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Review

Redox Remodeling of the Tumor Microenvironment by Reactive Oxygen Species

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Abstract

Reactive oxygen species (ROS) are increasingly recognized as important regulators of redox signaling rather than merely byproducts of cellular metabolism, contributing to the dynamic remodeling of the tumor microenvironment (TME). They originate from multiple intracellular and microenvironmental sources, including mitochondrial metabolism, NADPH oxidases, and inflammatory cells, while their biological effects are shaped by antioxidant systems such as the Keap1/Nrf2 axis and glutathione- and thioredoxin-dependent networks. This review examines the bidirectional relationship between ROS and the TME, focusing on redox-dependent communication among malignant, stromal, endothelial, and immune cells, and the extracellular matrix. Particular attention is given to the spatial and cellular heterogeneity of redox regulation and the context-dependent effects of ROS on tumor progression, metastatic dissemination, immune responses, and resistance to anticancer therapies. We also discuss how redox adaptation and antioxidant defenses contribute to tumor-cell survival under oxidative and therapeutic stress. Finally, we critically evaluate emerging strategies for exploiting tumor redox vulnerabilities and the potential of redox-related biomarkers while considering the limitations that currently hinder their clinical translation. By integrating molecular mechanisms with spatial TME organization, cellular crosstalk, and translational challenges, this review provides a framework for understanding how context-dependent redox remodeling shapes tumor behavior and therapeutic susceptibility.

Keywords: reactive oxygen species; tumor microenvironment; redox signaling; Nrf2; immune modulation; oxidative stress; cancer therapy resistance

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1. Introduction

ROS comprise a chemically heterogeneous group of oxygen-derived species that includes both free radicals, such as the superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), and alkoxyl radicals ($RO^{\bullet}$) and non-radical oxidants, namely hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), and organic hydroperoxides (ROOH) [1,2]. Although they were initially regarded as harmful by-products of aerobic metabolism, generated in part through electron leakage within the mitochondrial

electron transport chain (ETC) and subsequent reduction of molecular oxygen, ROS are now widely recognized as integral mediators of normal cellular physiology [3]. Indeed, at controlled concentrations, ROS contribute to host defense, stress responses, signal transduction, and cellular adaptation [3,4]. Among these species, H_2O_2 plays a pivotal role in redox signaling because of its relative chemical stability, tightly regulated enzymatic generation and elimination, and capacity to mediate reversible oxidation of protein thiol groups [5,6]. Such thiol-based modifications can modulate the activity of redox-sensitive proteins, thus influencing downstream signaling networks [3]. Accordingly, the biological effects of ROS cannot be attributed to their abundance alone; rather, they are governed by their chemical identity, site and mode of generation, local concentration and persistence, molecular targets, and the antioxidant capacity of the relevant cellular compartment [7,8].

This conceptual shift has refined the classical understanding of oxidative stress by distinguishing between physiological redox signaling and excessive oxidation. Under physiological conditions, controlled ROS production supports cellular signaling and adaptive responses, a state termed oxidative eustress. Conversely, excessive or prolonged oxidant exposure that exceeds antioxidant and repair capacity can disrupt redox regulation and cause molecular damage, a condition referred to as oxidative distress [9,10]. This distinction is consistent with the concept of redox homeodynamics, which describes the redox state as a dynamic process maintained through the coordinated generation, sensing, and elimination of reactive species rather than as a static equilibrium [11]. Importantly, redox regulation is also spatially organized, with distinct oxidant sources, antioxidant systems, and redox-sensitive targets operating across cellular compartments [12]. Thus, the transition from physiological signaling to oxidative injury depends on the chemical identity, localization, and persistence of ROS, and the capacity of local antioxidant defenses, rather than on a universal concentration threshold [13].

This dynamic balance is particularly relevant to cancer, where altered metabolism, oncogenic signaling, and interactions within the TME can increase cellular redox pressure [14,15]. Rather than being uniformly susceptible to oxidative damage, cancer cells can adapt by reinforcing antioxidant and metabolic pathways that maintain redox balance while allowing ROS-dependent signaling to support proliferation, survival, and stress adaptation [16–19]. These responses involve interconnected defense systems, including Kelch-like ECH-associated protein 1 (Keap1)/Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling, glutathione and thioredoxin networks, peroxiredoxins, superoxide dismutases, catalase, and nicotinamide adenine dinucleotide phosphate (NADPH)-generating pathways [20]. However, redox adaptation is not uniform across cancers: ROS abundance and biological effects vary according to tumor type, cellular state, subcellular localization, and the surrounding microenvironment [14]. The contextual nature of redox regulation becomes even more evident when tumors are viewed not as autonomous populations of transformed cells, but as dynamic multicellular ecosystems [21]. The TME comprises malignant cells embedded within a remodeled extracellular matrix and interacting with diverse non-malignant populations, including immune cells, cancer-associated fibroblasts (CAFs), endothelial cells, pericytes, and other tissue-resident cells [22]. Importantly, the cellular composition and functional state of these populations vary with tumor type, anatomical site, stage, and tumor-intrinsic properties, and are continuously reshaped during tumor growth, invasion, and metastatic dissemination [21]. Within this complex cellular landscape, ROS influence not only processes related to cancer cells but also the behavior of stromal and immune cells, thus contributing to reciprocal communication between malignant cells and their surrounding microenvironment [23,24]. In turn, TME components can modify the redox state of cancer cells by altering oxygen and nutrient availability, providing antioxidant precursors and metabolites, generating ROS, and modulating redox-sensitive signaling pathways [25].

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Consequently, the tumor redox landscape displays substantial variation both spatially and temporally. Redox microenvironments are not distributed uniformly throughout a tumor but are shaped by spatial differences in oxygen availability, vascular perfusion, metabolic activity, and cellular composition. Abnormal tumor vascularization produces uneven perfusion and oxygen delivery, generating regions exposed to different degrees of hypoxia. Consequently, hypoxic tumor cores, invasive margins, and stromal- or immune-cell niches experience distinct oxidative and antioxidant pressures, with different consequences for tumor-cell adaptation, intercellular signaling, and immune function during tumor growth [26]. These regional differences also mean that measurements obtained from bulk tumor tissue may obscure localized redox states and opposing responses among neighboring cell populations. Accordingly, understanding ROS-mediated remodeling requires consideration not only of which cells produce or respond to ROS, but also of where these processes occur within the tumor architecture [27,28].

Hypoxia is closely related to oxidative stress, as hypoxia-inducible factor (HIF)-dependent responses interact with Nrf2-mediated antioxidant pathways to reshape cellular metabolism, stress adaptation, and redox homeostasis [26]. In parallel, differences in nutrient availability, metabolic demand, inflammatory activity, cellular density, and distance from perfused blood vessels establish distinct biochemical niches within the tumor mass [25,29]. As a result, tumor cells, stromal cells, and infiltrating immune cells located in close proximity may experience significantly different redox pressures [7]. Redox heterogeneity extends to the subcellular level, since ROS generation, antioxidant enzymes, redox couples, and susceptible molecular targets are unevenly distributed among cellular compartments [12]. Such compartmentalization permits localized redox events to selectively modify nearby proteins or signaling pathways without necessarily causing a measurable change in the overall redox state of the cell [30]. Thus, tumors are better regarded as comprising multiple, dynamically interconnected redox microenvironments rather than as possessing a single, static ROS level [7]. Importantly, the specificity of redox signaling depends not only on the identity and abundance of ROS, but also on the chemical reactivity of their molecular targets and the post-translational modifications (PTMs) they induce [31–33]. Protein cysteine residues are particularly important redox sensors because their local chemical environment, solvent accessibility, and ionization state determine their susceptibility to oxidation [31,32]. H_2O_2 can promote reversible cysteine sulfenylation (S-sulfenylation), which may subsequently lead to S-glutathionylation or the formation of intra- or intermolecular disulfide bonds [31–34]. These modifications can alter protein conformation, catalytic activity, interaction partners, and subcellular localization, thereby functioning as molecular switches that regulate downstream signaling [33–35]. In contrast, prolonged or excessive oxidation can promote further cysteine oxidation, potentially compromising protein function [31,32]. Redox specificity is further enhanced by spatially restricted oxidant production and enzymatic redox relays, in which reactive sensor proteins, particularly peroxiredoxins, transmit oxidative equivalents to selected target proteins [36,37]. In parallel, reactive sulfur species (RSS) can modulate protein function through cysteine persulfidation, providing an additional layer of regulation within cellular redox networks [38]. Thus, ROS-mediated signaling is determined by the interplay between oxidant chemistry, protein-specific cysteine reactivity, local antioxidant systems, and the availability of molecular targets. In the TME, these mechanisms provide a molecular basis through which localized redox changes can selectively regulate signaling pathways in malignant, stromal, endothelial, and immune cells, ultimately influencing tumor progression and therapeutic responses.

This review examines the bidirectional relationship between ROS generation, antioxidant defense mechanisms, and TME. A targeted literature search was conducted primarily through PubMed, using combinations of core terms, including “reactive oxygen

species", "ROS", "oxidative stress", "redox signaling", "redox homeostasis", "cancer", and "tumor microenvironment", with additional keywords related to redox adaptation, tumor progression and metastasis, cellular crosstalk within TME, resistance to anticancer therapies, ferroptosis, targeting of redox susceptibility, redox biomarkers, and methods for assessing ROS and redox states. The literature was selected i) to provide a comprehensive and mechanistic overview of ROS biology within the TME, with particular emphasis on ROS sources and compartmentalization, redox homeodynamics, antioxidant defense systems, and redox-dependent communication among cancer cells, stromal fibroblasts, endothelial cells, and immune populations, and ii) to reflect recent developments in the field through careful selection of very recent studies (distribution of cited literature quartile 1: 2021, quartile 2 = 2024, quartile 3 = 2026).

Priority was given to seminal studies establishing fundamental concepts, recent mechanistic advances, and representative evidence across major cancer types and relevant cellular and molecular contexts. Literature addressing therapeutic exploitation of tumor redox vulnerabilities was selected to encompass strategies that induce lethal oxidative stress, disrupt antioxidant defenses, or selectively modulate redox-regulatory pathways, with emphasis on mechanistic rationale and translational relevance. Recent studies on redox-related biomarkers, analytical and experimental approaches for characterizing ROS and redox states, and emerging strategies for therapeutic translation were also considered. When multiple studies addressed closely overlapping mechanisms or therapeutic approaches, representative studies were prioritized according to mechanistic depth, relevance to the TME, experimental robustness, recency, and translational significance. Clinical and translational claims, including information on therapeutic development, clinical evaluation, biomarkers, and emerging redox-targeted interventions, were cross-checked against primary literature and, where applicable, clinical trial registries and regulatory sources.

Although previous reviews have extensively examined ROS in cancer development, TME regulation, and therapeutic targeting, the present review seeks to integrate these aspects within a unified framework of redox remodeling. Its distinctive contribution lies in connecting ROS sources and compartmentalized redox regulation with bidirectional communication among malignant, stromal, immune, endothelial, and extracellular matrix components, rather than treating these processes as independent mechanisms. Our approach brings together the spatial and cellular heterogeneity of redox responses, their implications for tumor progression and therapeutic resistance, and the emerging opportunities and limitations of redox-related biomarkers and therapeutic interventions. This integrated perspective aims to provide a clearer understanding of how context-dependent redox alterations collectively shape the TME and influence the therapeutic vulnerabilities of cancer.

2. ROS Sources and Regulation in Cancer

2.1. Endogenous ROS Generation

Cancer cells can generate ROS from multiple intracellular sources that are spatially compartmentalized and closely regulated by cellular metabolism. Although mitochondrial respiration represents a major contributor, largely through electron leakage from the ETC, ROS are also produced through the activity of NADPH oxidases (NOXs), oxidative protein folding within the endoplasmic reticulum (ER), metabolic processes occurring in peroxisomes, and iron-dependent reactions taking place in lysosomes [14,15]. Notably, these sources differ in the ROS they generate, their subcellular localization, production kinetics, and integration with metabolic processes and signaling pathways, thereby promoting the formation of discrete redox microdomains rather than a uniform oxidative state throughout the cell [7].

2.1.1. Mitochondrial ROS

Mitochondria, the primary sites of energy production in cells, are considered significant intracellular sources of ROS in cancer cells. During oxidative phosphorylation, occurring as part of aerobic metabolism, electron leakage from the respiratory chain can lead to the incomplete reduction of molecular oxygen (O_2), resulting initially in the formation of $O_2^{\bullet-}$, which is subsequently converted into H_2O_2 through the enzymatic activity of superoxide dismutases (SODs) [39,40]. While respiratory complexes I and III are considered the main sites of mitochondrial ROS generation, several other mitochondrial enzymes and redox-active centers can also contribute to the generation of oxidants, including $O_2^{\bullet-}$ and/or H_2O_2, with their relative contribution shaped by substrate availability, functional state of respiratory chain, the mitochondrial membrane potential, and the broader metabolic environment [39]. However, in cancer cells, the oncogenic signaling and metabolic reprogramming can alter electron flow through the mitochondrial respiratory chain, thus affecting ROS generation. Under these conditions, mitochondrial ROS are not merely byproducts of respiration but can also serve as signaling molecules influencing a number of cellular processes, such as proliferation, survival, metabolic adaptation, and responses to hypoxia [41,42]. Notably, the biological effects of mitochondrial ROS depend not only on the capacity of nearby antioxidant defense mechanisms to efficiently eliminate them, but also on whether their release is taking place in the mitochondrial matrix or the intermembrane space, further underscoring the spatial organization of mitochondrial redox signaling [43].

2.1.2. NADPH Oxidases

The mammalian NOX family consists of seven transmembrane enzymes, including five NOX isoforms (NOX1-5) and two dual oxidases (DUOX1 and DUOX2), which generate $O_2^{\bullet-}$ and/or H_2O_2 in an isoform-dependent manner, by transferring electrons from NADPH to molecular oxygen (O_2) through membrane-associated redox centers [44]. ROS derived from NOXs act as deliberate, site-specific signaling molecules, produced in response to external stimuli rather than accidental byproducts of metabolic electron leakage [45]. In malignant tissues, several NOX isoforms exhibit altered expression or activity and have been implicated in cell proliferation and migration, metabolic adaptation, interactions with the tumor stroma, and resistance to therapy, with their relative contributions varying considerably across tumor types [46]. Among these isoforms, NOX4 is of particular interest because it is frequently expressed in tumors and has been strongly associated with hypoxic signaling, metabolic remodeling, and interactions between tumor cells and the surrounding stroma [47].

2.1.3. Endoplasmic Reticulum

The endoplasmic reticulum (ER) provides another important intracellular source of ROS, with their generation arising predominantly from oxidative protein folding. Following the formation and rearrangement of disulfide bonds in newly synthesized proteins, the reduced protein disulfide isomerase (PDI) is reoxidized by endoplasmic reticulum oxidoreductin 1 (ERO1), which channels electrons to O_2, thereby generating H_2O_2 [48,49]. In rapidly proliferating tumors, the increased demand for protein synthesis and folding, along with hypoxia and nutrient scarcity, may disturb the redox balance of ER [50]. Elevated expression of ERO1α has been reported in several malignancies and associated with tumor progression, responses to hypoxia, and immune evasion, although these associations are not uniform and vary by tumor type and context [50].

2.1.4. Peroxisomes

Peroxisomes represent another significant contributor to cellular ROS homeostasis through their oxidative metabolic functions, particularly those involved in fatty-acid oxidation (FAO), which primarily generate H_2O_2 and other reactive species [51,52]. Concurrently, peroxisomes possess a robust antioxidant arsenal, most notably catalase, which decomposes the H_2O_2 generated during their oxidative reactions [53,54]. Accordingly, they contribute to redox equilibrium in a dual manner, functioning both as sources of oxidants and as sites of their detoxification. In cancer cells, the alterations observed in peroxisomal biogenesis and lipid metabolism further suggest that these subcellular organelles may support metabolic plasticity and redox remodeling during tumor progression [55,56].

2.1.5. Lysosomes

Lysosomes are an additional site of localized ROS generation, owing in part to their role in intracellular iron metabolism. The degradation of ferritin and other iron-containing macromolecules contributes to elevated ferrous iron (Fe^{2+}) levels within lysosomes, which in the presence of H_2O_2 can participate in Fenton reactions generating the highly reactive $OH^{\bullet}$ [57]. These radicals are characterized by extreme reactivity and a short half-life, thus reacting in close proximity to their site of formation [58]. Consequently, lysosomal iron accumulation may promote localized oxidative damage, including lipid peroxidation and lysosomal membrane permeabilization, processes whose close relationship is increasingly recognized as a crucial factor in the susceptibility of cancer cells to ferroptosis [59,60].

2.2. Microenvironmental and Metabolic Drivers of ROS in Tumors

In addition to constitutive intracellular ROS generation, the redox state of cancer cells is shaped by the biochemical and microenvironmental conditions prevailing within the tumor milieu. Hypoxia, prolonged inflammatory response, nutrient-driven metabolic reprogramming, and dysregulated iron homeostasis are, altogether, key determinants of ROS production and redox adaptation [45,61]. Rather than operating in isolation, these factors engage in reciprocal and interconnected feedback mechanisms. Indeed, changes in oxygen availability alter cellular metabolism; metabolic shifts modify antioxidant defenses and redox cofactor availability; inflammatory signaling can amplify ROS generation; and disrupted iron handling can expand the pool of redox-active iron [45,62]. Collectively, these interrelated processes determine both the extent of ROS production and the ability of cancer cells to preserve redox balance under adverse conditions [14,15,63].

Hypoxia is a defining feature of many solid tumors, arising largely as the oxygen demand imposed by rapid cellular proliferation exceeds the supply provided by inadequate and structurally abnormal tumor vasculature [63]. Nevertheless, reduced oxygen availability does not necessarily result in diminished ROS generation, as acute or intermittent hypoxia can transiently enhance their mitochondrial formation, particularly within the ETC, contributing to the stabilization and activation of HIFs [26]. From a mechanistic perspective, ROS may inhibit prolyl hydroxylases and limit oxygen-dependent HIF-α degradation, while, in turn, HIF-dependent transcription remodels cellular metabolism to reduce oxygen demand and facilitate adaptation to sustained hypoxia [64]. As a result, hypoxic and oxidative stress are tightly linked, with substantial crosstalk between HIF-dependent responses and antioxidant pathways, including Nrf2 signaling [65]. However, this interaction varies according to the severity and duration of hypoxia, as well as the cellular background, with Nrf2 activity increasing, remaining unchanged, or decreasing under different conditions [26].

Chronic inflammation constitutes a second major source of oxidative pressure within the TME. Infiltrating immune populations, including tumor-associated macrophages, neutrophils, and myeloid-derived suppressor cells, produce ROS during the course of

their normal effector activity, primarily through NADPH oxidases and mitochondrial metabolism [66,67]. Hence, ROS generated by these inflammatory cells can induce DNA damage and modulate redox-sensitive signaling pathways in adjacent cells, while ROS produced by tumor cells can affect the differentiation and function of immune cells, thereby sustaining a reciprocal relationship between inflammatory and oxidative circuits in the TME [68]. These effects depend on both the intensity and persistence of ROS exposure, with transient ROS formation supporting physiological immune activation, whereas prolonged oxidative exposure can impair immune function and favor exhaustion and development of immunosuppressive phenotypes [69]. Thus, inflammation-associated ROS serve not only as a source of oxidative stress but also as important mediators of redox-dependent cellular communication within the tumor ecosystem.

Metabolic reprogramming further influences redox balance in tumors by altering both ROS generation and the availability of reducing equivalents required to maintain antioxidant defenses. Although aerobic glycolysis is prominent in many tumors, cancer metabolism is highly plastic and extends beyond the Warburg effect. Depending on cellular requirements and nutrient availability, malignant cells dynamically redistribute carbon and nitrogen through glycolysis, the pentose phosphate pathway (PPP), glutaminolysis, the tricarboxylic acid (TCA) cycle, and one-carbon metabolism [70]. The direct result of such metabolic adaptations is both the promotion of ROS generation and the enhancement in the capacity for redox buffering. More elaborately, NADPH produced through the oxidative branch of PPP, malic enzyme, and $NADP^+$-dependent isocitrate dehydrogenases supplies the reducing equivalents required to regenerate glutathione and maintain thioredoxin-dependent antioxidant systems [16,17].

Glutamine metabolism also contributes substantially to redox adaptation. Following cellular uptake, glutamine is converted to glutamate, which supports the replenishment of TCA cycle intermediates, biosynthesis, and the maintenance of redox homeostasis [20]. Glutamate is a precursor of glutathione and is exchanged for extracellular cystine through the xCT/SLC7A11 antiporter. Therefore, glutamine availability is linked to antioxidant capacity and susceptibility to ferroptosis [71]. Together, metabolic pathways that sustain the pools of NADPH, glutamate, cysteine, and glutathione enable cancer cells to withstand increased oxidative stress, while preserving the biosynthetic activity required for proliferation.

Iron metabolism plays a pivotal role in regulating the redox state of tumor cells. Although iron is essential for various physiological processes, including mitochondrial respiration, DNA synthesis, and numerous enzymatic reactions, its redox activity can also promote oxidative damage through Fenton chemistry [72,73]. In order to meet their high metabolic needs, cancer cells frequently modify iron uptake, storage, utilization, and export, thereby expanding the iron pool and increasing their vulnerability to iron-dependent oxidative damage [74,75]. This is particularly relevant to ferroptosis, a distinct form of cell death driven by iron-dependent lipid peroxidation that occurs when the relevant antioxidant defense systems are unable to prevent its accumulation [76,77]. Hence, the close interactions among iron metabolism, ROS production, and lipid peroxidation contribute to the predominance of oxidative pressure, creating a potential vulnerability in malignant cells.

Taken together, hypoxia, chronic inflammation, metabolic reprogramming, and altered iron metabolism continually influence ROS generation in tumors and govern the ability of cancer cells to manage oxidative stress. The resulting redox environment reflects a shifting balance between elevated generation of oxidants and activation of adaptive redox responses, providing a solid explanation on why various forms of cancer rely heavily on their endogenous antioxidant defense systems [69].

2.3. Antioxidant Defense Systems in Cancer Cells

Cancer cells survive under elevated oxidative burden by relying on an orchestrated antioxidant network that limits excessive ROS accumulation, while preserving redox signaling [78]. This balance is particularly important for cancer cells because, rather than eliminating ROS completely, their endogenous defense systems maintain ROS levels within a critical range, which is compatible with cell proliferation, survival, and metabolic adaptation, rendering several tumors particularly dependent on their antioxidant capacity [4,79].

The Keap1/Nrf2 pathway is a central regulator of the cellular antioxidant response. Under basal conditions, Nrf2 is bound to its repressor, Keap1, in the cytosol. This protein acts as substrate adaptor to recruit the Cullin 3 (CUL3) ubiquitin ligase complex, which mediates the polyubiquitination and subsequent proteasomal degradation of Nrf2 [80]. Thus, basal Nrf2 protein levels remain low, accompanied by relatively low expression of its target genes. However, under oxidative or electrophilic stress, modification in reactive cysteine residues in Keap1 impairs Nrf2 ubiquitination, allowing Nrf2 to dissociate from its repressor, translocate into the nucleus, dimerize with small Maf (sMaf) proteins, bind to antioxidant response elements (AREs), and activate the expression of cytoprotective genes involved in antioxidant defense, xenobiotic detoxification, iron metabolism, and intermediary metabolism [81,82]. Target genes include those encoding enzymes participating in de novo biosynthesis and recycling of glutathione, glutathione peroxidases, components of thioredoxin system, NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase 1 (HMOX1), and various NADPH-generating enzymes [83].

The biological impact of Nrf2 activation in cancer varies significantly with the disease stage. More specifically, in normal and premalignant tissues, Nrf2-regulated antioxidant and detoxification mechanisms contribute to limit the potential oxidative and genotoxic damage, thereby reducing the probability of tumor initiation [82]. The situation differs substantially in established tumors, where mutations in *NFE2L2* and *KEAP1* genes, or other pathway regulators, along with non-genetic mechanisms, can sustain Nrf2 activity [84,85]. Under these circumstances, Nrf2 signaling may enable malignant cells to endure oxidative stress, reprogram their metabolism, and resist the selected therapeutic intervention. Consequently, Nrf2 highlights the dual nature of antioxidant signaling in cancer, acting protective during early carcinogenesis but driving the survival of malignant cells in established tumors [86,87].

The glutathione system is a principal intracellular redox-buffering network. Reduced glutathione (GSH), a tripeptide composed of glutamate, cysteine, and glycine, is synthesized in two sequential ATP-dependent steps catalyzed by glutamate-cysteine ligase (GCL) and glutathione synthetase (GS), with cysteine availability often limiting its synthesis [88]. GSH acts both as a redox-active thiol and as a substrate for glutathione peroxidases (GPxs), which use it to reduce H_2O_2 and organic hydroperoxides, concomitantly oxidizing GSH to glutathione disulfide (GSSG) [89]. Subsequently, glutathione reductase (GR) regenerates GSH from GSSG using NADPH as a reducing equivalent [89]. This dependence on NADPH links glutathione recycling to cellular metabolism and may help cancer cells maintain antioxidant capacity under conditions of elevated oxidative stress.

Glutathione peroxidase 4 (GPx4) is an antioxidant enzyme particularly relevant to cancer biology because it uses GSH to catalyze the reduction of phospholipid hydroperoxides in cellular membranes, thereby limiting ferroptosis [90]. GPx4 activity is supported by system x_c^-, in which SLC7A11/xCT subunit mediates the import of extracellular cystine in exchange for intracellular glutamate [91]. Once reduced to cysteine, imported cystine provides a precursor for GSH synthesis, linking nutrient uptake to GPx4-dependent protection against lipid peroxidation [92]. Collectively, SLC7A11, GSH, and GPx4 form an

interconnected defense axis against lipid peroxidation, which preserves membrane integrity and promotes resistance to ferroptosis in malignant cells.

Alongside the glutathione system, the thioredoxin system provides a complementary NADPH-dependent mechanism for preserving the reduced state of protein thiols [93]. More elaborately, thioredoxin reductase (TrxR) uses NADPH to reduce oxidized thioredoxin (Trx), which can subsequently reduce disulfide bonds in target proteins and transfer reducing equivalents to peroxiredoxins [94,95]. In malignant cells, this pathway may assist in counteracting increased ROS generation, while supporting redox-regulated processes involved in proliferation, survival, and angiogenesis [96]. This partial functional overlap between the thioredoxin and glutathione systems also provides cancer cells with metabolic flexibility, as impairment of one pathway may increase the reliance on the other in specific cellular or metabolic contexts [97].

Peroxiredoxins (PRDXs) are a ubiquitous family of thiol-dependent peroxidases, functioning in close association with the thioredoxin system [98]. By rapidly reducing H_2O_2 and other peroxides, they constrain local peroxide concentrations and shape the intensity and duration of redox signals [99]. Beyond peroxide removal, PRDXs can relay oxidizing equivalents to redox-sensitive target proteins, contributing to the spatial organization of H_2O_2-dependent signaling [100]. Thus, their high cellular abundance and reactivity with H_2O_2 position them at the interface between physiological redox regulation and protection against oxidative damage.

Superoxide dismutases (SODs) constitute the primary enzymatic defense against $O_2^{\bullet-}$ by catalyzing its conversion to H_2O_2 and O_2 [101]. Mammalian cells express cytosolic and intermembrane-space SOD1, mitochondrial SOD2, and extracellular SOD3, allowing $O_2^{\bullet-}$ levels to be regulated in distinct cellular locations [102]. This reaction does not abrogate redox signaling, but, instead, changes the chemical nature, lifetime, and diffusibility of reactive species produced. Altered SOD expression has been reported across several malignancies, with its biological consequences varying according to tumor type and disease stage [101,103,104].

Catalase (CAT) provides a complementary route for H_2O_2 removal by converting it to H_2O and O_2 [105]. This function is particularly important in peroxisomes, where oxidative metabolic reactions generate high concentrations of H_2O_2 [106]. Alongside GPxs and PRDXs, CAT contributes to the regulation of intracellular peroxide levels, although the contribution of each antioxidant enzyme varies with its subcellular localization and the local availability of H_2O_2 [107].

The capacity of glutathione and thioredoxin systems to sustain antioxidant defense depends on a continuous supply of NADPH, which provides the reducing equivalents to regenerate their reduced components [108]. In cancer cells, NADPH is produced through several metabolic pathways, including the oxidative PPP, malic enzymes, $NADP^+$-dependent isocitrate dehydrogenases, and folate-mediated one-carbon metabolism [109]. These pathways integrate antioxidant defense with metabolic reprogramming by supplying reducing equivalents, while also supporting biosynthetic processes required for rapid cell proliferation [109]. Additionally, Nrf2 reinforces this adaptation by transcriptionally upregulating various NADPH-generating enzymes, thereby coordinating the cellular response to oxidative stress with the metabolic requirements of malignant growth [82]. The major intracellular sources of ROS, the antioxidant defense mechanisms responsible for their regulation, as well as the principal microenvironmental factors shaping the redox landscape of tumors are illustrated in Figure 1.

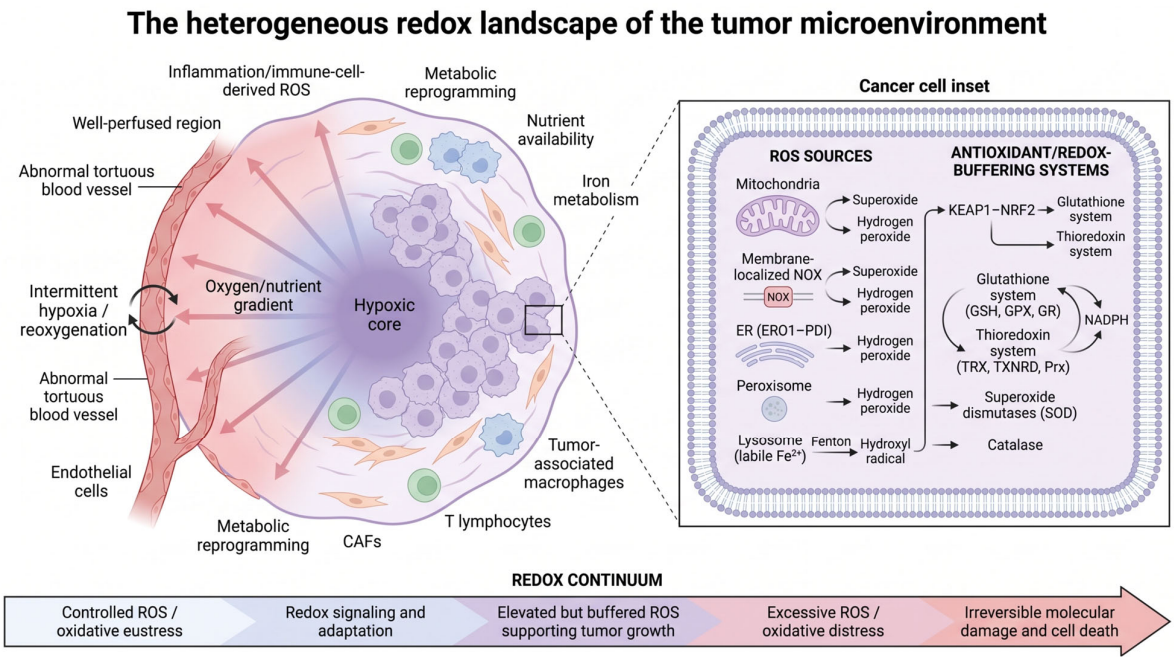


Figure 1. The heterogeneous redox landscape of the tumor microenvironment. This illustration depicts spatial and temporal variations in tumor redox conditions resulting from abnormal vascularization, uneven oxygen and nutrient delivery, intermittent hypoxia and reoxygenation, inflammation, metabolic reprogramming, and altered iron metabolism. Accordingly, malignant, stromal, endothelial, and immune cells are exposed to different local oxidative pressures. The enlarged cancer-cell inset highlights major sources of ROS, including mitochondria, NOXs, the endoplasmic reticulum ERO1–PDI system, peroxisomes, and lysosomal iron-dependent Fenton reactions. It also depicts the antioxidant and redox-buffering mechanisms that limit ROS accumulation, including the Keap1/Nrf2 pathway, glutathione and thioredoxin systems, peroxiredoxins, superoxide dismutases, catalase, and NADPH-dependent reducing capacity. The continuum at the bottom illustrates the context-dependent effects of ROS, progressing from controlled oxidative eustress and redox signaling, to elevated but buffered ROS levels that may promote tumor growth, and to oxidative distress, irreversible molecular damage, and ultimately to cell death when antioxidant capacity is overwhelmed. Created in BioRender. Vardakas, P. (2026) https://BioRender.com/x4xt8fa.

2.4. Redox-Sensitive Cysteine Modifications and Molecular Specificity of ROS Signaling

Redox signaling specificity is determined in large part by the selective modification in redox-sensitive amino acid residues, particularly cysteine thiols, whose reactivity is influenced by their local protein environment, accessibility, and ionization state [31,32,110]. Unlike nonspecific oxidative damage, reversible cysteine modifications can regulate protein function in a controlled and temporally restricted manner. H_2O_2, for example, can oxidize susceptible cysteine residues to sulfenic acid (S-sulfenylation; –SOH), which may subsequently form intramolecular or intermolecular disulfide bonds or react through appropriate redox pathways to generate protein S-glutathionylation (–SSG) [31–34,110]. These modifications can alter catalytic activity, protein conformation, protein–protein interactions, and subcellular localization. Their reversibility, mediated in part by the thioredoxin and glutaredoxin systems, enables protein activity to be dynamically adjusted according to cellular redox conditions [33,34,111]. However, sustained or excessive oxidation can promote further oxidation of sulfenic intermediates to sulfinic and sulfonic forms. Although sulfonic acid formation is generally irreversible under physiological conditions, sulfinic acid reduction is possible for specific proteins, notably typical 2-Cys peroxiredoxins, through sulfiredoxin-dependent mechanisms [31,36,110].

An illustrative example is the redox regulation of phosphatase and tensin homolog (PTEN), a negative regulator of phosphoinositide 3-kinase (PI3K)/AKT signaling. Oxidation of its catalytic cysteine can result in the formation of an intramolecular disulfide bond, reversibly inhibiting phosphatase activity and facilitating AKT activation [112,113]. Similarly, oxidation-sensitive protein tyrosine phosphatases can undergo reversible inactivation, thereby prolonging receptor- and kinase-mediated signaling [31,110]. These examples illustrate how localized oxidative events can influence defined signaling nodes rather than simply increasing the overall oxidative burden of the cell.

Redox signaling specificity is further enhanced by enzymatic redox relays and compartmentalization. Because many protein thiols react relatively slowly with H_2O_2, highly reactive peroxiredoxins can act as redox sensors and transmit oxidative equivalents to selected target proteins through thiol–disulfide exchange reactions [36,37,111]. For example, peroxiredoxin 2 has been shown to participate in a redox relay with signal transducer and activator of transcription 3 (STAT3), providing a mechanistic link between H_2O_2 and transcriptional regulation [37]. Localized production of H_2O_2 by NADPH oxidases and mitochondria, together with the spatial distribution of peroxiredoxins, catalase, glutathione-dependent enzymes, and thioredoxin systems, helps determine which targets are modified and the duration of their [36,111,114]. Thus, localized oxidant generation and the regulated activity of antioxidant enzymes can permit selective signaling without necessarily causing a substantial change in the overall cellular redox state [114,115].

In addition to oxygen-derived oxidants, reactive sulfur species contribute to the regulation of protein thiols. Cysteine persulfidation (–SSH), associated with hydrogen sulfide and related sulfur chemistry, can modify protein activity, stability, and interactions, although its formation and biological consequences depend on the prevailing chemical [38,116]. S-glutathionylation, persulfidation, and reversible disulfide formation should therefore be considered distinct but potentially interconnected regulatory mechanisms rather than interchangeable indicators of oxidative stress [33,34,38]. Together, these modifications establish a dynamic network of thiol-redox switches that links local chemical conditions to defined protein functions.

Within the TME, these mechanisms provide a molecular framework for understanding how redox changes regulate cancer-cell survival, stromal activation, endothelial responses, and immune-cell function. The biological consequences of ROS consequently depend not only on oxidant production but also on the identity of the modified proteins, the nature and reversibility of the resulting modifications, and the capacity of cellular antioxidant systems to restore their functional state [36,110,114]. This framework helps explain how spatially and temporally heterogeneous redox signals can produce distinct biological outcomes in different TME compartments.

3. ROS as Regulators of Tumor Microenvironment Components

3.1. Cancer Cells and Intratumoral Redox Signaling

The biological effects of ROS in malignant cells depend on their capacity to maintain sufficient antioxidant buffering while preserving redox-sensitive signaling. More specifically, ROS can play an important role in the regulation of major signaling pathways, such as NF-κB, PI3K/AKT, and mitogen-activated protein kinase/extracellular signal-regulated kinase pathway (MAPK/ERK) axes [117,118]. NF-κB is associated with reduced apoptotic responses and enhanced transcription of genes involved in cell proliferation, angiogenesis, and progression of tumor, such as interleukin-1 and cyclin D1 [118]. Notably, through the activation of PI3K/Akt pathway, cell proliferation is further amplified [118], along with alterations in MAPK signaling and ERK activation, which are related to migration, apoptosis and ROS generation [117,118].

Besides regulating proliferation and survival, ROS also participate in cancer-cell plasticity. ROS-dependent signaling can promote epithelial-to-mesenchymal transition (EMT) through transcription factors such as snail family transcriptional repressor (SNAIL), which results in increased invasive and metastatic properties [117]. Elevated ROS levels generated under hypoxic conditions are capable of activating transforming growth factor-beta (TGF-β)- and tumor necrosis factor-alpha (TNFα)-associated stress pathways in cancer stem cells and helping them maintain their undifferentiated state [119].

Beyond changes in cellular ROS abundance, redox-sensitive PTMs provide a mechanism through which oxidants regulate the functional state of individual TME components. Reversible oxidation of signaling proteins can modulate kinase–phosphatase balance, transcriptional responses, and cellular adaptation, whereas cysteine-dependent redox sensing through the Keap1/Nrf2 system supports the induction of antioxidant and cytoprotective programs [36,120,121]. These mechanisms may have distinct consequences across malignant, stromal, endothelial, and immune populations, depending on their antioxidant capacity, metabolic state, and repertoire of redox-sensitive proteins. Consequently, the effects of ROS cannot be explained solely by their extracellular or intracellular concentrations; the molecular targets and modifications involved must also be considered.

The extracellular matrix (ECM) is an important, yet relatively underexplored, component of redox remodeling in the TME. Its fibrous proteins, including collagens and elastin; glycoproteins, such as fibronectin and laminins; proteoglycans; and polysaccharides, including glycosaminoglycans such as hyaluronan, provide structural support while regulating cell adhesion, migration, and signaling. ROS can influence ECM remodeling by altering matrix protein expression, modifying extracellular macromolecules, and regulating matrix-degrading enzymes, including matrix metalloproteinases. Conversely, ECM composition and organization can influence cellular ROS production and redox-sensitive signaling through integrins and other cell-surface receptors. Oxidative modification or degradation of matrix components may further alter matrix integrity and generate bioactive fragments that affect inflammation, angiogenesis, and tumor-cell invasion. Thus, the ECM should be viewed not merely as a structural scaffold but as an active participant in bidirectional redox communication between tumor cells, stromal cells, and immune populations [122,123].

The effects of ROS on TME components should also be interpreted in their spatial context. Uneven vascular perfusion and oxygen diffusion create regions with different oxygen availability and metabolic constraints, while local differences in antioxidant capacity further influence ROS accumulation and signaling. Consequently, tumor cells in relatively oxygenated perivascular regions may experience redox conditions distinct from those in hypoxic or nutrient-deprived regions, whereas stromal and immune cells respond according to their location and local interactions with cancer cells. These differences can influence stress adaptation, immune-cell function, and therapeutic susceptibility; however, hypoxia should not be equated automatically with higher ROS, because ROS levels also depend on mitochondrial activity, oxidant-producing enzymes, and antioxidant defenses [27,124].

3.2. Cancer-Associated Fibroblasts (CAFs)

CAFs and ROS interact through a bidirectional crosstalk within the TME. This interaction occurs, as ROS can promote fibroblast activation, while activated CAFs can, in turn, contribute to oxidative stress in surrounding cells [117]. In a study involving skin squamous cell carcinoma, CAFs released higher amounts of H_2O_2 compared with normal fibroblasts. Moreover, exposure of normal fibroblasts to CAF-conditioned medium increased intracellular ROS levels and reduced the GSH/GSSG ratio, indicating oxidative stress. This was accompanied by increased expression of fibroblast-activated protein

(FAP), while antioxidant treatment reduced this response. In addition, exposure of keratinocytes to H_2O_2 resulted in oxidative modifications in phosphatase and tensin homolog (PTEN) and Src. However, an increase in intracellular ROS in cancer cells following exposure to CAF-derived signals was not directly demonstrated [125]. In particular, NOX4-derived ROS, in association with TGF-β signaling, contribute to the transformation of normal fibroblasts to myofibroblasts [118]. Another factor that has an important influence on this process is HIF-1α. HIF-1α accumulates in response to ROS and can promote fibroblast conversion into myofibroblastic CAFs (myCAFs), while it can also enhance the expression of TGF-β and other factors involved in stromal and ECM remodeling [119]. Notably, ROS-driven myofibroblast differentiation participates in ECM remodeling and creates a more pro-tumorigenic microenvironment that facilitates tumor growth and metastatic progression [118]. In addition to their role in ECM remodeling, CAFs also contribute to metabolic crosstalk with tumor cells [119]. In particular, H_2O_2 generated by cancer cells can promote oxidative stress and catabolic processes in nearby fibroblasts, which both result in increased lactate production [126]. This lactate can be used as a substrate and fuel for tumor cell metabolic activity [126]. This interaction is associated with the reverse Warburg effect, in which glycolytic CAFs produce and release lactate that is utilized by tumor cells for oxidative phosphorylation and energy production [118,119].

3.3. Endothelial Cells and Angiogenesis

Within the TME, ROS can promote tumor angiogenesis by modulating growth and transcription factors, including vascular endothelial growth factor (VEGF) and HIF-1α [127]. NOX-derived ROS contribute to this biological process, as increased ROS production is related to activation of the HIF-1α/VEGF axis [128]. H_2O_2 is necessary, as it inhibits the activity of prolyl hydroxylase and thereby promotes HIF-1α stabilization [128]. In some malignancies, such as ovarian cancer, H_2O_2 participates in the EGF-induced PI3K/AKT/p70S6K1 axis, leading to HIF-1 activation and enhanced VEGF mRNA expression [128]. Regarding HIF-1α, it has a significant role because it enhances the expression of VEGF and other pro-angiogenic factors, supporting the angiogenic response [42]. Under hypoxic conditions, VEGF binds to its receptors, promoting endothelial cell proliferation [119] as well as activating multiple signaling pathways, such as ERK/MAPK axis [117]. Besides endothelial cell proliferation, migration is also significant for the formation of new blood vessels, whereas vascularization can supply oxygen and nutrients to nearby tumor cells [118]. Thus, ROS-mediated signaling contributes to the communication between tumor and endothelial cells and supports the formation of new blood vessels within the TME [117,119]. However, the effects of ROS on angiogenesis depend on ROS levels, as excessive H_2O_2 concentration can oxidatively modify and inactivate VEGF receptor-2, subsequently inhibiting tumor angiogenesis [126].

3.4. Immune Cells in the TME

ROS have an important role in the regulation of immune cells within TME. Their effects are dependent on their concentration. In certain tumor contexts, sustained or spatially localized ROS production can impair immune-cell function and contribute to an immunosuppressive TME; however, ROS-dependent effects on immune cells vary with oxidant exposure, cell type, and activation state [117]. High ROS levels can reduce the function of cytotoxic T cells and NK cells and can also lead to T-cell apoptosis and exhaustion [117,118]. Macrophage polarization is also affected through the NF-κB signaling pathway and facilitate the creation of an immunosuppressive phenotype. In this process, programmed death-ligand 1 (PD-L1) expression is increased, and this leads to reduced cytotoxic T-cell activity [117]. In dendritic cells, elevated ROS levels can limit dendritic cell (DC) maturation and impair antigen presentation [129]. Myeloid-derived suppressor cells

(MDSCs) can suppress T-cell function through ROS-dependent mechanisms, while high ROS levels can increase the immunosuppressive activity of Tregs [68,117]. Neutrophil-derived H_2O_2 can contribute to metastatic tumor-cell killing or suppress T-cell and NK-cell activity [68]. Overall, increased ROS levels within the TME can reshape the immune compartment and promote an immunosuppressive environment that favors tumor immune evasion.

Importantly, the immunological effects of ROS are not exclusively immunosuppressive, as controlled ROS production also contributes to physiological immune activation and antitumor responses. In T cells, transient ROS generation following T-cell receptor (TCR) stimulation participates in intracellular signaling and supports T-cell activation, proliferation, and cytokine production, whereas excessive or sustained oxidative stress can impair these responses, promote apoptosis, and contribute to T-cell exhaustion [66,130]. Similarly, ROS generated by activated neutrophils and macrophages can contribute to antimicrobial and antitumor effector functions, although prolonged or excessive oxidant production may damage neighboring immune cells and favor chronic inflammation or immune [131]. Dendritic-cell responses are also context-dependent: ROS can participate in the regulation of cellular activation and inflammatory signaling, whereas excessive oxidative stress may interfere with antigen processing, maturation, and T-cell priming [132]. Thus, the outcome of ROS signaling depends not only on its magnitude but also on its source, subcellular localization, duration, and the antioxidant capacity and functional state of the responding immune cell. Consequently, controlled and transient ROS signaling within the physiological range of oxidative eustress may support antitumor immunity, whereas excessive or persistent oxidant exposure within the TME shifts the response toward oxidative distress contributing to immune suppression. The principal ROS sources and redox drivers across major cellular components of TME, along with their main biological consequences are presented in Table 1.

Table 1. Cell type-specific redox effects within the TME.

TME Cell Type	Main ROS Sources/Redox Drivers	Principal Effect in TME	References
Cancer cells	ROS-sensitive NF-κB, PI3K/AKT and MAPK/ERK signaling; Nrf2-dependent adaptation	Proliferation and survival; EMT, invasion and maintenance of stem-like phenotypes	[36,117–121]
CAFs	NOX4-derived ROS, TGF-β/HIF-1α signaling and cancer-cell-derived H_2O_2	Fibroblast activation, ECM remodeling and lactate-mediated metabolic crosstalk	[117–119,125,126]
Endothelial cells	NOX-derived ROS and H_2O_2-dependent HIF-1α/VEGF signaling	Promotion of endothelial proliferation and angiogenesis; excessive H_2O_2 may inhibit VEGFR2-dependent angiogenesis	[117–119,126–128]
T cells and NK cells	Transient ROS support immune signaling; sustained oxidative exposure suppresses effector function	T-cell activation under controlled ROS conditions versus impaired cytotoxicity, apoptosis and exhaustion under excessive ROS	[66,68,117,118,130]
Macrophages	ROS-dependent modulation of polarization and NF-κB signaling	Context-dependent antitumor activity or development of an immunosuppressive phenotype	[117,131]

Dendritic cells	Excessive oxidative stress	Impaired maturation and antigen presentation, with reduced T-cell priming	[129,132]
MDSCs/Tregs	ROS-dependent immunosuppressive activity	Suppression of T-cell responses and reinforcement of tumor immune evasion	[68,117]
Neutrophils	Neutrophil-derived H_2O_2	Context-dependent tumor-cell killing or suppression of T- and NK-cell activity	[68,131]

Figure 2 provides a complementary schematic overview of the major ROS-mediated effects on cell types of the TME.

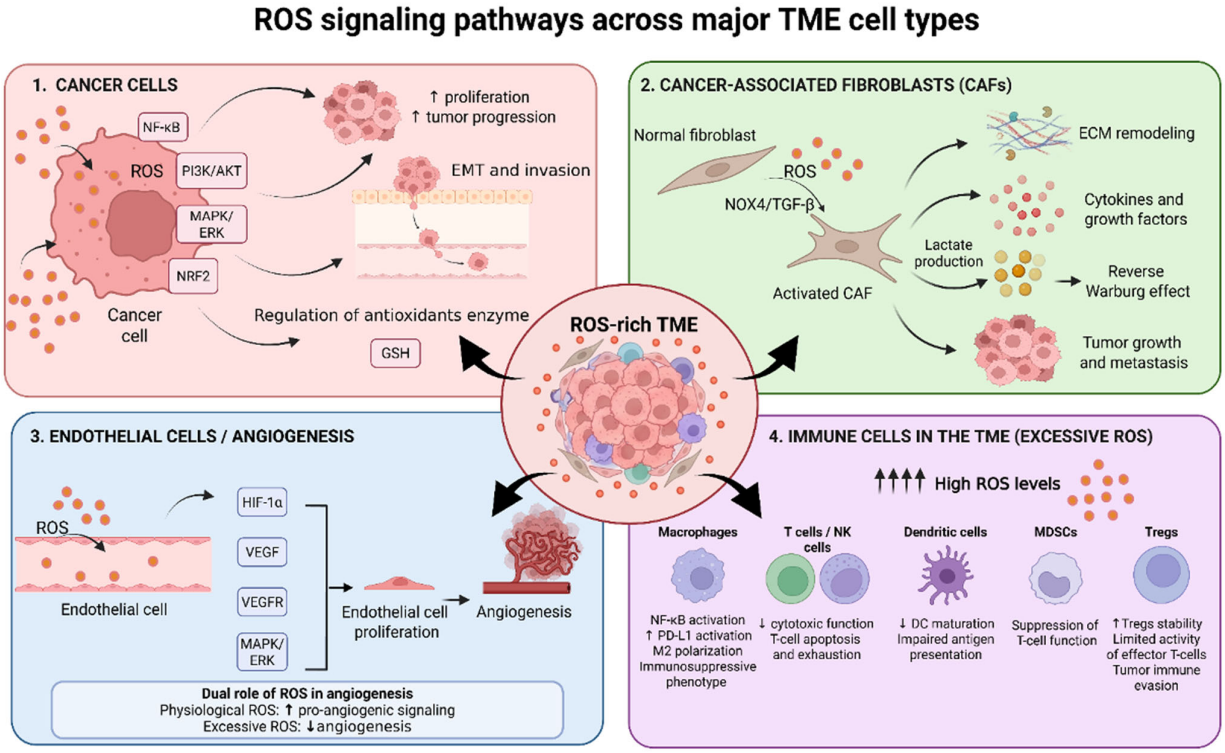


Figure 2. ROS signaling pathways across major TME cell types. In cancer cells, ROS have the ability to regulate major signaling pathways including NF-κB, PI3K/AKT, MAPK/ERK and NRF2 thereby supporting proliferation, tumor progression, EMT and invasion and antioxidant adaptation. Regarding CAFs, ROS promote fibroblast activation, ECM remodeling, secretion of cytokines and growth factors, and metabolic crosstalk through lactate production and the reverse Warburg effect. In endothelial cells, ROS have a dual role. High ROS levels contribute to endothelial cell proliferation and angiogenesis, while excessive ROS levels can impair angiogenesis. In immune cells, increased ROS levels create an immunosuppressive environment, thereby enhancing macrophage polarization, reducing T-cell and NK-cell function, impairing DC maturation, promoting MDSC-mediated T-cell suppression and increasing Treg immunosuppressive activity. Created in BioRender. ANATOLIOTAKI, C. (2026) https://BioRender.com/csb3yy8.

4. ROS-Mediated Intercellular Communication in the TME

ROS are involved not only in intracellular signaling but also in the communication between different cellular components of the tumor microenvironment. NADPH oxidases and other ROS-generating systems can produce localized H_2O_2 signals, while

peroxiredoxin-dependent redox relays facilitate the transfer of oxidative equivalents to selected signaling proteins [36,37,114,115]. Such mechanisms provide a basis for localized regulation of signaling pathways that control gene expression and cellular responses without requiring a uniform increase in ROS throughout the tissue. In the TME, this spatial selectivity may influence how malignant cells and neighboring stromal, endothelial, and immune populations respond to oxidative cues. Nevertheless, direct redox-dependent modification in intercellular mediators should be distinguished from indirect effects mediated through intracellular signaling and changes in gene expression. Another important mechanism is associated with soluble factors, such as cytokines and chemokines, whose production or biological activity can be regulated by ROS [129]. Several examples present the interaction between ROS and cytokine-mediated signaling within the TME [133]. ROS are capable of modulating the production and release of factors, including interleukin-10 (IL-10), VEGF, and HIF, by contributing to an immunosuppressive condition [129]. In addition, ROS-dependent modifications can also affect chemokines, such as C-C motif chemokine ligand 2 (CCL2), and alter their biological activity and capacity to recruit immune cells [129]. Another example is related to IL-1β signaling. Elevated mitochondrial ROS production induced by cholesterol in macrophages can promote NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation and subsequent IL-1β secretion. In a separate study, macrophage-derived IL-1β has been shown to activate the ROS/Src/MAPK/AP-1 pathway in breast cancer cells, leading to increased cyclooxygenase-2 (COX-2) expression [134]. This mechanism was investigated using macrophage–breast cancer cell co-cultures, where IL-1β neutralization, IL-1 receptor blockade, NADPH oxidase inhibition, and targeted gene silencing confirmed the involvement of IL-1β-dependent ROS production and downstream signaling. Furthermore, co-culture promoted IL-1β expression in both macrophages and breast cancer cells, while exogenous IL-1β induced its own expression in both cell types, supporting an autoamplification mechanism. However, a specific tumor cell-derived signal responsible for stimulating macrophages was not identified [134]. Importantly, cholesterol-induced mitochondrial ROS/NLRP3 activation and IL-1β-mediated signaling in breast cancer cells were examined in separate studies and were not experimentally validated as a single continuous pathway. These observations highlight the close interaction between redox regulation and cytokine-mediated intercellular communication [133].

Besides the modulation of soluble factors, preferential packaging of miRNAs into extracellular vesicles (EVs) is also affected [135]. MiRNA expression and packaging are influenced by oxidative stress. Depending on the redox conditions, specific redox-sensitive miRNAs, including miR-21 and miR-210, can be incorporated into EVs [135]. Once released, these vesicles have the ability to transfer their cargo to a variety of cell types, where miRNAs regulate gene expression and influence cellular functions. These ROS-sensitive exosomal miRNAs are involved in several biological processes, such as angiogenesis, immune responses and tumor progression [135]. ROS can also enhance tumor metastasis by promoting the secretion of EVs [136]. EV cargo contains multiple proteins, lipids and molecular mediators, which can affect crosstalk between tumor cells and the nearby TME [137]. ROS-mediated oxidative DNA damage facilitates the interaction of 8-oxoguanine DNA glycosylase (OGG1) with 8-oxo-7,8-dihydroguanine (8-oxoG), subsequently inducing NF-κB-dependent SYT7 transcription. Increased SYT7 expression enhances the secretion of E-cadherin-loaded EVs, reducing intracellular E-cadherin levels and consequently promoting EMT and metastatic progression [136]. Taken together, ROS-mediated changes in EV cargo and secretion represent an additional mechanism of intercellular communication within the TME.

Metabolic interactions represent another mechanism through which ROS participate in intercellular communication within the TME. ROS have crucial role in metabolic

reprogramming between tumors and immune cells, which may lead to metabolic alterations that can contribute to an immunosuppressive environment [129]. Among them, lactate represents an important mediator in these interactions. Signaling molecules derived from tumor cells, such as ROS, HIF-1α and TGF-β, promote CAF differentiation and result in lactate production through glycolysis. Lactate produced by CAFs can subsequently be transported to tumor cells through monocarboxylate transporters, such as MCT1. Through this process, metabolic substrates are provided to support tumor growth [138]. In prostate cancer models, this metabolic interaction was experimentally associated with increased lactate uptake, utilization of lactate for respiration and anabolic processes, and enhanced tumor cell proliferation. Although antioxidant treatment indicated the involvement of redox-sensitive HIF-1 signaling in MCT1 regulation, the study did not find a lactate-induced change in the intracellular redox state of tumor cells [139]. Notably, lactate can also participate in the communication between tumor cells and macrophages. Hepatocellular carcinoma and pancreatic cancer cells generate lactate, which stimulates ROS generation in macrophages, resulting in the stimulation of Nrf2 signaling and their transition to an M2 phenotype. Following M2 polarization, macrophages can contribute to the acquisition of an EMT phenotype in cancer cells and support tumor–immune crosstalk [133].

Under hypoxic conditions, cancer cells can alter both the secretion and molecular cargo of EVs, a process associated with the HIF-1α signaling pathway. A hypoxic TME drives tumor cells to release EVs containing hypoxia-related miRNAs, such as miR-210, which, as mentioned before, is related to angiogenesis [135]. Hypoxia-mediated EV communication influences the behavior of immune cells within the TME. For instance, EVs carrying miR-10a and miR-21 released by hypoxic glioma cells are capable of increasing MDSC abundance and immunosuppressive function [140]. Exposure to hypoxic glioma-derived EVs has also been associated with increased ROS, IL-10, TGF-β, ARG-1 and NO levels in MDSCs, accompanied by stronger suppression of CD8+ T-cell proliferation compared with EVs derived from normoxic cells [140]. The involvement of ROS was further investigated through inhibition of NF-κB signaling, which reduced ROS production and EV-induced MDSC expansion. The study did not directly examine whether reducing ROS levels in MDSCs affected their ability to suppress CD8+ T-cell proliferation [141]. Another study investigated the effects of MDSC-derived exosomes on CD8+ T cells. The researchers found that exposure to these exosomes increased intracellular ROS levels in CD8+ T cells. They also observed changes in T-cell activation and exhaustion markers, as well as a reduction in CD8+ T-cell numbers in vivo. Although a decrease in T-cell proliferation was observed in vitro, this difference was not statistically significant. The authors proposed that increased ROS production might contribute to T-cell death through the Fas/FasL pathway. However, this mechanism was not directly tested by inhibiting ROS production and checking whether T-cell proliferation was restored. Therefore, the involvement of ROS in the immunosuppressive effects of MDSC-derived exosomes remains a possible explanation rather than a confirmed mechanism [142]. These findings illustrate how hypoxia-associated alterations in redox signaling and EV-mediated communication can participate in the establishment of a tumor-supportive and immunosuppressive microenvironment.

5. ROS in Tumor Progression and Metastasis

At the molecular level, redox-dependent regulation of tumor progression can involve reversible modification in signaling proteins that control survival, proliferation, and cellular adaptation. For example, oxidation of PTEN can transiently inhibit its phosphatase activity through intramolecular disulfide formation, thereby favoring PI3K/AKT signaling under appropriate cellular conditions [112,113]. More broadly, redox-sensitive

cysteine modifications can alter the activity and interactions of proteins involved in kinase signaling, transcriptional regulation, and cytoskeletal organization [31,32,110]. These mechanisms provide a potential link between localized oxidative signals and phenotypes relevant to tumor progression.

During EMT, epithelial cells undergo changes in their morphology and gene expression patterns and acquire a mesenchymal-like phenotype [117,128]. This transition provides the essential characteristics associated with tumor progression and metastasis, including cytoskeletal remodeling, increased invasiveness and motility [117]. Recent studies have shown that elevated ROS levels are closely involved in the regulation of EMT and metastatic progression [143]. ROS have the ability to influence EMT by controlling EMT-related transcription factors, such as SNAIL, zinc finger E-box-binding homeobox 1 (ZEB1), and twist family bHLH transcription factor (TWIST), promoting the expression of mesenchymal markers and the acquisition of more invasive characteristics [117,143]. ROS activate several signaling pathways associated with EMT. For example, ROS-mediated activation of the MAPK pathway increases the expression of SNAIL and contributes to the formation of a more tumorigenic microenvironment [128]. Another example involves the growth factor TGF-β, which is induced by ROS and is a well-established inducer of the EMT process. TGF-β can activate the ROS/NRF2 pathway and subsequently Notch signaling, which results in increased snail family transcriptional repressor 1 (SNAI1) transcription and the induction of EMT [128,143]. ROS, also, have been demonstrated that upregulate integrin expression in tongue squamous cell carcinoma (TSCC), because integrins contribute to EMT and invasion through the TGF-β pathway [128]. In other words, these findings indicate that ROS modulate metastatic progression through multiple interconnected mechanisms that promote EMT.

Beyond promoting an invasive phenotype through EMT, ROS also support tumor cell spreading by regulating the remodeling and degradation of the ECM. The ECM consists of different components, including collagen, elastin, and proteoglycans and plays a significant role not only in cell survival but also in cancer progression and metastatic spread [117]. ECM remodeling includes the disruption of basement membrane through the degradation of numerous ECM proteins, enabling tumor cells to penetrate surrounding tissues with the help of proteolytic enzymes, such as matrix metalloproteinases (MMPs) and proteases [117,143]. ROS contribute to cell migration by increasing the expression of these enzymes through the modulation of different signaling pathways. For instance, ROS are capable of activating the MAPK pathway, as mentioned above, leading to elevated expression of MMP1, MMP3 and laminin subunit gamma 2 (LAMC2) [128]. ROS-activated pathways, including NF-κB, HIF-1 and TGF-β, contribute to the degradation and remodeling of the ECM by increasing the expression of urokinase type plasminogen activator (uPA) and MMP-9. In addition, ROS promote myofibroblast differentiation, which has a crucial role in ECM remodeling. Other studies revealed that ROS can modulate other ECM-related key enzymes, such as heparanase, which degrades heparan sulfate proteoglycans and promotes the release of growth factors, further contributing to tumor progression [117,143].

The influence of ROS in ECM remodeling can contribute to the formation of a supportive pre-metastatic niche. For instance, ROS can regulate collagen cross-linking through lysyl oxidase (LOX). This results in increased ECM stiffness and the formation of a scaffold that promote tumor cell invasion. ROS may also stimulate TGF-β signaling. Activation of TGF-β signaling enhances fibronectin and collagen deposition and creates a fibrotic environment that supports tumor cell adhesion and migration [143].

However, once tumor cells enter the circulation, oxidative stress becomes a major challenge to their survival. Circulating tumor cells (CTCs) are exposed to increased ROS levels as a result of different conditions (hemodynamic shear stress (HSS), oxygen

exposure and ECM detachment) [144]. To survive under those conditions, CTCs employ diverse strategies, enhance their antioxidant defense and limit ROS accumulation [145]. In particular, one of these strategies involves the upregulation of manganese superoxide dismutase (MnSOD), which protects CTCs from mitochondrial ROS generated in response to HSS [144]. Metabolic adaptations may also provide a crucial survival advantage. For example, HSS-induced expression of atonal bHLH transcription factor 8 (ATOH8) promotes HK2-dependent glycolysis in colorectal CTCs, thereby limiting ROS production [144]. Lactate and pyruvate metabolism can help maintain redox balance and resistance to oxidative stress. Lactate uptake through monocarboxylate transporter 1 (MCT1) has been associated with enhanced melanoma metastatic capacity by promoting pentose phosphate pathway function [145]. NADPH generated through this pathway contributes to the regeneration of the antioxidant GSH. Regarding pyruvate, it may function as an extracellular antioxidant [145]. Overall, the ability of CTCs to control ROS through antioxidant and metabolic adaptations appears to be essential for their survival during metastatic spread.

After surviving in circulation and completing extravasation, metastatic tumor cells also have to overcome oxidative stress to successfully colonize distant tissues [145]. However, oxidative stress remains a major challenge during this stage because it can cause cell death and limit metastatic colonization. Therefore, metastatic cells have to develop antioxidant mechanisms to control excessive ROS levels. In gastric cancer cells, rho GTPase-activating protein 15 (ARHGAP15) has been shown to contribute to this process by inhibiting Rac family small GTPase 1 (RAC1) activity and reducing intracellular ROS accumulation. In addition, elevated levels of ARHGAP15 were associated with better adaptation of the tumor cell to conditions with high ROS levels and ability to form colonies. These results show that the regulation of intracellular ROS through the ARHGAP15/RAC1 axis may support the survival of metastatic tumor cells and their subsequent colonization at distant sites [146]. The ROS-mediated mechanisms involved in tumor progression and metastatic spread, which were described above, are illustrated in Figure 3.

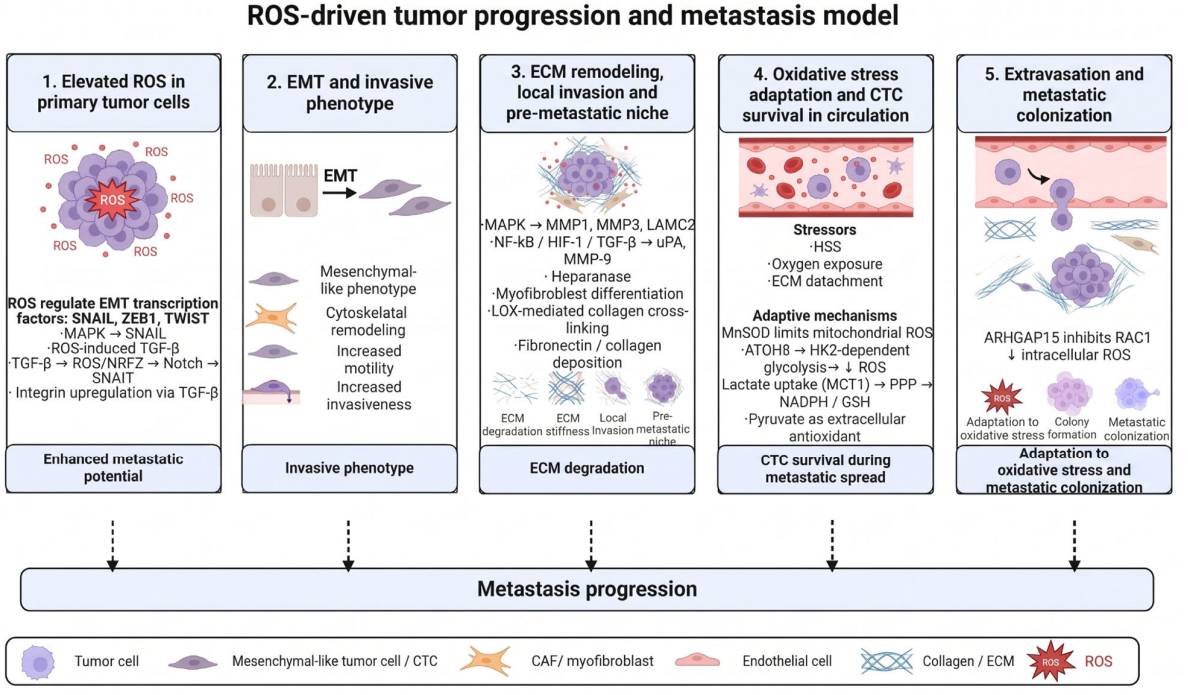


Figure 3. ROS-driven tumor progression and metastasis model. Elevated ROS levels promote tumor progression through multiple mechanisms. In primary tumor cells, ROS modulate the EMT

transcription factors (SNAIL, ZEB1, TWIST) and activate signaling pathways with metastatic potential and support an EMT and invasive phenotype. ROS also participate in ECM remodeling and degradation, and to the formation of a pre-metastatic niche. During circulation, CTCs are exposed to oxidative stress, and they develop adaptive mechanisms to enhance their survival. Following adaptation to oxidative stress, tumor cells are capable of surviving more efficiently and forming colonies at distant sites, promoting metastatic colonization. Created in BioRender. ANATOLIOTAKI, C. (2026) https://BioRender.com/e0hw0sr.

6. ROS and Therapy Resistance

A common feature of many anticancer therapies is the disruption of redox homeostasis, either directly by generating ROS or indirectly by altering mitochondrial function, damaging DNA, disturbing protein homeostasis, and diminishing antioxidant defenses [147]. When therapy-induced oxidative pressure surpasses the antioxidant buffering and repair capacity of tumor cells, they may enter a state of oxidative distress, thus contributing to their elimination. Nevertheless, repeated or sustained treatment can favor the survival of populations capable of restoring redox balance through the enhancement in antioxidant defenses, metabolic reprogramming, effective DNA repairing, altered drug transport, and interactions with the tumor microenvironment [148]. Therefore, redox adaptation should not be considered as an isolated resistance mechanism, but as part of a broader stress response that intersects with stemness, metabolic plasticity, DNA repair, drug detoxification, and immune evasion [149,150]. This adaptation is particularly relevant in case of tumors already exposed to elevated oxidative pressure, as treatment may further increase their reliance on Nrf2 signaling, the glutathione and thioredoxin systems, and metabolic pathways that produce NADPH [151].

6.1. Chemotherapy Resistance

Oxidative stress can contribute to the cytotoxic effects of conventional chemotherapy, although its importance varies significantly among the diverse agents [152]. Anthracyclines, platinum-based antineoplastics, and specific antimetabolites are capable of increasing intracellular ROS through several mechanisms that include redox cycling, mitochondrial dysfunction, DNA damage, and metabolic perturbations, with the resulting oxidative damage leading to apoptotic or other forms of cell death [153,154]. Nevertheless, tumor cells surviving the selected intervention may further strengthen their antioxidant defense mechanisms, indicating that the redox adaptations established following continued exposure can favor the emergence of populations highly resistant to oxidative burden [148].

During chemotherapy, sustained Keap1/Nrf2 signaling can contribute to resistance not only through redox adaptation but also by upregulating the expression of phase II detoxifying enzymes and ATP-binding cassette (ABC) transporters, thus enhancing drug detoxification and efflux and reducing exposure to susceptible agents [155,156]. Consequently, constitutive Nrf2 activity has been implicated in resistance to multiple cytotoxic agents, including platinum compounds, anthracyclines, taxanes, and antimetabolites, although its relative contribution to chemoresistance depends on tumor type, the administered drug, and the broader therapeutic context [157].

Increased GSH availability can further promote chemoresistance by supporting the conjugation of susceptible electrophilic drugs or their metabolites, through glutathione S-transferases, thereby reducing the effective intracellular drug exposure and attenuating the associated cytotoxic effects [158]. In parallel, the thioredoxin system can provide complementary redox protection by maintaining protein thiols in a reduced state and supplying reducing equivalents for peroxiredoxin-mediated peroxide removal. This functional compensation suggests that targeting a single antioxidant pathway may be insufficient to

compromise redox homeostasis and overcome treatment resistance in tumors with substantial redox plasticity [159].

Redox adaptation also intersects with mitochondrial plasticity and metabolic reprogramming. Chemoresistant cells may alter oxidative phosphorylation, glycolysis, glutaminolysis, and PPP activity to maintain ATP production and increase NADPH supply, supporting both biosynthesis and antioxidant regeneration [160,161]. These metabolic adjustments can reduce ROS accumulation after treatment, while also providing energy and metabolic intermediates for DNA repair and cell survival [162]. Importantly, ROS-sensitive transcriptional programs can promote stem-like phenotypes, epithelial-mesenchymal plasticity, and altered interactions with stromal cells, extending the effects of intracellular redox adaptation to wider mechanisms of acquired chemoresistance [163,164].

These findings indicate that chemoresistance emerges through a coordinated adaptive response in which antioxidant buffering, metabolic rewiring, and phenotypic switching reinforce one another to maintain redox homeostasis and support survival under treatment, rather than through enhanced ROS scavenging alone.

Cisplatin exemplifies the contrasting effects of antioxidant signaling on tumor response and non-malignant tissue toxicity during chemotherapy. Although the formation of DNA crosslinks is crucial to its antineoplastic activity, mitochondrial dysfunction and oxidative stress also contribute to the elicitation of cytotoxic effects [165]. In malignant cells, enhanced Nrf2 activity, increased GSH levels, and upregulated expression of antioxidant enzymes may reduce oxidative burden and promote cisplatin resistance [166]. Contrariwise, in normal tissues, Nrf2 activation may protect against treatment-associated toxicity. This divergence highlights a major therapeutic challenge; systemic suppression of antioxidant pathways may sensitize tumor cells to chemotherapy, while simultaneously reducing the protection of normal tissues, which emphasizes the need for tumor-selective redox targeting strategies [167].

6.2. Radiotherapy Resistance

Redox processes are integral to the cellular response to radiotherapy. Ionizing radiation damages cellular macromolecules both directly through energy deposition and indirectly through H_2O radiolysis [168]. Despite the fact that DNA double-strand breaks are among the most consequential lesions, ROS generated during irradiation can also impair mitochondrial function, oxidize membrane lipids and proteins, and sustain redox-sensitive signaling beyond the initial exposure [169].

The extent of oxidative damage caused by irradiation depends, in part, on oxygen availability at the time of exposure [170]. According to the oxygen fixation hypothesis (OFH), O_2 reacts with radiation-induced DNA radicals, converting some potentially reversible alterations into more persistent lesions [171]. In hypoxic tumor regions, this reaction is less efficient, allowing a greater proportion of DNA radicals to undergo chemical restitution before the damage becomes irreversible [63,172]. Therefore, tumor hypoxia represents a well-established microenvironmental determinant of radioresistance in solid tumors.

Reduced fixation of radiation-induced damage due to limited oxygen availability represents only one component of hypoxic radioresistance. Hypoxia also engages hypoxia-inducible factor (HIF) signaling, which can reshape glucose metabolism, promote angiogenesis and autophagy, favor cellular states associated with stemness, and modify mechanisms involved in the recognition and repair of DNA damage, thereby supporting tumor cell survival following irradiation [173,174]. However, hypoxia does not eliminate intracellular ROS production, as hypoxic tumor cells must still regulate oxidant levels and may rely on antioxidant defenses to preserve cellular viability [175]. Accordingly, hypoxia can contribute to radioresistance through two complementary mechanisms; reduced

fixation of radiation-induced lesions and continued reliance on adaptive redox buffering to support tumor cell survival under metabolic stress.

Following irradiation, adaptive responses driven by Nrf2 upregulation can strengthen antioxidant buffering and facilitate DNA damage recognition and repair, resulting in radioresistance [176,177]. In experimental models of lung cancer, irradiation increased Nrf2 abundance, while Nrf2 promoted replication protein A 32 kDa subunit (RPA32) phosphorylation and DNA topoisomerase II-binding protein 1 (TOPBP1) recruitment to damaged DNA, enhancing ataxia telangiectasia and Rad3-related—checkpoint kinase 1 (ATR–CHK1 signaling) [178,179]. These observations suggest that the contribution of Nrf2 to radioresistance involves not only antioxidant defense but also direct modulation of DNA damage response. Mechanistically, this is particularly important, linking redox adaptation with checkpoint activation and DNA repair capacity, rather than treating them as separate mechanisms of resistance.

Metabolic reprogramming further reinforces radioresistance. Increased flux through the PPP supplies NADPH for the regeneration of antioxidant systems and ribose phosphates for nucleotide synthesis, while glutamine metabolism provides substrates for GSH synthesis and supports the metabolic requirements of DNA repair processes [180]. Changes in mitochondrial metabolism may also help meet the increased energy demands of cell survival and repair following irradiation [181].

Collectively, these adaptations showcase that radioresistance reflects the interaction of reduced oxygen-dependent damage fixation, antioxidant buffering, metabolic reprogramming, and DNA-damage responses, not a single ROS-scavenging mechanism.

6.3. Targeted Therapy and Immunotherapy Resistance

Redox adaptation may also contribute to resistance to molecularly targeted therapies. Inhibition of an oncogenic pathway can impose metabolic stress, prompting tumor cells to alter mitochondrial function, nutrient use, and antioxidant defenses [182]. These changes may enable a subset of cells to persist despite continued target inhibition [183]. Therefore, resistance may arise not only through secondary mutations or pathway reactivation, but also through reversible, stress-tolerant cell states associated with altered redox metabolism.

This form of adaptation has been studied extensively in B-Raf proto-oncogene, serine/threonine kinase (BRAF)-mutant melanoma. BRAF and MEK inhibition can suppress MAPK-dependent glycolysis and increase mitochondrial oxidative metabolism in surviving cells [184]. In some experimental models, resistant cells also become more reliant on glutamine to fuel mitochondrial activity [185]. These metabolic changes alter ROS production and may increase the need for antioxidant defenses, helping cells persist during treatment. At the same time, the resulting dependence on mitochondrial and glutamine metabolism may create additional therapeutic vulnerabilities that could be exploited in resistant disease.

In residual tumor cells exposed to targeted therapy, sustained Nrf2 activity may facilitate persistence by supporting adaptation to treatment-induced oxidative and metabolic stress [186]. Although the primary oncogenic genotype dictates the initial therapeutic response, the remaining subpopulation may become progressively dependent on adaptive redox mechanisms that contribute to the subsequent emergence of acquired resistance. This shift highlights a critical biological divergence between the persistent residual disease and the treatment-naïve tumor architecture.

The relationship between redox regulation and immunotherapy resistance extends beyond the antioxidant capacity of malignant cells. T-cell activation depends on appropriately regulated ROS signaling, whereas sustained oxidative stress in the tumor microenvironment can compromise T-cell metabolism and effector function and contribute to

exhaustion [187,188]. Myeloid-derived suppressor cells further shape this environment through the production of ROS and reactive nitrogen species (RNS), which can interfere with T-cell signaling and antigen-specific recognition [189]. Oxidative stress within the tumor microenvironment may restrict the efficacy of immune-checkpoint blockade even when tumor antigens remain available for recognition.

Redox pathways may also influence checkpoint regulation more directly. Associations between Nrf2 activity and PD-L1 expression have been reported in experimental models, although their direction and strength vary with tumor context [190]. In lung squamous-cell carcinoma, Nrf2 pathway activation was associated with shorter progression-free survival among patients treated with programmed cell death protein 1 (PD-1) blockade and chemotherapy, with mechanistic analyses also implicated Nrf2 in constitutive PD-L1 expression [191]. These observations point to a relationship between redox adaptation and checkpoint regulation, rather than a uniform effect of ROS on PD-L1 expression across cancers.

Evidence from Keap1-mutant lung adenocarcinoma suggests an additional mechanism. Experimental Keap1 loss and consequent Nrf2 activation diminished dendritic-cell and T-cell responses and conferred resistance to immune-checkpoint therapy [192]. Glutaminase inhibition partially restored antitumor immunity and improved the response to checkpoint blockade, implicating altered tumor metabolism in the immunosuppressive effects of Keap1/Nrf2 activation [192].

7. Therapeutic Targeting of ROS in the TME

The elevated oxidative burden and high dependence on antioxidant defenses characteristic of many cancer cells create potential therapeutic vulnerabilities. One approach is to further increase ROS production or inhibit the mechanisms that regulate their levels pushing malignant cells toward oxidative distress and inducing cytotoxicity [193]. Conversely, the selective suppression of ROS levels may offer therapeutic benefits, particularly when redox signaling contributes to promoting inflammation, activating the tumor stroma, or causing oxidative damage to normal tissues as a consequence of anticancer therapy [69,194].

Therefore, therapeutic modulation of the redox state should not be limited to simply increasing or decreasing ROS; instead, it should selectively target the redox mechanisms upon which cancer cells depend, while preserving the physiological functions of ROS in normal and immune cells [195].

7.1. Pro-Oxidant Strategies and Disruption of Antioxidant Defenses

One of the key therapeutic approaches relies on increasing oxidative pressure to a level that exceeds the capacity of cancer cells for self-regulation. This can be achieved either by enhancing intracellular ROS production or by impairing the antioxidant systems that regulate the accumulation of oxidants from cellular metabolism or therapeutic interventions. This strategy is of particular interest because many cancer cells have less capacity to adapt to a further increase in oxidative load before suffering lethal cellular damage [15,196]. However, this preferential vulnerability is not absolute, as the therapeutic window is determined by factors such as the tumor's genetic background and metabolic state, the tissue microenvironment, as well as the distribution of the therapeutic agent [15].

Among pro-oxidant approaches, compounds that undergo redox cycling are of particular interest, as they can drive continuous ROS production and simultaneously deplete reducing equivalents. A prime example is found in certain quinones that serve as substrates for NQO1, an enzyme overexpressed in various cancer types. The reduction of suitable quinones by NQO1 is followed by the rapid re-oxidation of the resulting hydroquinones, establishing a continuous redox cycle accompanied by elevated ROS

production, particularly of H_2O_2 [197]. This intense oxidative stress can induce extensive cellular damage and ultimately cell death, while simultaneously allowing the elevated NQO1 expression in certain tumors to be exploited for more selective anticancer activity [198]. In preclinical models of triple-negative breast cancer, the bioactivation of isobutyl-deoxynyboquinone (IB-DNQ) by NQO1 increased ROS generation and exerted cytotoxic effects against cancer stem cells with either epithelial- or mesenchymal- like phenotypes, with inhibition of SOD1 further enhancing this activity [197]. Despite significant preclinical interest, therapeutic strategies based on NQO1-dependent redox cycling have not yet been widely established in clinical practice.

Mitochondria might represent an additional target for pro-oxidant interventions due to their central role in oxidative metabolism, ROS production, and the regulation of cell death. Disrupting mitochondrial respiration, electron transport chain function, membrane potential, or the mechanisms maintaining mitochondrial redox balance can promote ROS production, affect energy homeostasis, and ultimately trigger cell death pathways [41]. Researchers have also investigated targeting systems based on lipophilic cations, such as triphenylphosphonium (TPP^+) which accumulate within mitochondria by exploiting the negative potential of the inner mitochondrial membrane [199]. Consequently, conjugating therapeutic molecules with TPP^+ can boost their concentration within this specific organelle and, depending on the transported agent, locally increase oxidative stress [199]. Within this context, mitochondrially targeted tamoxifen (MitoTam) was investigated in an open-label, single-center phase I/lb trial in patients with metastatic solid tumors [200]. Phase I enrolled 37 patients aiming to characterize safety and to determine maximum tolerated dose [200]. Phase lb enrolled 38 patients to examine toxicity as the primary objective and antitumor activity as a secondary objective [200]. Among six patients with renal cell carcinoma in phase lb, one achieved a partial response and four had stable disease [200]. However, these preliminary findings are limited by the small subgroup and the absence of a comparator arm. More broadly, the clinical translation of mitochondria-targeted redox approaches is constrained by the heterogeneity of mitochondrial metabolism across different tumors, potential toxicity to non-cancerous tissues, and the difficulty of achieving sufficient tumor selectivity [41].

Another pro-oxidant approach relies on the utilization of metal ions to enhance ROS production within the tumor. This principle underlies chemodynamic therapy, which aims to locally increase oxidative stress and induce cytotoxicity within the TME using appropriate metal-containing systems [201]. Specific characteristics of certain tumors including lower pH, elevated H_2O_2 concentrations, and disrupted metal homeostasis can facilitate the action of these systems within the cancer microenvironment [201]. Nevertheless, its application remains primarily at the preclinical stage and often necessitates the use of nanomaterials for the effective delivery and activation of therapeutic agents [201].

The GSH dependency represents a potential therapeutic vulnerability in tumors that rely heavily on this pathway to sustain survival or develop resistance to treatment. Accordingly, reducing GSH availability, inhibiting its biosynthesis, or restricting cysteine supply can compromise antioxidant defense and sensitize cancer cells to treatment [202]. In particular, inhibition of cystine uptake through SLC7A11/system x_c^- can restrict GSH synthesis and increase susceptibility to lipid peroxidation and ferroptosis [203]. However, the therapeutic value of this strategy strongly depends on the degree of pathway dependence, as tumors with high metabolic and redox plasticity may compensate through alternative antioxidant mechanisms. This translational challenge is illustrated by early-phase clinical studies using buthionine sulfoximine (BSO) to inhibit GSH synthesis, including in combination with melphalan, demonstrating that substantial GSH depletion in peripheral blood and tumor tissue is achievable [204,205]. In a dose-escalation study enrolling 41 patients, the intermittent BSO administration at doses of ≥13 g/m^2 lowered intratumoral

GSH to ≤20% of baseline levels by day 3 in five of seven patients with evaluable biopsies [204]. Dose-related neutropenia required a reduction in the melphalan dose [204]. In a subsequent Phase I study involving 21 patients with advanced cancers, serial tumor biopsies were obtained from 10 patients [205]. The continuous BSO infusion reduced intratumoral GSH to less than 10% of pre-treatment levels, with consistent GSH depletion after 48–72 h of infusion [205]. BSO alone was associated with minimal toxicity, whereas occasional grade 4 myelosuppression occurred in combination with melphalan [205]. These studies primarily established pharmacological feasibility and safety rather than definitive antitumor efficacy, and BSO has not become an established anticancer treatment.

The thioredoxin system constitutes a complementary therapeutic target, particularly in tumors that maintain redox homeostasis despite the disruption of antioxidant defenses depending on GSH. TrxR inhibition can increase intracellular oxidative stress and is being investigated as a potential anticancer strategy [206]. For example, auranofin, an approved gold-based drug developed for the treatment of rheumatoid arthritis, has attracted scientific interest as a potential anticancer agent because of its ability to inhibit TrxR. However, its clinical evaluation in oncology remains at an early stage, primarily involving Phase I and II trials [207]. For instance, the combined use of auranofin with sirolimus, an mTOR inhibitor immunosuppressant, was investigated in a Phase II trial in patients with recurrent high-grade serous ovarian cancer as a potential strategy to target protein kinase C iota (PKCι) signaling [208]. Tumor response was the primary endpoint, with no objective responses observed among the [NO_PRINTED_FORM] 21 evaluable patients and at least one adverse event of grade 3 or higher reported in 14 patients [208]. Thus, current clinical evidence is insufficient to establish whether thioredoxin dependence can be exploited with an adequate therapeutic window in selected tumors.

The reliance of selected tumors on sustained Keap1/Nrf2 signaling provides a rationale for therapeutic intervention [209,210]. While various inhibitors and intervention strategies targeting the Nrf2 signaling pathway have been proposed, most remain at the preclinical stage, with selectivity, pharmacokinetics, and toxicity in normal tissues constituting significant limitations [211]. Direct pharmacological targeting is further complicated by the structural characteristics of this transcription factor and the limited specificity of several experimental compounds. For instance, brusatol, initially proposed as an Nrf2 inhibitor, also suppresses global protein synthesis, raising concerns regarding its specificity [212,213].

Consequently, although GSH metabolism, TrxR, and Nrf2 represent attractive therapeutic targets, their clinical exploitation remains constrained by pharmacological selectivity, toxicity, and the absence of validated biomarkers identifying tumors that are specifically dependent on these pathways.

The induction of ferroptosis has also attracted the attention of the scientific community as a potential pro-oxidant strategy. Its induction in cancer cells can be achieved by inhibiting the SLC7A11–GSH–GPx4 axis, increasing the intracellular labile iron pool, altering membrane lipid composition to favor peroxidation, or blocking alternative protective mechanisms such as FSP1 [214,215]. Notably, certain mesenchymal, dedifferentiated, or therapy-resistant cancer phenotypes exhibit an increased reliance on mechanisms that limit lipid peroxidation, a characteristic that may render them more susceptible to ferroptosis [216–218].

Despite growing therapeutic interest, the clinical application of ferroptosis in oncology still remains at an early stage. To date, no anticancer therapy based specifically on its induction has been established in clinical practice. Key issues requiring investigation include the selective targeting of cancer cells, the identification of reliable biomarkers of sensitivity, the effects of ferroptosis on immune and stromal cells, and the determination of a safe and effective therapeutic window [219,220].

7.2. Antioxidant and Redox-Normalizing Strategies: Context-Dependent Applications

Antioxidant and redox-normalizing interventions pose a distinct therapeutic challenge because suppression of oxidative processes may exert opposing effects in malignant and non-malignant cells. Therefore, their potential value hinges on selectivity, identifying and targeting the cellular sources and molecular pathways that drive pathological redox signaling within the relevant tissue compartment, rather than indiscriminately decreasing ROS levels [14,24].

Systemic antioxidant administration may simultaneously protect malignant cells against oxidative damage induced by chemotherapy or radiotherapy, thereby rendering non-selective antioxidant administration less effective [221,222]. Data from systematic reviews and clinical studies show significant heterogeneity, with observations ranging from potential benefit to a lack of effect or even potential interference with anticancer therapy [222,223]. These effects vary considerably depending on the cancer type, therapeutic approach, type of antioxidant agent, dosage, and timing of administration.

The limitations of systemic antioxidant administration are also illustrated by large randomized clinical trials. In the Beta-Carotene and Retinol Efficacy Trial (CARET), supplementation with β-carotene and retinyl palmitate in smokers and asbestos-exposed individuals failed to prevent lung cancer and was associated with increased lung cancer incidence and overall mortality, resulting in premature termination of the trial [224]. Similarly, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) demonstrated that vitamin E supplementation was associated with an increased incidence of prostate cancer during extended follow-up [225]. Although these studies investigated cancer prevention rather than treatment of established malignancies, they highlight the potential adverse consequences of assuming that systemic antioxidant supplementation necessarily provides cancer-related benefits. In Diet, Exercise, Lifestyle and Cancer Prognosis (DELCaP) study, a prospective ancillary study within SWOG S0221, 1134 patients with breast cancer provided information on their dietary supplement use before and during chemotherapy. Compared with patients who disclosed no antioxidant supplementation at either time point, those who reported use at both of them had adjusted hazard ratios of 1.41 for a disease-free survival event (95% Cl, 0.98–2.04; $p = 0.06$) and 1.40 for all-cause mortality (95% Cl, 0.90–2.18; $p = 0.14$) [221]. The disease-free survival endpoint included breast cancer recurrence, new breast cancer diagnosis, or death. Although the point estimates showed higher hazards among patients who reported antioxidant supplementation at both time points, the confidence intervals included 1.0 and were compatible with a range of associations. Indeed, the randomization in the parent trial applied to chemotherapy schedules, but not to supplement use, which was self-reported and observational. Thus, residual confounding and potential misclassification of supplement use limit causal inference and the observed differences cannot be confidently attributed to antioxidant supplementation. Collectively, these findings emphasize that the biological effects of antioxidants depend on the clinical setting, dose, timing, and underlying redox state, and reinforce the need to distinguish nonspecific antioxidant supplementation from selective pharmacological modulation of redox pathways.

Amifostine, an organic thiophosphate, provides an established clinical example of redox modulation, serving as a cytoprotective agent against specific adverse effects of chemotherapy and radiotherapy [226,227]. In contrast to redox-targeting strategies directed at tumors, its purpose is to protect normal tissues rather than promote oxidative injury in malignant cells. Randomized clinical trials have demonstrated that amifostine can mitigate nephrotoxicity and hematologic toxicity of cisplatin-based chemotherapy and reduce both acute and chronic xerostomia in patients undergoing radiotherapy for head and neck cancer [228–230]. Specifically, in a randomized trial involving 242 patients with advanced ovarian cancer treated with cisplatin and cyclophosphamide, a decline in

creatinine clearance of at least 40% from baseline occurred in 13% of patients who received amifostine and 30% of controls ($p = 0.001$) [229]. Furthermore, in a randomized phase III trial in patients undergoing radiotherapy for head and neck cancer, amifostine reduced the incidence of grade ≥2 acute xerostomia from 78% to 51% ($p < 0.0001$) and chronic xerostomia from 57% to 34% ($p = 0.002$) [230]. Amifostine did not reduce acute mucositis, while common adverse effects included nausea, vomiting, hypotension, and allergic reactions [230]. The observed decrease in xerostomia was maintained at the two-year follow-up, with no significant between-group differences in locoregional control, progression-free survival, or overall survival [228]. Consequently, its clinical use highlights the value of redox modulation in supportive oncology, whereas approaches directly exploiting specific redox vulnerabilities of tumors remain at a substantially earlier stage of development.

It is crucial to clearly distinguish between the modulation of redox status by potential pharmacological agents and the non-specific dietary administration of antioxidants. Targeted antioxidant agents or ROS-scavenging systems may be beneficial when directed at normal tissues to limit cancer therapy-related toxicity, or when specific ROS-dependent pathways contribute to the maintenance of pathological inflammation or stromal dysfunction [231]. Conversely, a generalized enhancement in the tumor's antioxidant capacity could increase its resistance to therapies that rely on inducing oxidative damage. Accordingly, the therapeutic impact of redox suppression strongly depends on the cellular compartment targeted, as reducing oxidative injury in normal cells may be beneficial, whereas enhancing antioxidant capacity in malignant cells may compromise anticancer efficacy [232,233].

Consequently, antioxidant approaches should not be considered universally anticancer. Their potential therapeutic value depends on which cells or tissues are protected, which ROS are targeted, the cellular compartment where the intervention takes place, and the stage of treatment at which it is applied. This distinction is particularly important within the TME, where excessive ROS accumulation can suppress the function of T lymphocytes and NK cells [234], whereas maintaining ROS at controlled levels is essential for the normal activation and signaling of immune cells [66]. Therefore, excessive suppression of ROS through antioxidant interventions can be just as undesirable as overwhelming oxidative stress [66].

7.3. Combination Therapies

One of the most promising therapeutic approaches targeting redox status involves combination therapies, in which redox modulators are primarily used to enhance the efficacy of established treatments or to counteract mechanisms of adaptive resistance, rather than as stand-alone therapeutic agents. Given that chemotherapy, radiotherapy, targeted therapies, and immunotherapy affect redox homeostasis through distinct mechanisms, combination approaches can be employed to exploit the oxidative vulnerabilities induced during treatment, while simultaneously allowing for a potential reduction in the required doses of the individual therapeutic agents [15,19].

Combining factors that disrupt redox homeostasis with chemotherapy can limit the antioxidant response and, consequently, increase the sensitivity of cancer cells to oxidative stress induced by chemotherapeutic agents. In this context, inhibiting Nrf2 signaling [209], reducing GSH levels [235], and targeting SLC7A11 [236] or TrxR [206] have been investigated as strategies to re-sensitize resistant cancer cells to cytotoxic therapies. The rationale is not merely to intensify oxidative stress, but to block the adaptive pathways on which residual tumor cells become progressively reliant for survival following treatment. A similar approach can be applied to radiotherapy, where compromising antioxidant defense can intensify the radiation-induced oxidative burden and limit the cells' ability to cope with the resulting damage [237]. However, since both chemotherapy and

radiotherapy can also cause oxidative damage to normal tissues, achieving sufficient tumor selectivity is essential for preventing exacerbating systemic toxicity [15,237].

Among them, combination approaches based on the induction of ferroptosis attract significant interest, particularly for addressing the treatment-resistance of cancer. Specifically, radiotherapy might induce lipid peroxidation and alter iron homeostasis and redox balance, while certain chemotherapeutic and targeted agents can compromise protective mechanisms against ferroptosis. Combining these effects with the inhibition of SLC7A11, GPx4, or other related protective pathways could, therefore, enhance the induction of ferroptotic cell death in cancer populations that otherwise would exhibit therapeutic resistance [238–240]. Analysis of tumor specimens from 30 patients with esophageal cancer showed that higher 4-hydroxynonenal (4-HNE) staining after radiotherapy was associated with better treatment response and longer disease-free survival [239]. However, these findings establish a biomarker-outcome association rather than demonstrate a clinical benefit from combining radiotherapy with a ferroptosis sensitizer.

The relationship between redox regulation and immunotherapy seems to be more complex. Excessive ROS accumulation within the TME can impair the function of effector lymphocytes and contribute to immunosuppression, supporting the notion that the selective restoration of oxidative load, whether extracellular or of myeloid origin, could potentially improve responses to immune checkpoint inhibitors. Conversely, ROS-induced cancer cell death can increase the release of damage-associated molecular patterns (DAMPs) and enhance immunogenic signaling [241]. Similarly, ferroptosis can either potentiate or limit anti-tumor immunity, depending on the cell population undergoing lipid peroxidation [242]; ferroptosis in cancer cells can influence the activation of the immune response, whereas its induction in $CD8^+$ T cells can impair anti-tumor immunity [243,244].

These observations suggest that non-selective systemic modification in redox status combined with immunotherapy may not be the most suitable therapeutic approach. On the contrary, the efficacy of such combinations likely requires interventions with cellular and spatial selectivity, for instance, to preferentially enhance oxidative stress in cancer cells while simultaneously preserving or restoring the redox functionality of anti-tumor immune cells. The requisite selectivity could be achieved through various approaches, such as the use of tumor-selectively activated prodrugs, nanoparticle systems, metabolic targeting, or patient selection based on appropriate biomarkers, although many of these strategies remain at the preclinical stage [245–247]. The main therapeutic approaches discussed herein, together with their principal redox targets or strategies, representative interventions, current stage of evaluation, and principal findings and key translational limitations, are summarized in Table 2.

Table 2. Redox-targeting therapeutic approaches in cancer therapy.

Therapeutic Approach	Redox Target or Strategy	Representative Intervention	Stage of Evaluation	Principal Finding and Key Limitation	References
Pro-oxidant/disruption of antioxidant defenses	NQO1-dependent redox cycling	IB-DNQ	Preclinical	NQO1-mediated activation of IB-DNQ increased ROS production and cytotoxicity in preclinical triple-negative breast cancer stem-cell models. NQO1 dependence may limit applicability across tumor types and clinical selectivity and safety remain unestablished.	[197,198]
	Mitochondria-targeted redox modulation	MitoTam	Open-label Phase I/lb	Phase I (n = 37) established safety and maximum tolerated dose. Phase Ib (n = 38) assessed toxicity (primary) and anti-tumor activity (secondary).	[200]

			Thromboembolic events occurred in 13% of patients; One patient had a partial response and 13 had stable disease. In the renal cell carcinoma subgroup (n = 6), 1 had a partial response and 4 had stable disease. The small subgroup and single-arm design limit interpretation.	
	Chemodynamic therapy	Metal-based Fenton-type systems	Predominantly preclinical	In preclinical models, the catalytic agent generated ROS and induced tumor cell death. Activity depends on catalyst delivery and local TME conditions.	[201]
	GSH depletion	BSO, including combination with melphalan	Phase I	At doses of ≥13 g/m^2, intermittent BSO reduced tumor GSH to ≤20% of baseline by day 3 in 5/7 evaluable patients; Continuous-infusion BSO reduced it to <10% and the combination with melphalan caused occasional grade 4 myelosuppression. Antitumor benefit remains unestablished.	[204,205]
	SLC7A11/system x_c$^-$ targeting	Inhibition of cystine uptake	Predominantly preclinical	Cystine uptake inhibition can limit GSH synthesis and sensitize cells to ferroptosis. Alternative cysteine sources and antioxidant pathways reduce susceptibility.	[202,203]
	TrxR inhibition	Auranofin, in combination with sirolimus	Phase II	Auranofin-sirolimus produced no objective responses on the primary endpoint and 14/21 patients experienced at least one grade ≥3 adverse event. The trial tested a PKCι-directed strategy; thus, intratumoral TrxR engagement and the clinical potential of TrxR inhibition remain unresolved.	[206–208]
	Keap1/Nrf2 pathway inhibition	Brusatol and experimental Nrf2 inhibitors	Predominantly preclinical	In experimental models, brusatol suppressed Nrf2 signaling and increased susceptibility to oxidative injury. Its limited specificity precludes attributing the sensitization to Nrf2 inhibition alone.	[209,210,212,213]
	Ferroptosis induction	Targeting SLC7A11–GSH–GPx$_4$, iron metabolism or FSP1	Predominantly preclinical	Ferroptosis was induced in susceptible tumor cells in experimental models. Tumor selectivity, predictive biomarkers, and the therapeutic window remain unresolved.	[203,214,215,219,220]
Antioxidant/redox normalizing	Selective suppression of pathological ROS signaling	Selective ROS scavenging or tissue-directed redox modulation	Investigational	ROS suppression requires cell and tissue selectivity because excessive suppression may protect tumor cells or impair antitumor immunity.	[14,24,66]
	Normal-tissue redox protection	Amifostine	Established clinical use in supportive oncology	Amifostine reduced the incidence of a ≥40% decline in creatinine clearance during cisplatin-based chemotherapy for advanced ovarian cancer (13% vs. 30% in controls). In a phase III head and neck radiotherapy trial, amifostine reduced grade ≥2 acute xerostomia (51% vs. 78%)	[228–230]

				and late xerostomia (34% vs. 57%). The benefit persisted at two years.	
Combination therapies	Redox-defense inhibition & chemotherapy/radiotherapy	Targeting GSH, TrxR, Nrf2, or SLC7A11 during conventional therapy	Investigational; stage varies by strategy	Limited tumor selectivity may increase oxidative injury and treatment-related toxicity in normal tissues.	[206,209,235–237]
	Ferroptosis-based combinations	Ferroptosis sensitization combined with radiotherapy or other anticancer therapies	Predominantly preclinical	Ferroptosis sensitizers enhanced radiation response in preclinical models. In 30 patients with esophageal cancer, stronger post-radiotherapy 4-HNE staining was associated with better response and longer disease-free survival. Clinical benefit of the combination remains unestablished.	[238–240]
	Redox modulation & immunotherapy	Selective modulation of tumor oxidative stress and immune redox function	Predominantly preclinical/mechanistic	In experimental models, tumor-cell ferroptosis contributed to the antitumor effects of immune checkpoint blockade. CD36-mediated lipid peroxidation and ferroptosis in tumor-infiltrating CD8⁺ T cells compromised their effector function.	[242–244]
	Tumor-selective redox delivery	Redox-responsive nanocarriers and tumor-activated prodrugs	Predominantly preclinical	Clinical translation remains limited by delivery, biodistribution, tumor selectivity, and biomarker-guided patient selection.	[245–247]

The principal therapeutic strategies designed to target redox vulnerabilities of tumor cells, along with their potential combinations and major translational barriers are illustrated in Figure 4.

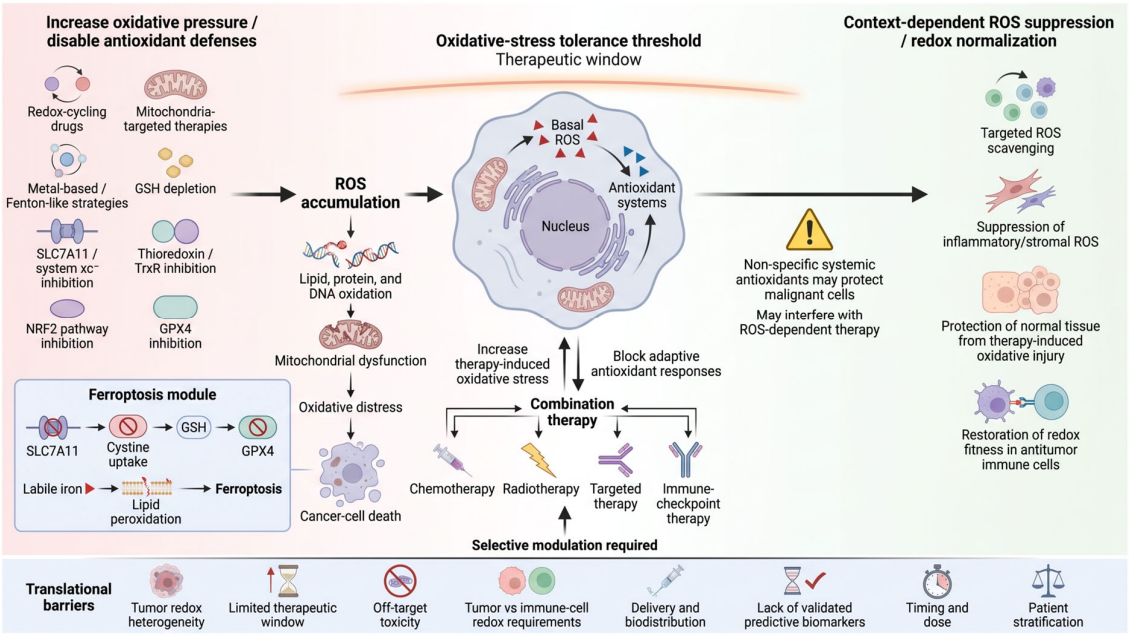


Figure 4. Therapeutic exploitation of redox vulnerabilities in cancer. Cancer cells frequently experience elevated oxidative stress and rely on antioxidant defenses to sustain redox homeostasis. Therapeutic strategies can target this reliance by enhancing ROS production or weakening antioxidant defense systems, including the SLC7A11–GSH–GPx4 axis, TrxR, and Nrf2 signaling. Excessive oxidative damage impairs cellular function and induces cancer cell death; disruption of the SLC7A11–GSH–GPx4 axis may also trigger ferroptosis via lipid peroxidation induced by iron accumulation. On the contrary, selective ROS suppression may protect normal tissues or preserve antitumor immune cell function, whereas nonspecific antioxidant administration may inadvertently protect tumor cells. Redox modulation shows the potential to complement conventional and immune-based therapies, although tumor heterogeneity, off-target effects, delivery challenges, and the absence of validated predictive biomarkers are key barriers to clinical translation. Created in BioRender. Vardakas, P. (2026) https://BioRender.com/n7dxt71.

8. Biomarkers of ROS Activity in the TME

The measurement of ROS levels is considered demanding and complex due to their limited stability, low cellular concentrations, and rapidly changing levels. Alternatively, ROS activity can be indirectly detected using oxidative damage markers, which provide information about ROS generation, as well as the cellular repair and damage-removal mechanisms [248]. The most common byproducts resulting from excessive ROS-mediated damage include 8-hydroxy-2′-deoxyguanosine (8-OHdG), 4-HNE modifications, and malondialdehyde (MDA), which are associated with oxidative damage to nucleic acid, protein and lipids [249]. Regarding lipid damage, ROS oxidize polyunsaturated fatty acids, leading to lipid peroxidation and the production of MDA, HNE and F2-isoprostanes (F2-IsoPs), which can be measured using different analytical methods, including LC-MS [248]. Notably, elevated MDA levels can be found in the blood serum of patients with different cancer types [250]. The most extensively investigated ROS-mediated DNA modification is the formation of 8-OHdG [251]. Due to its clinical relevance, 8-OHdG levels can be used to assess oxidative stress associated with pathological conditions and may also provide prognostic information. For example, increased serum 8-OHdG levels in epithelial ovarian cancer have been correlated with advanced disease stages and poorer survival [250]. Protein oxidation can be also measured through specific alterations, including protein carbonyl formation and they can be detected with ELISA and LC-MS assays [248]. However, oxidative damage can be easily overestimated or underestimated, underlining the importance of careful sample handling and data interpretation [248].

In addition to oxidative damage markers, researchers are now turning to redox-related gene signatures as another way to measure stress in cancer. Advances in sequencing technologies have enabled the identification of gene expression patterns associated with redox-related pathways. This strategy may provide information about tumor prognosis and the characteristics of the TME [252]. For instance, for colorectal cancer, a redox-driven prognostic signature (RDPS) consisting of two genes, alcohol dehydrogenase 5 (ADH5) and hydroxyacyl-CoA dehydrogenase (HADH), was developed based on the expression profiles of redox-related genes. The resulting risk score was associated with patient survival and could stand as a predictive factor for poor outcome [252]. Differences in immune cell populations and immune checkpoint profiles were observed between risk groups, suggesting a correlation of redox-related gene expression with the immune landscape of the TME [252]. Another similar example involves the use of oxidative stress-related genes (OSRGs) in patients with breast cancer [253]. OSRG scores were related to immune cell infiltration within the TME. Interestingly, patients with higher OSRG scores showed lower sensitivity to PD-1 and cytotoxic T-lymphocyte-associated protein (CTLA-4) according to the low-score group, implying the utility of OSRG in predicting immunotherapy outcomes [253]. Overall, these findings indicate that redox gene signatures may

provide information not only about cancer prognosis but also about the immune characteristics and potential therapeutic response of the TME.

Despite these promising findings, the clinical actionability of redox-related gene signatures remains uncertain. An association between a redox-driven prognostic signature (RDPS) or OSRG score and survival does not, by itself, establish that the score can predict benefit from a particular treatment. Similarly, differences in immune-cell infiltration or computationally inferred sensitivity to immune checkpoint blockade should be considered hypothesis-generating unless supported by clinical response data and independent validation in appropriately treated patient cohorts. Before these signatures can inform treatment selection, their predictive performance should be confirmed in independent, preferably multicenter cohorts, using prespecified scoring algorithms and clinically reproducible thresholds. Their added value beyond established clinicopathological variables and validated biomarkers should also be demonstrated. Accordingly, RDPS and OSRG scores are currently best regarded as investigational tools for prognostic assessment and TME characterization, rather than established biomarkers for selecting redox-targeted or immunotherapeutic interventions.

In recent years, a variety of methods are available for ROS detection. Fluorescence analysis, electron paramagnetic resonance (EPR or ESR) and chemiluminescence are the most popular. Among them, fluorescence analysis is one of the most widely used approaches [254]. More specifically, fluorescence imaging with probes provides major advantages, such as easy handling and high sensitivity to detect ROS in cellular systems [255]. Different probes can be employed depending on the ROS, which are under investigation [248]. For example, the 2,7-Dichlorofluorescin diacetate (DCF-DA) is applied for the detection of H_2O_2, hydroxyl radicals and ROO, whereas DHE can be used for superoxide measurement through the generation of a fluorescent signal [248]. However, fluorescent probes vary in ROS specificity, brightness and stability, which must be optimized [255]. Another approach for ROS detection is provided by genetically encoded fluorescent sensors. These sensors offer increased specificity and sensitivity, especially for real-time monitoring in living cells. A limitation of this strategy is that their expression can influence cellular processes, including ROS generation and intracellular redox regulation [248]. In addition, EPR is suitable for measuring ROS with unpaired electrons, such as O_2, and presents high ability even at smaller ROS levels. Its application in living cells and tissues remains difficult because EPR requires large sample volumes [248]. These challenges emphasize the need for advanced methods for accurate ROS detection and monitoring [255].

Circulating biomarkers represent another approach for assessing redox alterations in cancer patients. Several oxidative stress markers can be detected in biological fluids, including MDA and 8-OHdG in blood and 8-iso-prostaglandin F2α (8-iso-PGF2α) in blood and urine [250]. In non-small-cell lung cancer (NSCLC), plasma 8-OHdG levels were associated with tumor histology and tumor volume [251]. Notably, changes in 8-OHdG levels during radiotherapy were related to treatment response. This event supports its use as a future prognostic biomarker [251]. Of note, 8-iso-PGF2α has the ability to remain stable in urine and plasma, and researchers have demonstrated that excessive urinary levels were observed in patients with bladder cancer compared with healthy individuals [250]. Last but not least, decreased activity of the antioxidant enzyme paraoxonase 1 (PON1) has been detected in the serum of patients with endometrial, colorectal and bladder cancer, while reduced PON1 levels were further reported in cases of tumor recurrence [250].

At present, most oxidative damage markers are more suitable for research-level assessment of oxidative injury or longitudinal monitoring than for standalone treatment selection. Their interpretation depends on the biological compartment sampled, the timing of collection relative to treatment, and the balance between oxidative damage, repair, and clearance. For example, circulating 8-OHdG may reflect systemic processes and does not

necessarily capture spatially localized oxidative DNA damage within the tumor, while MDA and 4-HNE measurements can be affected by sample processing and assay specificity. Differences in analytical platforms, pre-analytical handling, normalization procedures, and threshold selection further limit comparisons across studies. Where feasible, validated chromatographic methods coupled with mass spectrometry can improve analytical specificity for selected oxidative damage products, although technical complexity and cost may constrain routine implementation [252,256,257]. Clinical translation will therefore require standardized assays, reproducible cutoffs, prospective validation in defined patient populations, and evidence that biomarker-guided decisions improve outcomes. Ultimately, the most useful approach may combine redox-related measurements with established clinical and molecular variables rather than rely on a single oxidative stress marker or gene-expression score.

9. Emerging Concepts and Future Directions

Apart from the GSH-GPx$_4$ antioxidant system, several other mechanisms have been identified that protect cells against lipid peroxidation and ferroptosis. One of these mechanisms involves ferroptosis suppressor protein 1 (FSP1), which acts independently of GPx$_4$ to protect cells against ferroptosis. FSP1 is recruited to the plasma membrane through myristoylation, where it reduces coenzyme Q10 (CoQ10) to ubiquinol. Ubiquinol is a lipophilic antioxidant that limits the propagation of lipid peroxidation. Interestingly, FSP1 expression was positively correlated with ferroptosis resistance across hundreds of cancer cell lines. These findings imply that its inhibition could increase the sensitivity of cancer cells to ferroptosis-inducing treatments [215].

The GTP cyclohydrolase 1 (GCH1)–tetrahydrobiopterin (BH4) pathway represents another mechanism of this category. GCH1 promotes the production of BH4 and dihydrobiopterin (BH2), which act as antioxidants and protect cells against lipid peroxidation. More specifically, increased GCH1 expression was shown to alter cellular lipid composition and protect phospholipids containing two polyunsaturated fatty acid chains from oxidative damage [258]. Dihydroorotate dehydrogenase (DHODH) is a mitochondrial enzyme involved in pyrimidine biosynthesis. It has also been identified as an important regulator of ferroptosis. Its protective function includes the reduction of ubiquinone to ubiquinol, thereby limiting lipid peroxidation in the inner mitochondrial membrane. This mechanism acts simultaneously with mitochondrial GPx4. The inhibition of DHODH was found to induce ferroptosis in cancer cells with low GPx4 expression. In experimental tumor models, the DHODH inhibitor brequinar suppressed the growth of GPx4-low tumors, while its combination with sulfasalazine (an FDA-approved drug with ferroptosis-inducing activity) reduced the growth of GPx4-high tumors. This approach could represent a potential therapeutic strategy for cancer management [259].

In addition to these antioxidant mechanisms, the lipid composition of cellular membranes also influences the sensitivity of cancer cells to ferroptosis. In dormant disseminated tumor cells (DTCs), increased de novo lipogenesis results in the activation and integration of monounsaturated fatty acids (MUFAs) into cellular membranes. The acyl-CoA synthetase long-chain family member 3 (ACSL3) participates to this process and contributes to protection of these cells against ferroptosis. In contrast, inhibition of de novo lipogenesis or ACSL3 knockdown was found to promote lipid peroxidation and ferroptotic cell death [260]. Another example concerns monocyte-to-macrophage differentiation-associated (MMD), which is a Golgi-associated protein. MMD was found to increase ferroptosis sensitivity in ovarian and renal carcinoma cells through its interaction with acyl-CoA synthetase long-chain family member 4 (ACSL4) and membrane-bound O-acyltransferase domain-containing 7 (MBOAT7). These enzymes promote the incorporation of arachidonic acid (AA) into phosphatidylinositol (PI) lipids, which results in an increased

abundance of AA-containing phospholipids. As an outcome, ovarian and renal carcinoma cells become more susceptible to ferroptosis [261].

The effects of ferroptosis within the TME also influence antitumor immunity. Activated CD8+ T cells were found to promote ferroptosis in cancer cells through the release of interferon-gamma (IFN-γ), which reduces the expression of SLC3A2 and SLC7A11. This reduction limits cystine uptake by cancer cells and leads to increased lipid peroxidation and ferroptotic cell death. In particular, in experimental mouse models, combining the enzyme cyst(e)inase with immune checkpoint blockade enhanced antitumor immunity and ferroptosis in cancer cells [242]. Ferroptosis can also impact non-malignant immune cells. High expression of CD36 in tumor-infiltrating CD8+ T cells was linked to tumor growth and poor patient survival. Moreover, CD36 promotes fatty acid uptake in these cells, which leads to increased lipid peroxidation and ferroptosis. This process reduces the production of cytotoxic cytokines and impairs their antitumor activity. Importantly, inhibition of CD36 or ferroptosis in CD8+ T cells was found to restore their antitumor function [243]. In other experimental models, early ferroptotic cancer cells were found to induce dendritic cell maturation and promote antitumor immune responses. This effect was related to the release of adenosine triphosphate (ATP) and high-mobility group box 1 (HMGB1), which function as damage-associated molecular patterns (DAMPs). However, the immunogenic effect was observed only in early ferroptotic cells, which may indicate that the immune response depends on the stage of ferroptosis. These observations are very promising for the evaluation of new cancer immunotherapy strategies based on ferroptosis [262]. However, it is important to take into consideration both the resistance mechanisms of cancer cells and the multiple effects of ferroptosis on malignant and non-malignant cells within the TME when developing and improving therapeutic approaches based on ferroptosis.

Tumors are characterized by considerable spatial heterogeneity due to the different distribution of cellular populations across the TME. Spatial analysis of oral squamous cell carcinoma (OSCC) demonstrated that the tumor core (TC) area exhibited a significant accumulation of epithelial cells, which extended towards the Transition (TS) zone. On the other hand, T/NK cells, macrophages and endothelial cells were in high concentration in leading edge (LE) area, while fibroblasts were absent in the tumor region [263]. Importantly, this spatial heterogeneity is also associated with differences in stress responses within the TME. For instance, stromal cells located closer to cancer cells have been shown to present higher levels of stress-response activation, indicating the presence of spatial stress gradients within tumors [264]. In breast and ovarian tumors, areas with high oxidative stress and hypoxia stress responses showed an accumulation of stromal cells and particularly CAFs [264]. Notably, these differences within tumors may be influenced by signals originating from cancer cells, such as cytokines, which can affect stress responses in neighboring cells [264].

In recent years, single-cell approaches have been investigated as useful tools for examination of cellular and molecular heterogeneity within the TME. Previously established methods, such as mass spectrometry, lacked resolution at the individual-cell level in contrast to single-cell methods, which can offer a more detailed analysis of intracellular conditions and a better understanding of different cellular phenotypes [265]. Regarding single-cell RNA sequencing (scRNA-seq), this approach enables the exploration of tissue heterogeneity by identifying different cell populations and their gene expression patterns [263]. In cancer, scRNA-seq can reveal distinct cellular states and molecular pathways within the TME [266]. Despite its significance, scRNA-seq does not reveal how different cells are spatially organized within the tissue. Spatial transcriptomics (ST) can contribute by offering insights into spatial distribution and single-cell localization [263]. Development of both methods has improved the investigation of gene expression by allowing for

the examination of transcriptional changes at single-cell and spatial levels [267]. A characteristic example is signaling network under redox stress profiling (SN-ROP). SN-ROP represents a method based on single-cell mass cytometry that enables the analysis of redox-dependent signaling pathways in individual cells. Its application in T cells demonstrated dynamic alterations in the regulation of redox processes during T-cell activation and exhaustion [265]. Future advances in single-cell methods could improve our understanding of redox heterogeneity and its complex role within the TME [265].

Over the past few years, machine learning and computational approaches have been increasingly used for the analysis of extensive omics information and the evaluation of redox-related gene signatures in cancer. These approaches can play an important role in the creation of predictive models and benefit patient prognosis. For instance, in breast cancer, a variety of bioinformatic tools, such as Random Forest and Gradient Boosting Machine, and TCGA and GEO datasets were combined to develop a redox-related signature with prognostic value [268]. The results showed promising performance in predicting patient outcomes [268]. Similar approaches have also been applied to colon cancer. Multivariable cox regression analysis led to a model with the ability to predict patient survival, while survival analysis of 6 important genes demonstrated variations among the patients with different expression of these genes [269]. In lung adenocarcinoma, similar analyses resulted in six gene redox-related signature, while high-risk patients presented poorer overall survival [266]. In the future, the combination of these computational approaches could contribute to the identification of prognostic biomarkers in cancer.

10. Clinical Translation Challenges

Although there is strong molecular evidence supporting the targeting of redox sensitivity in cancer, clinical translation remains challenging; ROS do not exert uniform effects on tumor cells, with their biological impact varying according to several factors, including concentration, chemical identity, exposure duration, metabolic conditions, subcellular localization, and the compromised cellular population. As a result, the same intervention may disrupt physiological signaling or stimulate adaptive responses in one context, while inhibiting tumor-promoting redox signaling in another. This level of complexity underscores the need for adopting redox interventions in each specific context rather than pursuing a general approach focusing solely on increasing or decreasing total ROS levels [270]. While limited data currently support reliable inter-individual redox stratification and its clinical relevance, the notion of individualized redox biology is particularly pertinent in this case [270].

Defining an appropriate therapeutic window continues to represent a key barrier to clinical translation. Pro-oxidants therapies tend to selectively push malignant cells beyond their tolerance threshold, as many cancer cells experience redox disturbances and rely heavily on antioxidant defenses [271]. However, distinguishing between tumoricidal oxidative stress and damage to healthy tissues is difficult because of variability across organs, treatment regimens, and individual patients. Importantly, non-malignant cells within the TME, such as effector T cells, endothelial cells, NK cells, fibroblasts, and myeloid populations, each have distinct redox demands [272]. Therefore, interventions designed to elevate ROS levels in cancer cells may also impair antitumor immunity or disrupt vascular and stromal functions. To expand the therapeutic window of redox-related therapies, the parallel implementation of other strategies, such as tumor-selective drug delivery, conditional activation within the TME, and cell-type-specific targeting might be essential [273].

Another important challenge is the profound spatial, temporal, and phenotypic heterogeneity of tumors. Indeed, distinct regions within the same lesion may differ in oxygen and nutrient requirements, iron metabolism, antioxidant capacity, and exposure to therapy. Additionally, therapeutic interventions can induce transient stress-tolerant states and

select for cell populations with different metabolic and redox characteristics. Drug-tolerant persister cells exemplify this phenomenon: these populations demonstrate substantial phenotypic and metabolic plasticity, and multiple persister states may coexist and evolve during treatment within a single tumor [274]. Notably, reliable biomarkers for identifying these populations in patients are still scarce. This limitation poses a major obstacle for redox-targeted cancer therapy, as a single tumor biopsy or circulating sample may not accurately reflect the dynamic redox dependencies of the entire tumor ecosystem.

This heterogeneity further complicates effective patient stratification. Potential biomarkers, including Keap1/Nrf2 pathway status, SLC7A11 expression, GPx4 dependence, GSH metabolism, hypoxia signatures, and iron- and lipid-metabolic profiles, may help identify tumors with specific redox vulnerabilities. However, most of these candidates have not been validated as predictors of response to redox-directed therapies. Clinical observations indicate that such stratification may be feasible. For instance, in patients with KRAS^G12C-mutant non-small-cell lung cancers, the presence of KEAP1 alterations has been associated with poorer outcomes following treatment with KRAS^G12C inhibitors [275,276]. Nevertheless, these associations should not be interpreted as evidence that Keap1 is a predictive redox biomarker, as prognostic and treatment-specific predictive effects remain challenging to distinguish and may vary across tumor genotypes and treatment settings.

Several of these complications are also emerging during the clinical development of therapies designed to induce ferroptotic death. While ferroptosis represents a promising approach to target iron and lipid peroxidation in case of aggressive and therapy-resistant cancers, the therapeutic sensitivity appears to depend on tumor lineage, metabolic state, lipid composition, antioxidant systems, and the TME. Unfortunately, pharmacodynamic assessment remains difficult due to the absence of validated biomarkers for ferroptosis that are suitable for routine clinical application. Recent studies demonstrate that the effective clinical implementation of ferroptosis-based therapies requires reliable biomarkers, appropriate patient selection, evidence of tumor-selective ferroptosis, and rigorously designed clinical trials [220,277].

Another significant limitation involves the evaluation of redox status. In contrast to direct measurements of specific ROS within defined tumor compartments, commonly applied redox biomarkers, including byproducts of lipid peroxidation, oxidized nucleic acids, protein carbonyls, antioxidant enzyme activities, and circulating redox metabolites, generally provide indirect or integrated results. Considering that many reactive species are extremely short-lived and highly localized within cells, measurements from blood or bulk tumor tissue may not accurately reflect the biologically relevant redox events occurring within specific cells or organelles. Emerging approaches, such as spatial metabolomics, redox proteomics, genetically encoded sensors, single-cell profiling, and multimodal imaging may address some of these challenges. However, these methods, at present, lack sufficient analytical standardization, reproducibility, accessibility, and clinical validation. This limitation is particularly critical for precision redox medicine, as patient selection requires biomarkers that not only indicate oxidative stress but also identify individuals whose tumors depend on specific redox pathways that can be targeted therapeutically [270,274].

Clinical experience with antioxidant supplementation further demonstrates the complexity of redox interventions, which cannot be adequately interpreted in terms of antioxidant or prooxidant effects. Antioxidants have been proposed to prevent treatment-associated toxicity by protecting healthy tissues; however, this protective effect may also extent to malignant cells, shielding them from ROS-dependent components of chemotherapy or radiotherapy. Available clinical evidence regarding antioxidant supplementation during cancer treatment remains highly heterogeneous. The substantial inconsistency

among studies can be attributed to variations in antioxidant compound, dosage, treatment modality, tumor type, study design, and clinical endpoints [278]. For instance, in the previously discussed prospective DELCaP ancillary study of SWOG S0221, patients with breast cancer who reported using antioxidant supplements before and during chemotherapy had higher estimated hazards of a disease-free survival and of all-cause mortality than those who disclosed no use at either time point [221]. The 95% confidence intervals included 1.0. and the estimates reflect observational associations because supplement use was not randomized. These findings reinforce the importance of distinguishing pharmacologically targeted redox modulation from nonspecific systemic antioxidant supplementation, and of considering dose, timing, tissue distribution, and treatment mechanism when evaluating clinical outcomes.

Despite the substantial preclinical evidence supporting redox-targeted interventions, their limited clinical success reflects several interconnected challenges. First, the therapeutic window is often narrow because the redox differences between malignant and normal cells are relative rather than absolute, and systemic disruption of antioxidant defences may also compromise normal tissues and antitumor immune populations. Second, inhibition of a single antioxidant pathway may be insufficient because cancer cells can activate compensatory mechanisms to restore redox homeostasis. In particular, the glutathione and thioredoxin systems exhibit substantial functional overlap, allowing one pathway to compensate for inhibition of the other [96]. Similarly, metabolic adaptation and Nrf2-dependent transcriptional responses may reinforce alternative antioxidant defenses, thereby limiting the effectiveness of single-target interventions [279]. Third, achieving adequate drug accumulation and target engagement within heterogeneous tumor regions remains challenging. Poor vascularization, elevated interstitial pressure, stromal barriers, and heterogeneous nanoparticle distribution can limit intratumoral drug penetration, while systemic exposure may increase toxicity in non-malignant tissues [280]. These limitations are particularly relevant to experimental approaches such as chemodynamic therapy and mitochondria-targeted pro-oxidant strategies, whose efficacy depends on appropriate intracellular delivery and local biochemical conditions.

The clinical experience with antioxidant and pro-oxidant interventions further illustrates this translational gap. Large, randomized prevention trials, including CARET and SELECT, failed to demonstrate a protective effect of antioxidant supplementation and, in some settings, identified increased cancer risk [224,225]. Although these findings cannot be directly extrapolated to pharmacological treatment of established tumors, they emphasize that systemic manipulation of redox status may produce unexpected biological consequences. Similarly, clinical investigations of glutathione depletion using BSO and TrxR inhibition using auranofin have established early pharmacological feasibility but have not yet resulted in broadly validated redox-targeted anticancer treatments [204,205,207]. Although Nrf2 modulation has been extensively investigated, selective inhibition of this pathway remains predominantly preclinical, and compounds such as brusatol have raised concerns regarding off-target effects [212]. Importantly, the clinical development of Nrf2-activating agents for other indications should not be interpreted as evidence for the clinical success of Nrf2 inhibition in oncology. Collectively, these experiences indicate that the principal obstacle is not the lack of biologically relevant redox targets, but the difficulty of identifying the appropriate patients, achieving selective target engagement, overcoming compensatory antioxidant responses, and demonstrating a favorable therapeutic index in clinical settings.

To advance clinical translation, it is essential to move beyond the concept of a single "oxidative-stress phenotype" and instead focus on mechanistically defined redox vulnerabilities. Future clinical trials should incorporate molecular and metabolic stratification, longitudinal assessment of treatment-induced redox adaptation, pharmacodynamic

markers of target engagement, and analysis of both malignant and non-malignant TME compartments. Such approaches may enable the identification of patients whose tumors are specifically dependent on Nrf2, GSH, thioredoxin, GPx4, iron metabolism, or other redox-regulatory pathways, thereby allowing for an appropriate selection of therapeutic combinations. Until such biomarkers and trial designs are established, the clinical applicability of redox-directed therapy will remain constrained by the same characteristics that confer biological potency to ROS and, to be more specific, their chemical diversity, compartmentalization, dynamic regulation, and highly context-dependent effects.

11. Conclusions and Perspectives

ROS are important regulators of tumor biology whose effects depend on their chemical identity, abundance, duration, and site of generation, and the antioxidant capacity of the surrounding cellular environment. Within the TME, these factors are further shaped by hypoxia, inflammation, metabolic activity, and interactions among malignant, stromal, endothelial, immune, and extracellular matrix components. Consequently, tumor redox regulation is spatially and temporally heterogeneous, and cannot be adequately represented by a single measure of oxidative stress.

This context dependence underlies the dual effects of ROS in cancer. Controlled redox signaling can support tumor-cell adaptation, proliferation, invasion, and immune modulation, whereas excessive oxidative stress can cause molecular damage and promote cell death. The ability of cancer cells to adapt their antioxidant and metabolic defenses further contributes to resistance against anticancer therapies and provides a rationale for interventions that exploit tumor-specific redox vulnerabilities. Nevertheless, the clinical translation of these strategies remains limited by heterogeneous redox dependencies, narrow therapeutic windows, compensatory antioxidant mechanisms, and the lack of validated predictive biomarkers.

Several important questions remain unresolved. These include how redox states vary among individual tumor-cell populations and neighboring TME components, how specific ROS sources and localized cysteine modifications regulate distinct signaling targets, how these redox interactions change during tumor progression and treatment, and which antioxidant dependencies are functionally essential for tumor survival rather than simply associated with oxidative stress. Addressing these questions will require combining single-cell and spatial profiling with compartment-specific measurements of redox activity and functional perturbation of candidate pathways to establish causal relationships between localized redox alterations, molecular signaling events, and tumor phenotypes. Longitudinal studies will also be important for distinguishing pre-existing redox vulnerabilities from adaptive responses that emerge during treatment. Finally, the development of standardized, compartment-sensitive biomarkers and prospective biomarker-guided clinical studies will be necessary to identify patients most likely to benefit from redox-targeted interventions. Integrating these approaches may help establish when, where, and in which cellular populations redox modulation can be exploited therapeutically while limiting toxicity to normal tissues.

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