOX2 may contribute to the immunosuppression exerted by myeloid cells, in part by producing extracellular ROS that trigger dysfunction in adjacent lymphocytes. Recent studies show that NOX2 promotes tumor growth and metastasis and that intact NOX2 is crucial for self-tolerance, thus fulfilling the criteria of an immune checkpoint [83]. Inhibition of NOX2-derived ROS may thus relieve immunosuppression in cancer and may act in synergy with cancer immunotherapies such NK and T cell-activating cytokines or checkpoint inhibitors.

Conflicts of Interest

Authors HGW, KH, and AM hold issued or pending patents that protect the use of NOX2-inhibitors in cancer.

References

[1] Y. Nisimoto, B. A. Diebold, D. Cosentino-Gomes, and J. D. Lambeth, "Nox4: a hydrogen peroxide-generating oxygen sensor," Biochemistry, vol. 53, no. 31, pp. 5111–5120, 2014.

[2] K. Bedard and K. H. Krause, "The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology," Physiological Reviews, vol. 87, no. 1, pp. 245–313, 2007.

[3] J. W. Heinecke, W. Li, G. A. Francis, and J. A. Goldstein, "Tyrosyl radical generated by myeloperoxidase catalyzes the oxidative cross-linking of proteins," Journal of Clinical Investigation, vol. 91, no. 6, pp. 2866–2872, 1993.

[4] K. H. Krause, "Tissue distribution and putative physiological function of NOX family NADPH oxidases," Ipn J Infect Dis, vol. 57, no. 5, pp. S28–S29, 2004.

[5] A. Panday, M. K. Sahoo, D. Osorio, and S. Batra, "NADPH oxidases: an overview from structure to innate immunity-associated pathologies," Cell Mol Immunol, vol. 12, no. 1, pp. 5–23, 2015.

[6] Q. Xu, S. Choksi, J. Qu et al., "NADPH oxidases are essential for macrophage differentiation," Journal of Biological Chemistry, vol. 291, no. 38, pp. 20030–20041, 2016.

[7] J. S. Moon, K. Nakahira, K. P. Chung et al., "NOX4-dependent fatty acid oxidation promotes NLRP3 inflammasome activation in macrophages," Nature Medicine, vol. 22, no. 9, pp. 1002–1012, 2016.

[8] B. Adane, H. Ye, N. Khan et al., "The hematopoietic oxidase NOX2 regulates self-renewal of leukemic stem cells," Cell Reports, vol. 27, no. 1, pp. 238–254.e6, 2019.

[9] I. Á. Kovács, M. Horváth, Á. Lányi, G. L. Petheó, and M. Geiszt, "Reactive oxygen species-mediated bacterial killing by B lymphocytes," Journal of Leukocyte Biology, vol. 97, no. 6, pp. 1133–1137, 2015.

[10] A. Savina, C. Jancic, S. Hugues et al., "NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells," Cell, vol. 126, no. 1, pp. 205–218, 2006.

[11] A. R. Mantegazza, A. Savina, M. Vermeulen et al., "NADPH oxidase controls phagosomal pH and antigen crosspresentation in human dendritic cells," Blood, vol. 112, no. 12, pp. 4712–4722, 2008.

[12] I. Dingjan, D. R. J. Verboogen, L. M. Paardekooper et al., "Lipid peroxidation causes endosomal antigen release for cross-presentation," Scientific Reports, vol. 6, no. 1, 2016.

[13] D. Xian, R. Lai, J. Song, X. Xiong, and J. Zhong, "Emerging perspective: role of increased ROS and redox imbalance in skin carcinogenesis," Oxidative Medicine and Cellular Longevity, vol. 2019, Article ID 8127362, 11 pages, 2019.

[14] H. P. Wang, X. Wang, L. F. Gong et al., "Nox1 promotes colon cancer cell metastasis via activation of the ADAM17 pathway," European Review for Medical and Pharmacological Sciences, vol. 20, no. 21, pp. 4474–4481, 2016.

[15] A. Juhasz, S. Markel, S. Gaur et al., "NADPH oxidase 1 supports proliferation of colon cancer cells by modulating reactive oxygen species-dependent signal transduction," Journal of Biological Chemistry, vol. 292, no. 19, pp. 7866–7877, 2017.

[16] J. Aurelius, F. B. Thorén, A. A. Akhiani et al., "Monocytic AML cells inactivate antileukemic lymphocytes: role of NADPH oxidase/gp91phox expression and the PARP-1/PAR pathway of apoptosis," Blood, vol. 119, no. 24, pp. 5832–5837, 2012.

[17] J. Aurelius, A. Martner, R. E. Riise et al., "Chronic myeloid leukemia cells trigger poly(ADP-ribose) polymerase-dependent inactivation and cell death in lymphocytes," Journal of Leukocyte Biology, vol. 93, no. 1, pp. 155–160, 2013.

[18] U. H. Mellqvist, M. Hansson, M. Brune, C. Dahlgren, S. Hermodsson, and K. Hellström, "Natural killer cell dysfunction and apoptosis induced by chronic myelogenous leukemia cells: role of reactive oxygen species and regulation by histamine," Blood, vol. 96, no. 5, pp. 1961–1968, 2000.

[19] K. Hellstrand, A. Asea, C. Dahlgren, and S. Hermodsson, "Histaminergic regulation of NK cells: role of monocytic-derived reactive oxygen metabolites," Journal of Immunology, vol. 153, no. 11, pp. 4940–4947, 1994.

[20] S. M. Kim, D. Y. Hur, S. W. Hong, and J. H. Kim, "EBV-encoded EBNA1 regulates cell viability by modulating mir34a-NOX2-ROS signaling in gastric cancer cells," Biochem Biophys Res Commun, vol. 494, no. 3–4, pp. 550–555, 2017.

[21] S. W. Hong, N. S. Park, M. H. Noh et al., "Combination treatment with erlotinib and ampelopsin overcomes erlotinib resistance in NSCLC cells via the Nox2-ROS-Bim pathway," Lung Cancer, vol. 106, pp. 115–124, 2017.

[22] B. Zhang, Z. Liu, and X. Hu, "Inhibiting cancer metastasis via targeting NAPDH oxidase 4," Biochem Pharmacol, vol. 86, no. 2, pp. 253–266, 2013.

[23] S. H. Kim, K. Y. Kim, S. N. Yu et al., "Silibinin induces mitochondrial NOX4-mediated endoplasmic reticulum stress response and its subsequent apoptosis," BMC Cancer, vol. 16, no. 1, p. 452, 2016.

[24] B. Bánfi, R. A. Clark, K. Steger, and K.-H. Krause, "Two novel proteins activate superoxide generation by the NADPH oxidase NOX1," Journal of Biological Chemistry, vol. 278, no. 6, pp. 3510–3513, 2003.

[25] X. L. Cui, D. Brockman, B. Campos, and L. Myatt, "Expression of NADPH oxidase isoform 1 (Nox1) in human placenta: involvement in preeclampsia," Placenta, vol. 27, no. 4–5, pp. 422–431, 2006.
[26] Y. A. Suh, R. S. Arnold, B. Lassegue et al., "Cell transformation by the superoxide-generating oxidase Mox1," *Nature*, vol. 401, no. 6748, pp. 79–82, 1999.

[27] M. Kato, M. Marumo, J. Nakayama, M. Matsumoto, C. Yabes-Nishimura, and T. Kamata, "The ROS-generating oxidase Nox1 is required for epithelial restitution following colitis," *Experimental Animals*, vol. 65, no. 3, pp. 197–205, 2016.

[28] I. Szanto, L. Rubbia-Brandt, P. Kiss et al., "Expression of NOX1, a superoxide-generating NADPH oxidase, in colon cancer and inflammatory bowel disease," *The Journal of Pathology*, vol. 207, no. 2, pp. 164–176, 2005.

[29] M. Fukuyama, K. Rotkut, T. Sano, H. Miyake, M. Shimada, and S. Tashiro, "Overexpression of a novel superoxide-producing enzyme, NADPH oxidase 1, in adenoma and well differentiated adenocarcinoma of the human colon," *Cancer Lett.*, vol. 221, no. 1, pp. 97–104, 2005.

[30] E. Laurent, J. W. McCoy, R. A. Macina et al., "Nox1 is overexpressed in human colon cancers and correlates with activating mutations in K-Ras," *International Journal of Cancer*, vol. 123, no. 1, pp. 100–107, 2008.

[31] S. D. Lim, C. Sun, J. D. Lambeth et al., "Increased Nox1 and hydrogen peroxide in prostate cancer," *Prostate*, vol. 62, no. 2, pp. 200–207, 2005.

[32] A. Martner, H. G. Wiktorin, B. Lenox et al., "Histamine promotes the development of monocytic-derived dendritic cells and reduces tumor growth by targeting the myeloid NADPH oxidase," *The Journal of Immunology*, vol. 194, no. 10, pp. 5014–5021, 2015.

[33] B. M. Babior, J. D. Lambeth, and W. Nauseef, "The neutrophil NADPH oxidase," *Arch Biochem Biophys*, vol. 397, no. 2, pp. 342–344, 2002.

[34] A. R. Cross and A. W. Segal, "The NADPH oxidase of professional phagocytes—prototype of the NOX electron transport chain systems," *Biochim Biophys Acta*, vol. 1657, no. 1, pp. 1–22, 2004.

[35] R. Kiffin, H. Grauers Wiktorin, M. S. Nilsson et al., "Antileukemic properties of histamine in monocytic leukemia: the role of NOX2," *Front Oncol.*, vol. 8, p. 218, 2018.

[36] J. Aurelius, A. Hallner, O. Werlenius et al., "NOX2-dependent immunosuppression in chronic myelomonocytic leukemia," *Journal of Leukocyte Biology*, vol. 102, no. 2, pp. 459–466, 2017.

[37] E. Aydin, J. Johansson, F. H. Nazir, K. Hellstrand, and A. Martner, "Role of NOX2-derived reactive oxygen species in NK cell-mediated control of murine melanoma metastasis," *Cancer Immunology Research*, vol. 5, no. 9, pp. 804–811, 2017.

[38] T. Kelkka, A. Pizzolla, J. P. Laurila et al., "Mice lacking NCF1 exhibit reduced growth of implanted melanoma and carcinoma tumors," *PLoS One*, vol. 8, no. 12, p. e84148, 2013.

[39] B. Bànfi, B. Malgrange, J. Knisz, K. Steger, M. Dubois-Dauphin, and K.-H. Krause, "NOX3, a superoxide-generating NADPH oxidase of the inner ear," *Journal of Biological Chemistry*, vol. 279, no. 44, pp. 46065–46072, 2004.

[40] R. Paffenholz, R. A. Bergstrom, F. Pasutto et al., "Vestibular defects in head-tilt mice result from mutations in Nox3, encoding an NADPH oxidase," *Genes & Development*, vol. 18, no. 5, pp. 486–491, 2004.

[41] G. Cheng, Z. Cao, X. Xu, E. G. V. Meir, and J. D. Lambeth, "Homologs of gp91 phox : cloning and tissue expression of Nox3, Nox4, and Nox5," *Gene*, vol. 269, no. 1-2, pp. 131–140, 2001.

[42] S. Carnesecchi, J. L. Carpentier, M. Foti, and I. Szanto, "Insulin-induced vascular endothelial growth factor expression is mediated by the NADPH oxidase NOX3," *Experimental Cell Research*, vol. 312, no. 17, pp. 3413–3424, 2006.

[43] M. Geiszt, J. B. Kopp, P. Varmai, and T. L. Leto, "Identification of renox, an NAD(P)H oxidase in kidney," *Proceedings of the National Academy of Sciences*, vol. 97, no. 14, pp. 8010–8014, 2000.

[44] A. Shiose, J. Kuroda, K. Tsuru et al., "A novel superoxide-producing NADPH oxidase in kidney," *Journal of Biological Chemistry*, vol. 276, no. 2, pp. 1417–1423, 2001.

[45] K. Block, Y. Gorin, P. Hoover et al., "NAD(P)H oxidases regulate HIF-2alpha protein expression," *Journal of Biological Chemistry*, vol. 282, no. 11, pp. 8019–8026, 2007.

[46] J. P. Fitzgerald, B. Nayak, K. Shanmugasundaram et al., "Nox4 mediates renal cell carcinoma cell invasion through hypoxia-induced interleukin 6 and 8 production," *PLoS One*, vol. 7, no. 1, p. e30712, 2012.

[47] C. Xia, Q. Meng, L. Z. Liu, Y. Rojanasakul, X. R. Wang, and B. H. Jiang, "Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor," *Cancer Research*, vol. 67, no. 22, pp. 10823–10830, 2007.

[48] T. Shono, N. Yokoyama, T. Uesaka et al., "Enhanced expression of NADPH oxidase Nox4 in human gliomas and its roles in cell proliferation and survival," *International Journal of Cancer*, vol. 123, no. 4, pp. 787–792, 2008.

[49] M. Yamaura, J. Mitsushita, S. Furuta et al., "NADPH oxidase 4 contributes to transformation phenotype of melanoma cells by regulating G2-M cell cycle progression," *Cancer Research*, vol. 69, no. 6, pp. 2647–2654, 2009.

[50] B. Bànfi, G. Molnár, A. Maturana et al., "A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes," *Journal of Biological Chemistry*, vol. 276, no. 46, pp. 37594–37601, 2001.

[51] B. Musset, R. A. Clark, T. E. DeCourcey et al., "NOX5 in human spermatozoa: expression, function, and regulation," *Journal of Biological Chemistry*, vol. 287, no. 12, pp. 9376–9388, 2012.

[52] W. C. Huang, X. Li, J. Liu, J. Lin, and L. W. K. Chung, "Activation of androgen receptor, lipogenesis, and oxidative stress converged by SREBP-1 is responsible for regulating growth and progression of prostate cancer cells," *Molecular Cancer Research*, vol. 10, no. 1, pp. 133–142, 2012.

[53] S. S. Brar, Z. Corbin, T. P. Kennedy et al., "NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells," *American Journal of Physiology-Cell Physiology*, vol. 285, no. 2, pp. C353–C369, 2003.

[54] J. Hong, M. Resnick, J. Behar et al., "Acid-induced p16 hypermethylation contributes to development of esophageal adenocarcinoma via activation of NADPH oxidase NOX5-S," *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 299, no. 3, pp. G706–G706, 2010.

[55] X. De Deken, D. Wang, M.-C. Many et al., "Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family," *Journal of Biological Chemistry*, vol. 275, no. 30, pp. 23227–23233, 2000.

[56] A. W. Boots, M. Hristova, D. I. Kasahara, G. R. M. M. Hennen, A. Bast, and A. van der Vliet, "ATP-mediated activation of the NADPH oxidase DUOX1 mediates airway epithelial
responses to bacterial stimuli,” *Journal of Biological Chemistry*, vol. 284, no. 26, pp. 17858–17867, 2009.

[57] R. Forteza, M. Salathe, F. Miot, R. Forteza, and G. E. Conner, “Regulated hydrogen peroxide production by Duox in human airway epithelial cells,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 32, no. 5, pp. 462–469, 2005.

[58] M. Pulcrano, H. Boukerhis, M. Talbot et al., “Poorly differentiated follicular thyroid carcinoma: prognostic factors and relevance of histological classification,” *Thyroid*, vol. 17, no. 7, pp. 639–646, 2007.

[59] R. Ameziane-El-Hassani, M. Talbot, M. C. de Souza Dos Santos et al., “NADPH oxidase DUOX1 promotes long-term persistence of oxidative stress after an exposure to irradiation,” *Proceedings of the National Academy of Sciences*, vol. 112, no. 16, pp. 5051–5056, 2015.

[60] A. C. Little, D. Sham, M. Hristova et al., “DUOX1 silencing in lung cancer promotes EMT, cancer stem cell characteristics and invasive properties,” *Oncogenesis*, vol. 5, no. 10, p. e261, 2016.

[61] S. Luxen, S. A. Belinsky, and U. G. Knaus, “Silencing of DUOXNDPH oxidases by promoter hypermethylation in lung cancer,” *Cancer Research*, vol. 68, no. 4, pp. 1037–1045, 2008.

[62] C. Dupuy, M. Pomerance, R. Ohayon et al., “Thyroid oxidase (THOX2) gene expression in the rat thyroid cell line FRTL-5,” *Biochemical and Biophysical Research Communications*, vol. 277, no. 2, pp. 287–292, 2000.

[63] R. A. El Hassan, N. Benfares, B. Caliou et al., “Dual oxidase2 is expressed all along the digestive tract,” *American Journal of Physiology-Gastrointestinal and Liver Physiology*, vol. 288, no. 5, pp. G93–G942, 2005.

[64] H. Grasberger, J. Gao, H. Nagao-Kitamoto et al., “Increased expression of DUOX2 is an epithelial response to mucosal dysbiosis required for immune homeostasis in mouse intestine,” *Gastroenterology*, vol. 149, no. 7, pp. 1849–1859, 2015.

[65] F. Sommer and E. Backhed, “The gut microbiota engages different signaling pathways to induce Duox2 expression in the ileum and colon epithelium,” *Mucosal Immunol*, vol. 8, no. 2, pp. 372–379, 2015.

[66] D. V. Bann, Q. Jin, K. E. Sheldon et al., “Genetic variants implicate dual oxidase-2 in familial and sporadic Nonmedullary thyroid cancer,” *Cancer Research*, vol. 79, no. 21, pp. 5490–5499, 2019.

[67] Y. Wu, S. Antony, A. Juhasz et al., “Up-regulation and sustained activation of Stat1 are essential for interferon-gamma (IFN-gamma)-induced dual oxidase 2 (Duox2) and dual oxidase A2 (DuoxA2) expression in human pancreatic cancer cell lines,” *Journal of Biological Chemistry*, vol. 286, no. 14, pp. 12245–12256, 2011.

[68] Y. Wu, J. Lu, S. Antony et al., “Activation of TLR4 is required for the synergistic induction of dual oxidase 2 and dual oxidase A2 by IFN-γ and lipopolysaccharide in human pancreatic cancer cell lines,” *The Journal of Immunology*, vol. 190, no. 4, pp. 1859–1872, 2013.

[69] T. Mochizuki, S. Furuta, J. Mitsushita et al., “Inhibition of NADPH oxidase 4 activates apoptosis via the AKT/apoptosis signal-regulating kinase 1 pathway in pancreatic cancer Panc-1 cells,” *Oncogene*, vol. 25, no. 26, pp. 3699–3707, 2006.

[70] E. C. Vaquero, M. Edderkouai, S. J. Pandol, I. Guuskovskaya, and A. S. Gukovskaya, “Reactive oxygen species produced by NAD(P)H oxidase inhibit apoptosis in pancreatic cancer cells,” *Journal of Biological Chemistry*, vol. 279, no. 33, pp. 34643–34654, 2004.

[71] J. K. Lee, M. Edderkouai, P. Truong et al., “NADPH oxidase promotes pancreatic cancer cell survival via inhibiting JAK2 dephosphorylation by tyrosine phosphatases,” *Gastroenterology*, vol. 133, no. 5, pp. 1637–1648, 2007.

[72] J. Li, T. Lan, C. Zhang et al., “Reciprocal activation between IL-6/STAT3 and NOX4/Akt signalings promotes proliferation and survival of non-small cell lung cancer cells,” *Oncotarget*, vol. 6, no. 2, pp. 1031–1048, 2015.

[73] C. Zhang, T. Lan, J. Hou et al., “NOX4 promotes non-small cell lung cancer cell proliferation and metastasis through positive feedback regulation of PI3K/Akt signaling,” *Oncotarget*, vol. 5, no. 12, pp. 4392–4405, 2014.

[74] R. Rastogi, X. Geng, F. Li, and Y. Ding, “NOX activation by subunit interaction and underlying mechanisms in disease,” *Frontiers in Cellular Neuroscience*, vol. 10, 2017.

[75] B. A. Diebold and G. M. Bokoch, “Molecular basis for Rac2 regulation of phagocyte NADPH oxidase,” *Nat Immunol*, vol. 2, no. 3, pp. 211–215, 2001.

[76] M. T. Quinn, C. A. Parkos, and A. J. Jesaitis, “[48] Purification of human neutrophil NADPH oxidase cytochrome b-558 and association with Rap 1A,” *Methods Enzymol.*, vol. 255, pp. 476–487, 1995.

[77] M. Takahashi, T. J. Dillon, C. Liu, Y. Kariya, Z. Wang, and P. J. S. Stork, “Protein kinase A-dependent phosphorylation of Rap1 regulates its membrane localization and cell migration,” *Journal of Biological Chemistry*, vol. 288, no. 39, pp. 27712–27723, 2013.

[78] F. Kotsias, E. Hoffmann, S. Amigorena, and A. Savina, “Reactive oxygen species production in the phagosome: impact on antigen presentation in dendritic cells,” *Antioxid Redox Signal*, vol. 18, no. 6, pp. 714–729, 2013.

[79] D. E. Arnold and J. R. Heimall, “A review of chronic granulomatous disease,” *Advances in Therapy*, vol. 34, no. 12, pp. 2543–2557, 2017.

[80] R. L. Baehner and D. G. Nathan, “Leukocyte oxidase: defective activity in chronic granulomatous disease,” *Science*, vol. 155, no. 3764, pp. 835–836, 1967.

[81] P. G. Quic, J. G. White, B. Holmes, and R. A. Good, “In vitro bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood,” *Journal of Clinical Investigation*, vol. 46, no. 4, pp. 668–679, 1967.

[82] A. Pizzolla, M. Hultqvist, B. Nilson et al., “Reactive oxygen species produced by the NADPH oxidase 2 complex in monocyes protect mice from bacterial infections,” *The Journal of Immunology*, vol. 188, no. 10, pp. 5003–5011, 2012.

[83] A. Martner, E. Aydin, and K. Hellstrand, “Association of NOX2 subunits with autoimmunity, tumor growth and metastasis,” *The Journal of Pathology*, vol. 247, no. 2, pp. 151–154, 2019.

[84] J. Zhong, L. M. Olsson, V. Urbonaviciute, M. Yang, L. Bäckdahl, and R. Holmdahl, “Association of NOX2 subunits with arthritis, in *Oxidative Medicine and Cellular Longevity*, vol. 133, no. 5, pp. 1637–1648, 2007.

[85] S. Stuberius, “Estrogen and 2-methoxyestradiol: regulation of arthritis, inflammation and reactive oxygen species,” in *Department of Rheumatology and Inflammation Research*, University of Gothenburg, Gothenburg, 2014.
Oxidative Medicine and Cellular Longevity

[86] M. Vulcano, S. Dusi, D. Lissandrini et al., “Toll receptor-mediated regulation of NADPH oxidase in human dendritic cells,” The Journal of Immunology, vol. 173, no. 9, pp. 5749–5756, 2004.

[87] N. R. Madamanchi and M. S. Runge, “Mitochondrial dysfunction in atherosclerosis,” Circulation Research, vol. 100, no. 4, pp. 460–473, 2007.

[88] M. Jastrow, A. S. Divakaruni, S. Mookerjee, J. R. Treberg, and M. D. Brand, “Mitochondrial proton and electron leaks,” Essays Biochem, vol. 47, pp. 53–67, 2010.

[89] L. B. Sullivan and N. S. Chandel, “Mitochondrial reactive oxygen species and cancer,” Cancer Metab, vol. 2, no. 1, p. 17, 2014.

[90] R. Kiessling, K. Wasserman, S. Horiguchi et al., “Tumor-induced immune dysfunction,” Cancer Immunology Immunotherapy, vol. 48, no. 7, pp. 353–362, 1999.

[91] M. Hansson, A. Asa, U. Ersson, S. Hermodsson, and K. Hellstrand, “Induction of apoptosis in NK cells by monocyte-derived reactive oxygen metabolites,” Journal of Immunology, vol. 156, no. 1, pp. 42–47, 1996.

[92] M. Schieber and N. S. Chandel, “ROS function in redox signaling and oxidative stress,” Current Biology, vol. 24, no. 10, pp. R453–R462, 2014.

[93] C. C. Winterbourn and M. B. Hampton, “Thiol chemistry and specificity in redox signaling,” Free Radical Biology and Medicine, vol. 45, no. 5, pp. 549–561, 2008.

[94] L. He, T. He, S. Farrar, L. Ji, T. Liu, and X. Ma, “Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species,” Cell Physiol Biochem, vol. 44, no. 2, pp. 532–553, 2017.

[95] B. Marengo, M. Nitti, A. L. Furfaro et al., “Redox homeostasis and cellular antioxidant systems: crucial players in cancer growth and therapy,” Oxidative Medicine and Cellular Longevity, vol. 2016, Article ID 6253641, 16 pages, 2016.

[96] A. Kobayashi, M. I. Kang, H. Okawa et al., “Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2,” Molecular and Cellular Biology, vol. 24, no. 16, pp. 7130–7139, 2004.

[97] R. Venugopal and A. K. Jaiswal, “Nrf1 and Nrf2 positively regulate c-Fos and FRA1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quione oxidoreductase 1 gene,” Proceedings of the National Academy of Sciences, vol. 93, no. 25, pp. 14960–14965, 1996.

[98] T. Jarmi and A. Agarwal, “Heme oxygenase and renal disease,” Current Hypertension Reports, vol. 11, no. 1, pp. 56–62, 2009.

[99] W. A. Solis, T. P. Dalton, M. Z. Dieter et al., “Glutamate-cysteine ligase modifier subunit: mouse Gclm gene structure and regulation by agents that cause oxidative stress,” Biochem Pharmacol, vol. 63, no. 9, pp. 1739–1754, 2002.

[100] C. A. Neumann, J. Cao, and Y. Manevich, “Peroxiredoxin 1 and its role in cell signaling,” Cell Cycle, vol. 8, no. 24, pp. 4072–4078, 2014.

[101] J. Chaudiere and R. Ferrari-Ilhou, “Intracellular antioxidants: from chemical to biochemical mechanisms,” Food Chem Toxicol, vol. 37, no. 9-10, pp. 949–962, 1999.

[102] J. Limón-Pacheco and M. E. Gomsebatt, “The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress,” Mutation Research - Genetic Toxicology and Environmental Mutagenesis, vol. 674, no. 1-2, pp. 137–147, 2009.

[103] P. Chiarugi, “From anchorage dependent proliferation to survival: lessons from redox signalling,” JUBMB Life, vol. 60, no. 5, pp. 301–307, 2008.

[104] M. R. Porembska, J. B. Mitchell, B. A. Belt et al., “Pancreatic adenocarcinoma induces bone marrow mobilization of myeloid-derived suppressor cells which promote primary tumor growth,” Cancer Immunology, Immunotherapy, vol. 61, no. 9, pp. 1373–1385, 2012.

[105] L. Wang, E. W. Y. Chang, S. C. Wong, S. M. Ong, D. Q. Y. Chong, and K. L. Ling, “Increased myeloid-derived suppressor cells in gastric cancer correlate with cancer stage and plasma S100A8/A9 proinflammatory proteins,” Journal of Immunology, vol. 190, no. 2, pp. 794–804, 2013.

[106] C. M. Diaz-Montero, M. L. Salem, M. I. Nishimura, E. Garrett-Mayer, D. J. Cole, and A. J. Montero, “Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy,” Cancer Immunology, Immunotherapy, vol. 58, no. 1, pp. 49–59, 2009.

[107] C. Bergenfelz, A. M. Larsson, K. von Stedingk et al., “Systemic monocytic-MDSCs are generated from monocytes and correlate with disease progression in breast cancer patients,” PLoS ONE, vol. 10, no. 5, 2015.

[108] S. J. Antonia, N. Mirza, I. Fricke et al., “Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer,” Clinical Cancer Research, vol. 12, no. 3, pp. 878–887, 2006.

[109] Y. Tsuchiya, M. Igarashi, R. Suzuki, and K. Kumagai, “Production of colony-stimulating factor by tumor cells and the factor-mediated induction of suppressor cells,” Journal of Immunology, vol. 141, no. 2, pp. 699–708, 1988.

[110] Y. Mao, I. Poschke, and R. Kiessling, “Tumour-induced immune suppression: role of inflammatory mediators released by myelomonocytic cells,” Journal of Internal Medicine, vol. 276, no. 2, pp. 154–170, 2014.

[111] A. Martner, F. B. Thorén, J. Aurelius, and K. Hellstrand, “Immunotherapeutic strategies for relapse control in acute myeloid leukemia,” Blood Reviews, vol. 27, no. 5, pp. 209–216, 2013.

[112] Y. Kondo, S. Ariti, A. Mori, M. Furutani, T. Chiba, and M. Imamura, “Enhancement of angiogenesis, tumor growth, and metastasis by transfection of vascular endothelial growth factor into LoVo human colon cancer cell line,” Clinical Cancer Research, vol. 6, no. 2, pp. 622–630, 2000.

[113] A. M. Roberts, L. R. Watson, A. J. Evans, D. A. Foster, M. S. Irwin, and M. Ohh, “Suppression of hypoxia-inducible factor 2 Restores p53 activity via Hdm2 and reverses chemoresistance of renal carcinoma cells,” Cancer Research, vol. 69, pp. 9056–9064, 2009.

[114] J. T. Ender, K. L. Bennewith, M. Nicolau et al., “Lysyl oxidase is essential for hypoxia-induced metastasis,” Nature, vol. 440, no. 7088, pp. 1222–1226, 2006.

[115] A. K. Azab, J. Hu, P. Quang et al., “Hypoxia promotes dissemination of multiple myeloma through acquisition of epithelial to mesenchymal transition-like features,” Blood, vol. 119, no. 24, pp. 5782–5794, 2012.

[116] B. Muz, P. de la Puente, F. Azab, and A. K. Azab, “The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy,” Hypoxia, vol. 3, pp. 83–92, 2015.

[117] S. Movafagh, S. Crook, and K. Vo, “Regulation of hypoxia-inducible factor-1α by reactive oxygen species: new
developments in an old debate,” *Journal of Cellular Biochemistry*, vol. 116, no. 5, pp. 696–703, 2015.

[118] E. Bolton-Gillespie, M. Schemionek, H. U. Klein et al., “Genomic instability may originate from imatinib-refractory chronic myeloid leukemia stem cells,” *Blood*, vol. 121, no. 20, pp. 4175–4183, 2013.

[119] M. Sattler, S. Verma, G. Shrikhande et al., “The BCR/ABL tyrosine kinase induces production of reactive oxygen species in hematopoietic cells,” *Journal of Biological Chemistry*, vol. 275, no. 32, pp. 24273–24278, 2000.

[120] U. E. Martinez-Outschoorn et al., “BRCA1 mutations drive oxidative stress and glycolysis in the tumor microenvironment: implications for breast cancer prevention with antioxidant therapies,” *Cell Cycle*, vol. 11, no. 23, pp. 4402–4413, 2014.

[121] A. Salazar-Ramiro, D. Ramírez-Ortega, V. Pérez de la Cruz et al., “Role of redox status in development of Glioblastoma,” *Frontiers in Immunology*, vol. 7, no. APR, 2016.

[122] K. Roy, Y. Wu, J. L. Meitzler et al., “NADPH oxidases and cancer,” *Clin Sci (Lond)*, vol. 128, no. 12, pp. 863–875, 2015.

[123] J. L. Meitzler, M. M. Konate, and J. H. Doroshow, “Hydrogen peroxide-producing NADPH oxidases and the promotion of migratory phenotypes in cancer,” *Arch Biochem Biophys*, vol. 675, p. 108076, 2019.

[124] N. Hempel, P. M. Carrico, and J. A. Melendez, “Manganese superoxide dismutase (Sod2) and redox-control of signaling events that drive metastasis,” *Anti-Cancer Agents in Medicinal Chemistry*, vol. 11, no. 2, pp. 191–201, 2011.

[125] P. Chiariugi, G. Pani, E. Giannoni et al., “Reactive oxygen species as essential mediators of cell adhesion,” *Journal of Cell Biology*, vol. 161, no. 5, pp. 933–944, 2003.

[126] L. Tochhawng, S. Deng, S. Pervaiz, and C. T. Yap, “Redox regulation of cancer cell migration and invasion,” *Mitochondrion*, vol. 13, no. 3, pp. 246–253, 2013.

[127] H. Peshavariya, G. J. Dusting, F. Jiang et al., “NADPH oxidase isoform selective regulation of endothelial cell proliferation and survival,” *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 380, no. 2, pp. 193–204, 2009.

[128] Y. Li, N. Han, T. Yin et al., “Lentivirus-mediated Nox4 shRNA invasion and angiogenesis enhances radiosensitivity in human glioblastoma,” *Oxidative Medicine and Cellular Longevity*, vol. 2014, 9 pages, 2014.

[129] M. Wang, J. S. Kirk, S. Venkataraman et al., “Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1α and vascular endothelial growth factor,” *Oncogene*, vol. 24, no. 55, pp. 8154–8166, 2005.

[130] Z. A. Sibenaller, J. L. Welsh, C. du et al., “Extracellular superoxide dismutase suppresses hypoxia-inducible factor-1α in pancreatic cancer,” *Free Radical Biology and Medicine*, vol. 69, pp. 357–366, 2014.

[131] C. R. Hoyal, A. Gutierrez, B. M. Young et al., “Modulation of p47PHox activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase,” *Proceedings of the National Academy of Sciences*, vol. 100, no. 9, pp. 5130–5135, 2003.

[132] B. Govindarajan, J. E. Sligh, B. J. Vincent et al., “Overexpression of Akt converts radial growth melanoma to vertical growth melanoma,” *Journal of Clinical Investigation*, vol. 117, no. 3, pp. 719–729, 2007.

[133] S. K. Sastry and L. A. Elferink, “Checks and balances: interplay of RTKs and PTPs in cancer progression,” *Biochem Pharmacol*, vol. 82, no. 5, pp. 435–440, 2011.

[134] H. J. Forman, P. Ursini, and M. Maiorino, “An overview of mechanisms of redox signaling,” *Journal of Molecular and Cellular Cardiology*, vol. 73, pp. 2–9, 2014.

[135] K. Block and Y. Gorin, “Aiding and abetting roles of NOX oxidases in cellular transformation,” *Nature Reviews Cancer*, vol. 12, no. 9, pp. 627–637, 2012.

[136] R. Gopalakrishna and S. Jaken, “Protein kinase C signaling and oxidative stress,” *Free Radical Biology and Medicine*, vol. 28, no. 9, pp. 1349–1361, 2000.

[137] L. Lignitto, S. E. LeBoeuf, H. Homer et al., “Nrf2 activation promotes lung cancer metastasis by inhibiting the degradation of Bach1,” *Cell*, vol. 178, no. 2, pp. 316–329.e18, 2019.

[138] C. Wiel, K. le Gal, M. X. Ibrahim et al., “BACH1 stabilization by antioxidants stimulates lung cancer metastasis,” *Cell*, vol. 178, no. 2, pp. 330–345.e22, 2019.

[139] M. A. Ligtenberg, O. Çınar, R. Holmdahl, D. Mougiaskakos, and R. Kiessling, “Methyloleontanethrene-induced sarcomas develop independently from NOX2-derived ROS,” *PLoS ONE*, vol. 10, no. 6, 2015.

[140] F. Okada, M. Kobayashi, H. Tanaka et al., “The role of nicotinamide adenine dinucleotide phosphate oxide-derived reactive oxygen species in the acquisition of metastatic ability of tumor cells,” *American Journal of Pathology*, vol. 169, no. 1, pp. 294–302, 2006.

[141] L. van der Weyden, A. O. Speak, A. Światkowska et al., “Pulmonary metastatic colonisation and granulomas in NOX2-deficient mice,” *The Journal of Pathology*, vol. 246, no. 3, pp. 300–310, 2018.

[142] A. Betten, C. Dahlgren, U. H. Mellqvist, S. Hermodsson, and K. Hellstrand, “Oxygen radical-induced natural killer cell dysfunction: role of myeloperoxidase and regulation by sertonin,” *Journal of Leukocyte Biology*, vol. 75, no. 6, pp. 1111–1115, 2004.

[143] S. Chatterjee, E. A. Browning, N. K. Hong et al., “Membrane depolarization is the trigger for PI3K/Akt activation and leads to the generation of ROS,” *American Journal of Physiology-Heart and Circulatory Physiology*, vol. 302, no. 1, pp. H105–H114, 2012.

[144] A. Betten, C. Dahlgren, S. Hermodsson, and K. Hellstrand, “Histamine inhibits neutrophil NADPH oxidase activity triggered by the lipoxin A4 receptor-specific peptide agonist Trp-Lys-Tyr-Met-Val-Met,” *Scand J Immunol*, vol. 58, no. 3, pp. 321–326, 2003.

[145] B. A. Babbin, A. J. Jesaitis, A. I. Ivanov et al., “Formyl peptide receptor-1 activation enhances intestinal epithelial cell restitution through phosphatidylinositol 3-kinase-dependent activation of Rac1 and Cdc42,” *The Journal of Immunology*, vol. 179, no. 12, pp. 8112–8121, 2007.

[146] A. A. Akhiani, A. Hallner, R. Kiffin et al., “Idelalisib promotes anti CD20-mediated ADCC by inhibiting immunosuppressive ROS production in monocytes,” in *International Congress of Immunology*, Melbourne, Australia, 2016.

[147] F. B. Thoren, J. Aurelius, and A. Martner, “Antitumor properties of histamine in vivo,” *Nature Medicine*, vol. 17, no. 5, pp. 537–537, author reply 538, 2011.

[148] H. Grauers Wiktorin, M. S. Nilsson, R. Kiffin et al., “Histamine targets myeloid-derived suppressor cells and improves the anti-tumor efficacy of PD-1/PD-L1 checkpoint blockade,” *Cancer Immunol Immunother*, vol. 68, no. 2, pp. 163–174, 2019.
from bone marrow stromal cells to leukemic blasts,” *Blood*, vol. 130, no. 14, pp. 1649–1660, 2017.

[150] E. Aydin, A. Hallner, H. Grauers Wiktorin, A. Staffas, K. Hellstrand, and A. Martner, “NOX2 inhibition reduces oxidative stress and prolongs survival in murine KRAS-induced myeloproliferative disease,” *Oncogene*, vol. 38, no. 9, pp. 1534–1543, 2019.

[151] H. Grauers Wiktorin, T. Nilsson, E. Aydin, K. Hellstrand, L. Palmqvist, and A. Martner, “Role of NOX2 for leukemic expansion in a murine model of BCR-ABL-leukemia,” *British Journal of Haematology*, vol. 182, no. 2, pp. 290–294, 2018.

[152] N. E. Thomas, K. J. Busam, L. From et al., “Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment and melanoma study,” *Journal of Clinical Oncology*, vol. 31, no. 33, pp. 4252–4259, 2013.

[153] T. Donnem, S. M. Hald, E. E. Paulsen et al., “Stromal CD8+ T-cell density—a promising supplement to TNM staging in non-small cell lung cancer,” *Clinical Cancer Research*, vol. 21, no. 11, pp. 2635–2643, 2015.

[154] M. V. Dieci, M. C. Mathieu, V. Guarneri et al., “Prognostic and predictive value of tumor-infiltrating lymphocytes in two phase III randomized adjuvant breast cancer trials,” *Annals of Oncology*, vol. 26, no. 8, pp. 1698–1704, 2015.

[155] M. Okabe, U. Toh, N. Iwakuma et al., “Predictive factors of the tumor immunological microenvironment for long-term follow-up in early stage breast cancer,” *Cancer Science*, vol. 108, no. 1, pp. 81–90, 2017.

[156] E. Sato, S. H. Olson, J. Ahn et al., “Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 51, pp. 18538–18543, 2005.

[157] G. Esendagli, K. Bruderek, T. Goldmann et al., “Malignant and non-malignant lung tissue areas are differentially populated by natural killer cells and regulatory T cells in non-small cell lung cancer,” *Lung Cancer*, vol. 59, no. 1, pp. 32–40, 2008.

[158] S. Coca, J. Perez-Piqueras, D. Martinez et al., “The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma,” *Cancer*, vol. 79, no. 12, pp. 2320–2328, 1997.

[159] F. R. Villegas, S. Coca, V. G. Villarrubia et al., “Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer,” *Lung Cancer*, vol. 35, no. 1, pp. 23–28, 2002.

[160] L. Jiang, Z. Zhao, S. Jiang et al., “Immunological markers predict the prognosis of patients with squamous non-small cell lung cancer,” *Immunologic Research*, vol. 62, no. 3, pp. 316–324, 2015.

[161] M. F. Chevalier, S. Trabanieli, J. Racle et al., “ILC2-modulated T cell-to-MDSC balance is associated with bladder cancer recurrence,” *Journal of Clinical Investigation*, vol. 127, no. 8, pp. 2916–2929, 2017.

[162] S. Han, Y. Liu, Q. Li, Z. Li, H. Hou, and A. Wu, “Pre-treatment neutrophil-to-lymphocyte ratio is associated with neutrophil and T-cell infiltration and predicts clinical outcome in patients with glioblastoma,” *BMC Cancer*, vol. 15, no. 1, p. 617, 2015.

[163] F. Donskov, M. Hokland, N. Marcussen, H. H. Torp Madsen, and H. von der Maase, “Monocytes and neutrophils as “bad guys” for the outcome of interleukin-2 with and without histamine in metastatic renal cell carcinoma—results from a randomised phase II trial,” *British Journal of Cancer*, vol. 94, no. 2, pp. 218–226, 2006.

[164] M. D. Iglesias, J. S. Parker, A. E. Hoadley, C. M. Perou, and B. G. Vincent, “Genomic Analysis of Immune Cell Infiltrates Across 11 Tumor Types,” *Journal of the National Cancer Institute*, vol. 108, no. 11, p. djw144, 2016.

[165] W. Q. Wang, L. Liu, H. X. Xu et al., “Infiltrating immune cells and gene mutations in pancreatic ductal adenocarcinoma,” *British Journal of Surgery*, vol. 103, no. 9, pp. 1189–1199, 2016.

[166] T. M. Nywening, B. A. Belt, D. R. Cullinan et al., “Targeting both tumour-associated CXCR2(+) neutrophils and CCR2(+) macrophages disrupts myeloid recruitment and improves chemotherapeutic responses in pancreatic ductal adenocarcinoma,” *Gut*, vol. 67, no. 6, pp. 1112–1123, 2018.

[167] A. J. Templeton, M. G. McNamara, B. Šeruga et al., “Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis,” *JNCI: Journal of the National Cancer Institute*, vol. 106, no. 6, p. dju124, 2014.

[168] C. Groth, X. Hu, R. Weber et al., “Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression,” *British Journal of Cancer*, vol. 120, no. 1, pp. 16–25, 2019.

[169] D. I. Gabrilovich and S. Nagaraj, “Myeloid-derived suppressor cells as regulators of the immune system,” *Nature Reviews Immunology*, vol. 9, no. 3, pp. 162–174, 2009.

[170] A. H. Zea, P. C. Rodriguez, M. B. Atkins et al., “Arginase-producing myeloid suppressor cells in renal cell carcinoma patients: a mechanism of tumor evasion,” *Cancer Research*, vol. 65, no. 8, pp. 3044–3048, 2005.

[171] E. Teycanov, J. Mastio, E. Chen, and D. I. Gabrilovich, “Plasticity of myeloid-derived suppressor cells in cancer,” *Curr Opin Immunol*, vol. 51, pp. 76–82, 2018.

[172] Y. Zhu, J. M. Herndon, D. K. Sojka et al., “Tissue-resident macrophages in pancreatic ductal adenocarcinoma originate from embryonic hematopoiesis and promote tumor progression,” *Immunity*, vol. 47, no. 2, pp. 323–338.e6, 2017.

[173] T. Condamine, I. Ramachandran, J. I. Youn, and D. I. Gabrilovich, “Regulation of tumor metastasis by myeloid-derived suppressor cells,” *Annual Review of Medicine*, vol. 66, no. 1, pp. 97–110, 2015.

[174] Y. Lin, J. Xu, and H. Lan, “Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications,” *Journal of Hematology & Oncology*, vol. 12, no. 1, p. 76, 2019.

[175] K. Movahedi, D. Laoui, C. Gysemans et al., “Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes,” *Cancer Research*, vol. 70, no. 14, pp. 5728–5739, 2010.

[176] K. Y. Gerrick, E. R. Gerrick, A. Gupta, S. J. Wheelan, S. Yegnasubramanian, and E. M. Jaffe, “Transcriptional profiling identifies novel regulators of macrophage polarization,” *PLoS One*, vol. 13, no. 12, p. e0208602, 2018.

[177] G. Khayrullina, S. Bermudez, and K. R. Byrnes, “Inhibition of NOX2 reduces locomotor impairment, inflammation, and oxidative stress after spinal cord injury,” *Journal of Neuroinflammation*, vol. 12, no. 1, p. 172, 2015.
immunosuppressor cell abundance in a murine model of spontaneous medulloblastoma,” *Journal of Leukocyte Biology*, vol. 95, no. 2, pp. 357–367, 2014.

[194] Y. Nefedova, M. Fishman, S. Sherman, X. Wang, A. A. Beg, and D. I. Gabrilovich, “Mechanism of all-trans retinoic acid effect on tumor-associated myeloid-derived suppressor cells,” *Cancer Research*, vol. 67, no. 22, pp. 11021–11028, 2007.

[195] B. Almand, J. I. Clark, E. Nikitina et al., “Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer,” *Journal of Immunology*, vol. 166, no. 1, pp. 678–689, 2001.

[196] D. I. Gabrilovich, M. P. Velders, E. M. Sotomayor, and W. M. Kast, “Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells,” *Journal of Immunology*, vol. 166, no. 9, pp. 5398–5406, 2001.

[197] M. Mohity, S. Morbelli, D. Isnaridon et al., “All-trans retinoic acid skews monocyte differentiation into interleukin-12-secreting dendritic-like cells,” *British Journal of Hematology*, vol. 122, no. 5, pp. 829–836, 2003.

[198] A. Gervais, J. Leveque, F. Bouet-Toussaint et al., “Dendritic cells are defective in breast cancer patients: a potential role for polyclone in this immunodeficiency,” *Breast Cancer Research*, vol. 7, no. 3, pp. R326–R335, 2005.

[199] C. Hegeduš, K. Kovács, Z. Polgár et al., “Redox control of cancer cell destruction,” *Redox Biology*, vol. 16, pp. 59–74, 2018.

[200] W. J. Blot, J. Y. Li, P. R. Taylor et al., “Nutrition intervention trials in linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and diseasespecific mortality in the general population,” *Journal of the National Cancer Institute*, vol. 85, no. 18, pp. 1483–1491, 1993.

[201] B. Li, P. R. Taylor, J. Y. Li et al., “Linxian nutrition intervention trials design, methods, participant characteristics, and compliance,” *Annals of Epidemiology*, vol. 3, no. 6, pp. 577–585, 1993.

[202] S. M. Wang, P. R. Taylor, J. H. Fan et al., “Effects of nutrition intervention on total and cancer mortality: 25-year post-trial follow-up of the 5:25-year linxian Nutrition Intervention Trial,” *Journal of the National Cancer Institute*, vol. 110, no. 11, pp. 1229–1238, 2018.

[203] The Alpha-Tocopherol Beta Carotene Cancer Prevention Study, G, “The effect of vitamin e and beta carotene on the incidence of lung cancer and other cancers in male smokers,” *New England Journal of Medicine*, vol. 330, no. 15, pp. 1029–1035, 1994.

[204] F. Lo-Coco, G. Arvisati, M. Vignetti et al., “Retinoic acid and arsenic trioxide for acute promyelocytic leukemia,” *New England Journal of Medicine*, vol. 369, no. 2, pp. 111–121, 2013.

[205] S. Kusmartsev, F. Cheng, B. Yu et al., “All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination,” *Cancer Research*, vol. 63, no. 15, pp. 4441–4449, 2003.

[206] N. Mirza, M. Fishman, I. Fricke et al., “All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients,” *Cancer Research*, vol. 66, no. 18, pp. 9299–9307, 2006.

[207] C. Ilozoan, S. Antonia, A. Chiappori, D. T. Chen, and D. Gabrilovich, “Therapeutic regulation of myeloid-derived suppressor cells and immune response to cancer vaccine in patients with extensive stage small cell lung cancer,” *Cancer...*
[208] M. Brune, S. Castaigne, J. Catalano et al., “Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial,” Blood, vol. 108, no. 1, pp. 88–96, 2006.

[209] J. Aurelius, A. Martner, M. Brune et al., “Remission maintenance in acute myeloid leukemia: impact of functional histamine H2 receptors expressed by leukemic cells,” Haematologica, vol. 97, no. 12, pp. 1904–1908, 2012.

[210] E. Wallhult, J. Whisnant, J. M. Rowe et al., “Impact on quality of life of postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myelogenous leukemia,” in American Society of Hematology 49th Annual Meeting and Exposition, Atlanta, Georgia, 2007.