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Redox regulation of cell state and fate

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ABSTRACT

The failure in effective cancer treatment is thought to be attributed to a subpopulation of tumor cells with stem cell-like properties. These cancer stem cells (CSCs) are intimately linked to tumor initiation, heterogeneity, maintenance, recurrence and metastasis. Increasing evidence supports the view that a tight redox regulation is crucial for CSC proliferation, tumorigenicity, therapy resistance and metastasis in many cancer types. Since the distinct metabolic and epigenetic states of CSCs may influence ROS levels, and hence their malignancy, ROS modulating agents hold promise in their utility as anti-CSC agents that may improve the durability of current cancer treatments. This review will focus on (i) how ROS levels are regulated for CSCs to elicit their hallmark features; (ii) the link between ROS and metabolic plasticity of CSCs; and (iii) how ROS may interface with epigenetics that would enable CSCs to thrive in a stressful tumor microenvironment and survive therapeutic insults.

1. Redox homeostasis and signaling

Reactive oxygen species (ROS) is a collective term used to describe oxygen-containing, chemically reactive molecules [1]. The tight control of ROS generation and elimination is of paramount importance to normal and cancer cells since ROS (low to moderate levels)-mediated cell signaling can significantly impact a variety of cellular pathways, including cell growth, differentiation, survival and angiogenesis [2–4]. Interestingly, cancer cells and CSCs appear to have distinct redox profiles, with CSCs exhibiting redox patterns that are more similar to normal stem cells. We will briefly touch upon the basics of redox homeostasis and signaling, and refer the reader elsewhere for a comprehensive review of this topic [5–7].

1.1. ROS generation

The mitochondria is the primary endogenous source of ROS in mammalian cells as ROS is a by-product of oxidative phosphorylation (OXPHOS) [8]. The enzyme complexes of the electron transport chain, mainly complex I and complex III, leak free electrons which drive the monoelectronic O_2 reduction to superoxide ($O_2^{\bullet-}$), that is rapidly reduced by superoxide dismutases (SODs) to H_2O_2 (a non-radical ROS) [9,10]. Simultaneously, by the well-described Fenton reaction, Fe^{2+} and H_2O_2 can react with each other to yield •OH radicals [11]. The NADPH oxidase (NOX) family of membrane-bound enzymes represents another major endogenous source of ROS [12]. All members of the NOX family are able to drive the NADPH-dependent reduction of O_2 to $O_2^{\bullet-}$ [12,13]. In addition to $O_2^{\bullet-}$, NOX4, dual oxidase 1 (DUOX1) and DUOX2 generate regulated levels of H_2O_2 [14,15]. Other endogenous sources of ROS include enzymes such as oxidases (e.g. xanthine oxidase) and oxygenases (e.g. cytochrome P450), peroxisomal oxidative metabolism and oxidative protein folding in the endoplasmic recticulum [16–18]. Lastly, ROS is also produced by exogenous agents, including chemotherapy, radiation, heavy metals (or metal complexes), atmospheric pollutants, chemicals, drugs and xenobiotics [19,20].

The current paradigm is that cancer cells generate higher levels of ROS than normal cells due to the activation of oncogenes, inactivation of tumor suppressor genes, aberrant metabolism, mitochondrial malfunction, inflammation or genotoxic stress [3,21,22], and this is compensated for by a more robust antioxidant system (Fig. 1) [23,24]. Consequently, cancer cells have a lower buffering capacity against disruptions in ROS levels [23,25].

1.2. ROS scavenging

When ROS production exceeds the activity of antioxidant defense, oxidative stress ensues and this is associated with many disease states, including autoimmunity and cancer [5,11]. Optimal ROS scavenger

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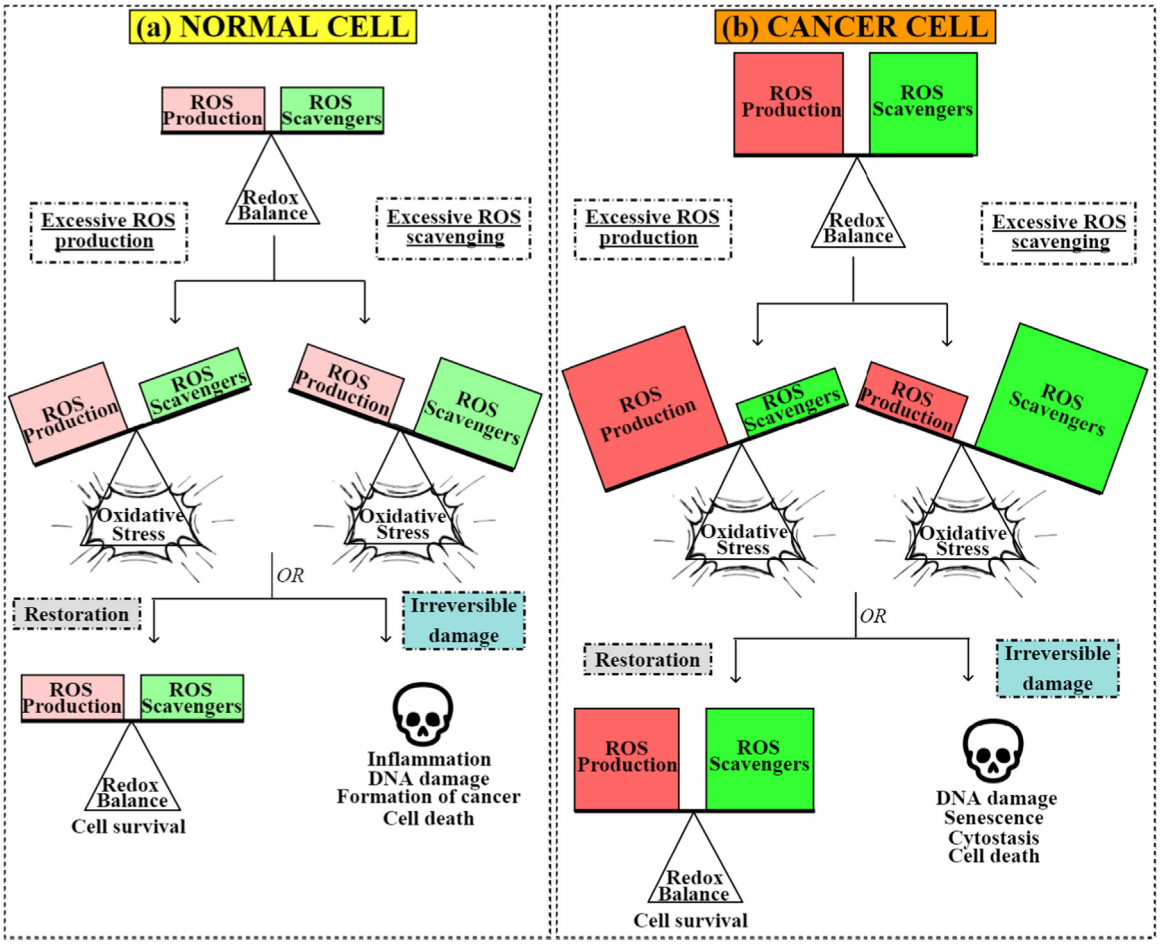

Fig. 1. Redox homeostasis in normal and cancer cells. (a) Normal cells maintain a low level of ROS for physiological function and constantly scavenge to remove excess ROS. Oxidative stress is induced if excessive ROS or antioxidants disrupts the delicate redox balance; this could result in cell death and damage. (b) Cancer cells inherently produce higher level of ROS than normal cells that is counterbalanced by greater ROS scavenging activity. Several therapies such as chemotherapy, pro-oxidant treatment and antioxidant inhibition or treatment target redox homeostasis in cancer cells to induce cytotoxic cell death.

systems are required to keep ROS levels in check and include enzymatic antioxidants such as SODs, catalases, thioredoxins, peroxiredoxins, glutathione peroxidases, p38-mitogen-activated protein kinases (MAPKs) and various sirtuins (SIRTs) [26–30]. Non-enzymatic antioxidants such as glutathione (GSH), vitamin C (ascorbate), vitamin E (tocopherols) and polyphenols, also act directly on oxidative agents [31].

1.3. Redox signaling

The deployment of ROS in cell signaling is known as redox signaling [32]. At redox-sensitive amino acid residues such as cysteine and methionine, ROS can oxidize cellular proteins to allosterically change their conformation and function [33,34]. With a longer half-life than other ROS agents, H_2O_2 acts as a second messenger for intracellular signaling through cysteine-based modifications [35], while other ROS agents, including $O_2^{\bullet-}$ and •OH, are more associated with cellular damage [7].

Redox sensors detect changes in ROS levels and initiate an appropriate cellular response that culminate in antioxidant responses, gene transcription, differentiation, cell growth, cell proliferation and apoptosis [33,36]. Several transcription factors have established roles in redox sensing, including members of the forkhead box O (FOXO) family, hypoxia inducible factors (HIFs), kelch-like ECH-associated protein 1 (KEAP1) with nuclear factor erythoid 2 (NRF2) and the p53 tumor suppressor. The direct and indirect effect of ROS on these molecules have been widely reported and reviewed [37–41]. Diverse enzyme families are also amenable to redox modulation, including kinases such as AKT kinases, MAPKs, ataxia-telangiectasia mutated (ATM), and mammalian target of rapamycin (mTOR), as well as phosphatases like phosphate and tensin homolog (PTEN) and SIRTs [25,33,41,42]. The downstream pathways of these enzymes are often involved in the mediation of ROS levels, for instance, through modulation of transcription factors (e.g FOXOs) [38,43].

2. Redox regulation in cancer stem cells

Cancer is a disease of heterogeneity at the genetic, phenotypic and functional levels. CSCs represent a subpopulation of cancer cells with robust self-renewal capacity, multipotency and tumorigenic potential, and contribute to tumor heterogeneity by perpetuating themselves and generating various differentiated progenitors (Fig. 2). The CSCs are widely associated with various clinical hallmark features of cancer, including therapy resistance, tumor recurrence, invasiveness and metastasis [44]. An emerging theme is that CSCs are not homogeneous even within the same tumor; rather, they may be heterogeneous in their cell cycle, metabolic and redox profiles, which may explain for the lack of a congruent correlation between ROS levels and CSC function from various studies. Here, we will summarize the findings that highlight the importance of redox regulation in supporting the hallmark features of CSC and attempt to crystallize a coherent view from these studies.

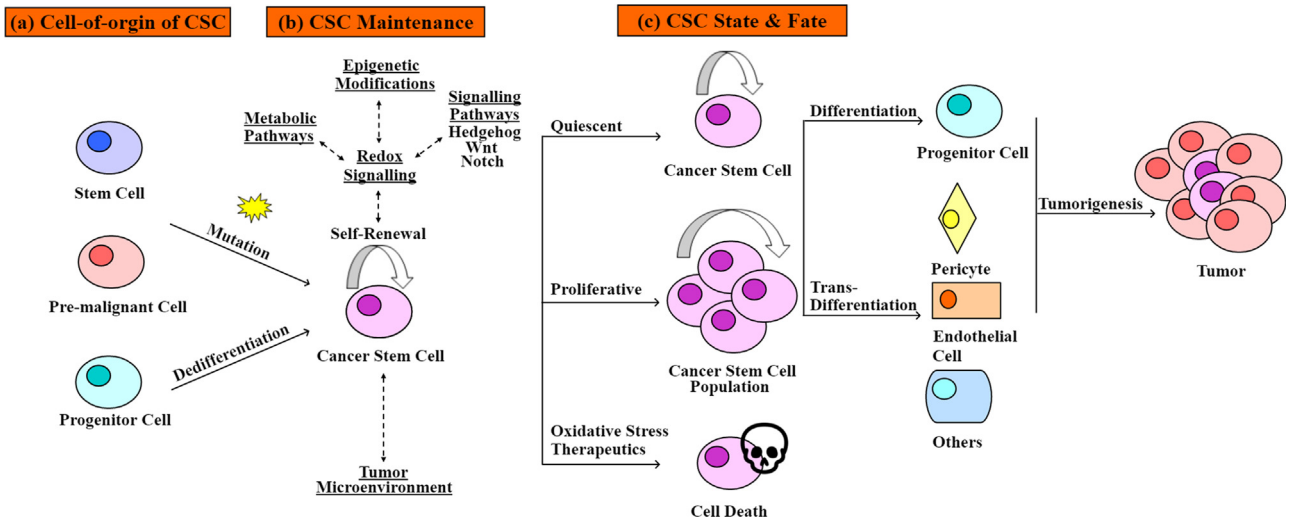

Fig. 2. Cancer stem cell origin, state and fate. (a) CSCs may arise from the accumulation of gene mutations in normal stem cells or the acquisition of a stem cell-like state ("dedifferentiation") in progenitor cells or pre-malignant cancer cells due to cumulative gene mutations. (b) In order to self-renew and remain in an undifferentiated state, CSCs interact with the tumor microenvironment and rely on unique redox, epigenetic and metabolic states, as well as a variety of cell signaling pathways (e.g. Hedgehog, Wnt and Notch). (c) Depending on the extracellular and intracellular signaling, CSCs may exist in a slow-cycling (or quiescent) state or proliferative state. Induction of CSC death could occur due to environmental stressors or CSC-targeted therapies. CSCs can also generate differentiated progenies or undergo transdifferentiation into cells of different lineages such as pericytes and endothelial cells. Tumor heterogeneity is thus maintained by quiescent and proliferative CSCs with long term self-renewal potential, as well as more differentiated CSCs. Dotted arrows indicate interaction between groups.

2.1. Robust self-renewal/proliferation and tumorigenic potential

CSCs are thought to possess robust self-renewal and tumorigenic potential, which is often assessed by the ability of CSCs to generate tumor xenograft (in vivo) or tumorspheres (in vitro) either with or without limiting dilutions. In T-cell acute lymphoblastic leukemia, the ROS^{low}, $CD44^{+}$ CSCs are highly enriched in leukemia-initiating cells [45]. Low levels of ROS in the CSCs are due to the downregulation of PKC-θ that is repressed by Notch1 through RUNX3 and RUNX1. Similarly, in *Hoxa9+Meis1*-induced acute myeloid leukemia (AML) the frequency of CSCs positively correlates with the expression of Gpx3 (a ROS scavenging enzyme) and low levels of ROS [46]. Consistent with the idea that low levels of ROS are required for the maintenance of leukemia CSCs, disulfiram/copper (an aldehyde dehydrogenase inhibitor) selectively eliminates CSCs by increasing ROS levels through the downregulation of NRF2 and upregulation of JNK pathway [47].

In hepatocellular carcinoma (HCC) cells, disulfiram treatment reduces CSC marker expression, tumorsphere formation and tumorigenicity in xenograft experiments in a ROS-p38 MAPK pathway-dependent manner [48]. These suggest that liver CSCs may prefer a low ROS cellular environment. Indeed, liver CSCs reduce mitochondrial OXPHOS and ROS production through NANOG that is in turn regulated by the Toll-like receptor 4 (TLR4)-E2F1 axis [49]. Notably, paraquat (an inducer of ROS) treatment or NANOG silencing decreases tumorsphere formation of liver CSCs.

While CSCs in leukemia and liver cancer favor a ROS^{low} environment, the opposite appears to apply for CSCs in glioblastoma and breast cancer. In glioblastoma, $CD133^{+}$ CSCs have higher levels of ROS than non-CSCs, although the pharmacologic and genetic modulation of superoxide levels did not affect CSC growth and viability [50,51]. However, glioblastoma CSCs overexpress GTP cyclohydrolase 1 (GCH1), a rate-limiting enzyme in a biosynthetic pathway for the production tetrahydrobiopterin (BH4), a cofactor for nitric oxide synthase [52]. The depletion of GCH1 reduces CSC growth and tumorigenicity, suggesting that high NO levels may be crucial for CSC activity in glioblastoma. In triple-negative breast cancer, CSCs have higher levels of ROS than non-CSCs due to elevated mitochondria biogenesis that is regulated by MYC and MCL1 [53]. High levels of ROS stabilize HIF1α and hence increased mammosphere formation.

In summary, the most compelling evidence is that ROS levels are lower in leukemia and liver CSCs when compared to non-CSCs, and this is required for the proliferation/self-renewal and tumorigenicity of CSCs. More rigorous experiments that take into account the potential cell cycle and redox heterogeneity of CSCs, as well as cancer subtype differences will be necessary to evaluate if ROS levels may directly impact CSC activity in other cancer types.

2.2. Therapy resistance

The role of dysregulated ROS levels in CSC therapy resistance is well supported in numerous studies. In AML, the ROS^{low}, quiescent leukemic cells exhibit CSC properties and overexpress B-cell lymphoma 2 (Bcl-2), an anti-apoptotic protein [54]. The inhibition of Bcl-2 increased mitochondrial ROS levels, decreased GSH levels and selectively eliminated therapy-resistant, quiescent CSCs. In colorectal cancer, ROS^{low} CSCs with low proteasome activity are enriched after radiation and chemotherapeutic treatment [55]. These CSCs overexpress *EID3* which upon depletion overcomes therapy resistance of colorectal cancer cells.

In HCC, chemotherapeutic drugs or radiation treatment invariably result in the enrichment of CSCs with a ROS^{low} profile that is accompanied with either the ability to reduce ROS-induced DNA damage after genotoxic insult, increased GSH levels or MAPK/PI3K activation [56–58]. Importantly, the inhibition of CD13 (a liver CSC marker) or treatment of $CD133^{+}$ CSCs with sulfasalazine (a potent xCT inhibitor) overcomes resistance to chemotherapeutic and radiation treatment by increasing ROS levels in the resistant cells [56,58,59]. In pancreatic adenocarcinoma, ROS^{low} CSC are also implicated in radioresistance [60,61]. Glutamine deprivation or the inhibition of non-canonical glutamine metabolism sensitizes pancreatic CSCs to radiation treatment in vitro and in tumor xenograft experiments via intracellular ROS accumulation [60].

Stem cell maintenance pathways can also contribute to therapy resistance. For example, radiation induces the expression of Jagged-1 and intracellular Notch-ICD in $CD24^{-/low}/CD44^{+}$-enriched breast CSCs, indicating the activation of the developmental Notch1 signaling pathway [62]. These breast CSCs exhibited higher radioresistance and lower ROS levels (suggesting higher reactive species scavenger levels) than non–breast CSCs. Collectively, all the above studies converge to a

Table 1
Identified metabolic phenotypes for various cancers.

Types of cancer	Metabolic processes involved	Effect on ROS levels and CSC state and fate	Ref.
Breast CSCs	Glycolysis	Low FBP1 expression in basal-like breast cancer promotes glycolysis while suppressing OXPHOS, thereby reducing ROS levels and maintaining CSC population.	[77,81]
		Breast CSCs rely on fermentative glycolysis and are sensitive to glycolysis inhibitor treatment. They overexpress several antioxidant enzymes such as mitochondrial SOD to counteract excessive ROS production.	[82]
	Glycolysis and OXPHOS	Mesenchymal-like breast CSCs have enhanced glycolysis and require a low level of ROS to maintain their quiescent state. On the other hand, epithelial-like breast CSCs are OXPHOS-dependent and have a higher level of mitochondrial ROS.	[64]
Brain CSCs	OXPHOS	Induction of H_2O_2 and $O_2^{\bullet-}$ generation in glioma stem cells occurred through electron transport chain activation.	[83]
	FAO	Glioblastoma stem and progenitor cells are less glycolytic than differentiated glioma cells. GSCs consume less glucose and produce less lactate while maintaining higher ATP levels than their differentiated progeny.	[84]
		In glioblastoma, inhibition of FAO causes a profound drop in NADPH levels and an increase in ROS levels.	[85]
Colon CSCs	Glycolysis	The colon CSC secretome is enriched in proteins involved in glycolysis and gluconeogenesis, and have enhanced anti-oxidant networks, suggesting that the maintenance of low ROS levels contributes to their intrinsic drug resistance.	[86]
Leukemia CSCs	OXPHOS	In acute myeloid leukemia, ROS^{low} CSCs are defined by quiescent cell cycle status, low energy production and Bcl-2 overexpression. However, these CSCs are paradoxically dependent on OXPHOS. Bcl-2 inhibition suppresses OXPHOS and increases mitochondrial ROS.	[54]
Liver CSCs	Glycolysis and FAO	In HCC, CSCs with repressed ROS generation have increased glycolysis and FAO accompanied by lower OXPHOS.	[49]
Ovarian CSCs	OXPHOS	More stem-like $CD44^+/CD117^+$ ovarian CSCs contain higher levels of H_2O_2 than $CD44^+/CD117^-$ cells. The epithelial ovarian CSCs privilege OXPHOS and inhibition of the mitochondrial respiratory chain induces cell death.	[87]
Pancreatic CSCs	Glycolysis	In gemcitabine-resistant pancreatic CSCs, the up-regulation of glycolysis and maintenance of low ROS promotes stemness, EMT and therapeutic resistant phenotypes.	[65]
	Glutamine metabolism	ROS^{low} CSCs are reliant on the non-canonical glutamine metabolic pathway and glutamine deprivation significantly inhibited CSC self-renewal and sensitizes CSC to irradiation.	[60]

model whereby high ROS induction may be useful in the eradication of therapy-resistant CSCs across multiple cancer types.

2.3. Epithelial-mesenchymal transition

In epithelial-mesenchymal transition (EMT), a polarized epithelial cell that typically associates with the basement membrane, undergoes multiple biochemical changes that enables it to transform into a mesenchymal cell phenotype, including increased migratory capacity, invasiveness, elevated resistance to apoptosis, and enhanced production of extracellular matrix components [63]. An emerging view is that ROS signaling mechanisms could influence the EMT-like phenotype of CSCs. In breast cancer, the ROS^{low} mesenchymal CSC are more sensitive to glycolysis inhibitor than the ROS^{high} epithelial CSC, which are more oxidative and reliant on the NRF2 antioxidant response [64]. Hypoxic and metabolic stressors promote the mesenchymal to epithelial state transition through ROS-mediated activation of the AMPK-HIF1α axis. Importantly, co-inhibition of glycolysis and antioxidant (e.g. thioredoxin and GSH) pathways targets both mesenchymal and epithelial CSC.

In pancreatic cancer, 2-deoxy-D-glucose or H_2O_2 treatment enhances cytotoxicity of gemcitabine, and suppresses CSC (including expression of CSC markers) and EMT phenotypes (including EMT marker expression and migration) of a gemcitabine-resistant pancreatic cell line, which could be reversed by N-Acetyl cysteine (NAC) treatment [65]. In lung cancer, $CD24^{low}$ CSC express low levels of DUOX1 when compared to the $CD24^{high}$ non-CSC [66]. Silencing DUOX1 (which should presumably decrease H_2O_2 levels) increases CSC frequency, mesenchymal gene expression, tumor invasiveness and resistance to tyrosine kinase inhibitor.

In HCC, the treatment of liver cancer cell lines with transforming growth factor-beta (TGF-β) increases the expression of mesenchymal markers and CD13, and tumorigenicity [67]. Higher ROS levels and stem cell maintenance gene (BMI1 and Notch1) expression are reported in the $CD13^+$/N-cadherin$^+$ cells than the $CD13^+$/N-cadherin$^-$ cells, reinforcing the idea of CSC and hence ROS heterogeneity. In general, the majority of studies have revealed a trend implicating low levels of ROS and the EMT phenotype of CSCs.

3. Metabolism and redox in CSCs

The reciprocal crosstalk between redox balance and metabolism has been gaining attention due to their implications in malignant progression and therapy resistance in cancer [23]. Recent studies support the view that the metabolic state of CSCs differ between cancer types, subtypes of the same cancer and even cycling states within the same tumor [44,54,68,69]. To add on to this complexity, the CSCs readily switch their metabolic profile according to their needs (i.e. metabolic plasticity) [68]. An interesting study by Sancho et al. [70] demonstrated metabolic heterogeneity within pancreatic CSCs by characterizing a pre-existing pro-glycolytic subpopulation of CSCs with enhanced metformin resistance. Treatment with metformin resulted in the expansion of the pro-glycolytic subpopulation, which suggested a metabolic switch of the OXPHOS-dependent CSCs.

In addition, secondary metabolic processes such as glutaminolysis, fatty acid oxidation (FAO) and one-carbon metabolism, can be activated in CSCs as additional means of energy generation, contributing to the complexity of CSC metabolism [6,71–75]. Chen et al. [49] demonstrated that NANOG contributes to HCC progression in mice by repressing OXPHOS activity and mitochondrial ROS generation, while activating FAO to support CSC self-renewal and drug resistance [49,76]. Notably, the restoration of OXPHOS activity and inhibition of FAO renders CSCs susceptible to sorafenib, highlighting a potential strategy to combat chemoresistance of HCC.

In basal-like breast CSCs, pro-glycolytic metabolic reprogramming decreases ROS production and enhances stem-like properties and in vitro tumorsphere formation [77]. Luo et al. [64] later showed that mesenchymal-like breast CSCs (M-BCSCs) similarly prefer glycolysis and maintain low ROS levels. Additionally, the study demonstrated that H_2O_2 treatment would force M-BCSCs into an epithelial-like, OXPHOS-enriched phenotype and application of NAC reverses this metabolic state-switch [64]. This serves as evidence supporting metabolic plasticity of breast CSCs and underscores the intimate link between CSC metabolism and ROS regulation [68,78–80].

It is noteworthy that the maintenance of low ROS levels does not always correspond to a preference for glycolysis. Lagadinou et al. [54] demonstrated that leukemia CSCs have characteristically low levels of $O_2^{\bullet-}$, but are surprisingly reliant on BCL-2-mediated OXPHOS for

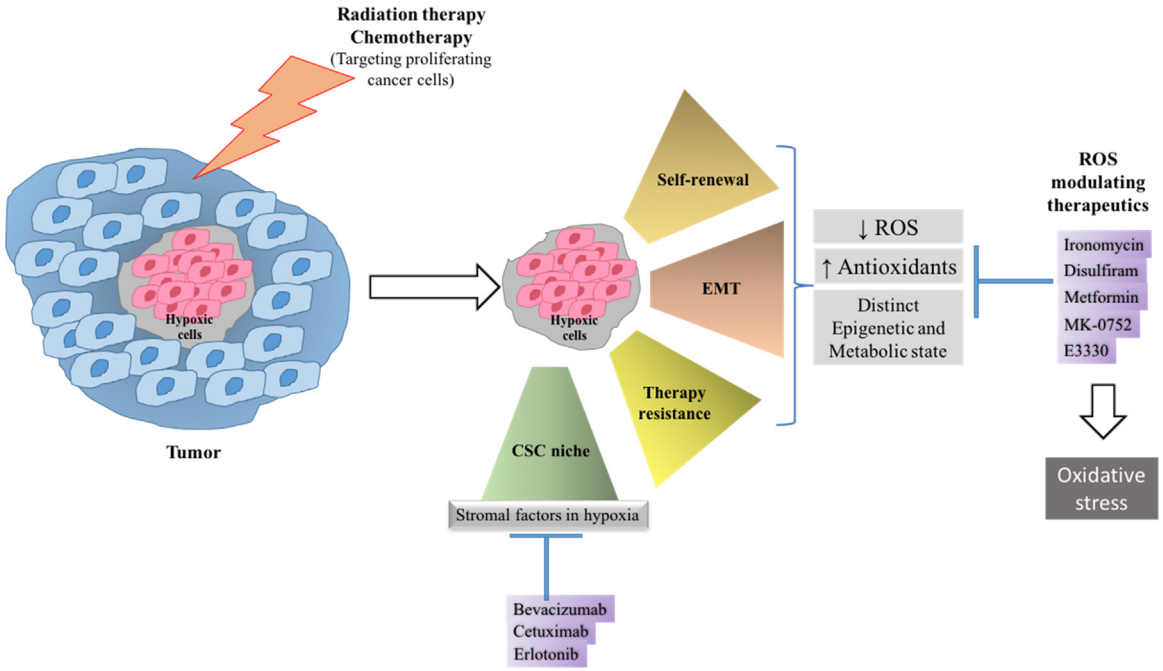

Fig. 3. Targeting the hallmarks of cancer stem cell. Redox regulation plays a crucial role in maintenance of the CSCs via downregulating the ROS or upregulating the antioxidants. Targeting the regulatory factors involved in these pathways seems promising to overcome therapy resistance and relapse. In addition, developing drugs or molecules that would aid in the delivery of anti-cancer therapeutics at the hypoxic CSC niche for example, nanoparticles and promotor drugs should aid in the efficacy of these drugs.

Table 2
Anti-CSCs agents that operate by directly or indirectly perturbing ROS levels.

Types of cancer	Potential drugs/inhibitors	Mechanism of action	Ref.
Breast cancer	A Copper(II) Phenanthroline Metallopeptide	Disrupts mitochondrial function by ROS generation in CSCs	[111]
	KPT-6566, peptidyl-prolyl isomerase inhibitor	Generates ROS and DNA damage by release of a quinone-mimicking drug upon covalently binding to the catalytic site of PIN1	[112]
	Ironomycin (AM5), Synthetic derivative of salinomycin	Causes depletion of iron in cytoplasm and sequestration of iron in lysosomes, leading to iron-mediated production of ROS in lysosomes, lysosomal membrane permeabilization and cell death by ferroptosis in CSCs.	[113]
	anti-xCT DNA vaccination	Anti-xCT can alter CSC redox balance, impairing metastasis and increasing CSC chemosensitivity.	[114]
Leukemia	E3330, redox-specific inhibitor	Inhibitor of Ref-1 which is crucial in cellular response to DNA damage and redox regulation resulting in potent inhibition of viability of leukemia T cells	[115]
	Disulfiram + Cu	Induces simultaneous ROS-JNK pathway activation and inhibition of the pro-survival NRF2 and NF-κB pathways in CSCs.	[116]
Lung cancer	LBL21, synthetic analogue of naturally occurring phenethyl isothiocyanate (PEITC)	Induces apoptosis partly by ROS accumulation and activating endoplasmic recticulum stress sensors and partly by depleting GSH in CSCs	[61]
Pancreatic cancer	MK-0752, γ-secretase inhibitor (GSI)	GSIs inhibit the proteolytic function of presenilin enzymes, resulting in intact form of Notch. ROS has been suggested to modulate Notch signaling.	[117,118]
Glioblastoma	Napabucasin (BBI608) in Combination With Temozolomide	BBI608 suppresses cancer stemness by targeting STAT3-driven gene transcription. STAT3 transcription factor can be activated by ROS.	Mason WP http://clinicaltrials.gov/show/NCT02315534
Breast cancer stem cells	Bevacizumab (humanized anti-VEGF monoclonal IgG_1 antibody)	Selectively inhibits circulating VEGF from binding to its cell surface receptors. Known to also increase ROS levels.	[119]
Advanced solid tumors	Amcasertib (BBI503) cancer cell stemness kinase inhibitor	Potential antineoplastic activity, known to target NANOG. Inhibits CSC survival pathways that assist in CSC and heterogeneous cancer cell growth. These pathways may be involved in metabolism and ROS modulation.	Boston Biomedical, Inc https://clinicaltrials.gov/ct2/show/NCT01781455

survival and maintenance of a quiescent, stem-like state. A summary of how specific metabolic processes may influence CSC state and fate in a variety of cancer types is shown in Table 1. Notwithstanding the complexity of CSC metabolism and ROS regulation, this area clearly warrants further investigation as the modulation of CSC metabolism represents an attractive and actionable avenue for novel CSC therapeutics.

4. ROS, epigenetics and CSCs

The acquisition of a stem cell-like phenotype in cancer cells is often accompanied by the accumulation of driver mutations in a wide range

of epigenetic regulators, resulting in the uncontrolled self-renewal and repression of cellular differentiation of the resulting cells [88,89]. Indeed, epigenetic alterations can affect the expression of metabolism and antioxidant defense genes, deregulating ROS levels that are conducive for CSC growth. In basal-like breast cancer, the Snail-G9a-DNMT1 complex decreases ROS levels by repressing fructose-1,6-biphosphatase transcription, increasing CSC-like characteristics [77]. In AML, TXNIP is downregulated due to PRC2-mediated gene silencing [90]. Disruption of PRC2, either by 3-Deazaneplanocin A (DZNep; a histone methyltransferase inhibitor) treatment or EZH2 knockdown, reactivates TXNIP, inhibits TXN activity, increases ROS and apoptosis of leukemia CSCs. Moreover, ROS can directly alter the expression and thus activity of DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs) or microRNAs (miRNA) [91–93], resulting in gene expression changes that may or may not be compatible with CSC function.

MiRNAs are an emerging class of epigenetic regulators which control gene expression at the post-transcriptional level through mRNA translation and stability [94–96]. They play critical roles in fine-tuning a wide array of biological processes and have been increasingly suggested to direct the fate of stem cells and CSCs into specific lineages [97–100]. ROS levels upregulate several miRNAs, including miR-21, miR-146a, miR-200 family and miR-210 [100–102]. For instance, members of the miR-200 family are upregulated by oxidative stress, and can decrease CSC self-renewal through downregulated expression of proteins in CSC maintenance pathways, including BMIl, Suz12, and Notch1 [103]. Conversely, ROS levels could reduce the activity of miRNAs such as miR-let-7 family, which may negatively regulate EMT and CSC features [104,105].

5. Future outlook

According to the CSC model, stem cell-like cancer cells with the greatest proliferative and tumorigenic potential, reside at the apex of cellular hierarchy, leading to the promise that the eradication of CSCs should reduce cancer relapse and treatment failure. Given the emerging view that ROS levels can impact CSC stemness, metabolism and epigenome, ROS modulating agents may have efficacy in anti-CSC therapy. Indeed, there are a variety of strategies that exploit ROS perturbations, either directly or indirectly, to target the hallmark features of CSCs (Fig. 3 and Table 2). Given the importance of the tumor microenvironment on CSC plasticity and therapy resistance, the effectiveness of anti-CSC therapies may be further enhanced by the concurrent targeting of angiogenic and stromal factors, including anti-vasculature therapies (such as anti-VEGF antibodies-bevacizumab, anti-EGFR antibodies-cetuximab or small molecules like erlotonib) that lead to nutrient and oxygen starvation, and reduce tumor interstitial pressure aid in enhanced cytotoxic drug uptake [106–108]. Lastly, the development of nanoparticles and promoter drugs to enhance the delivery of anti-CSC agents into the hypoxic CSC niche should also improve the efficacy of these drug [109,110].

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