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Targeting Cancer Stem Cell Redox Metabolism to Enhance Therapy Responses

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Cancer has long been viewed as a disease of altered metabolism. Although it has long been recognized that the majority of cancer cells display increased dependence on glycolysis, the metabolism of "cancer stem-like cells" (CSCs) that drive tumor growth and metastasis is less well characterized. In this chapter, we review the current state of knowledge of CSC metabolism with an emphasis on the development of therapeutic strategies to exploit the metabolic vulnerabilities of these cells. We outline emerging evidence indicating distinct metabolic pathways active in the proliferative, epithelial- (E) and quiescent, mesenchymal-like (M) CSC states in triple negative breast cancer. These CSC states are characterized by their different redox potentials and divergent sensitivities to inhibitors of glycolysis and redox metabolism. We highlight the roles of two redox-regulated signaling pathways, hypoxia-inducible factor 1α and nuclear factor erythroid 2-related factor 2, in regulating CSC epithelial–mesenchymal plasticity during metabolic and/or oxidative stress, and discuss clinical strategies using combinations of pro-oxidant-based therapeutics simultaneously targeting E- and M-like CSCs. By specifically targeting CSCs of both states, these strategies have the potential to increase the therapeutic efficacy of traditional chemotherapy and radiation therapy.
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Introduction

There is substantial evidence that many cancers, including breast cancer, are hierarchically organized and driven by a small population of tumor cells displaying stem cell properties.[1-3] These "cancer stem-like cells (CSCs)" or "tumor initiating cells," which reside at the apex of tumor heterogeneity, have the capacity for self-renewal, tumor initiation, and generation of differentiated tumor progeny constituting the tumor bulk. In addition to their tumorigenic potential, CSCs are inherently resistant to traditional cancer therapies including chemotherapy and ionizing radiation, leading to treatment failure, metastases, and cancer relapse.[4-10]

As discussed in several recent reviews, tumor cells including the small population of CSCs display remarkable genetic and/or epigenetic heterogeneity and cellular plasticity.[11-13] Adding another layer of complexity, recent studies demonstrate that BCSCs maintain the plasticity to transition between proliferative, epithelial-like (E) and quiescent, mesenchymal-like (M) states.[14] These distinct E- and M-like BCSCs exhibit discrete patterns of marker expression characterized by elevated aldehyde dehydrogenase (ALDH) activity and $CD24^{-}CD44^{+}$ expression respectively.[14] The transition of CSCs from the E- to M-like state closely resembles the epithelial-to-mesenchymal transition, which is associated with the acquisition of stem cell properties.[15] The equilibrium of these dynamic CSC states is regulated by the tumor microenvironment through mechanisms including, but not limited to, cytokine and/or chemokine signaling, genetic and/or epigenetic regulation of key transcription factors as well as growth factor receptors and microRNA and/or LncRNAs.[13,16,17] Most recently, we demonstrated that the dynamics of BCSCs in distinct E- and M-like states is tightly controlled by changing reduction–oxidation (redox) states induced by metabolic stressors.[18] In this chapter, we highlight the emerging knowledge regarding redox-regulated CSC plasticity, the underlying signaling mechanisms governing redox regulation of CSC state dynamics, and the different metabolic pathways contributing to their differential redox potentials as well as divergent responses to inhibitors of glycolysis and redox metabolism. We emphasize the implications of these findings for the development of novel combinatory therapeutic strategies to effectively target CSCs

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of distinct phenotypic states, and discuss how these strategies may enhance tumor responses to the conventional treatment approaches including chemo- and radiation therapies.

CSC Plasticity as a Mechanism Conferring Treatment Resistance and Metastatic Relapse

Current cancer therapies are mainly aimed at reducing tumor mass by targeting rapidly proliferating bulk tumor cells. Although these therapeutic strategies are effective in reducing the size of tumors, they frequently fail to eradicate advanced tumors and are associated with metastatic relapse. The CSC hypothesis suggests that conventional antitumor strategies targeting rapidly proliferating cells may fail to target CSCs, which divide infrequently and are endowed with multiple mechanisms accounting for their therapeutic resistance.[19] Indeed, a large body of studies has indicated that BCSCs are resistant to ionizing radiation and chemotherapy.[9,20-22] This has been demonstrated in cultured cancer cell lines, in primary mammary tumor cells,[7,23,24] in patient derived tumor xenografts,[9,25,26] as well as in the clinical setting of breast cancer patients undergoing neoadjuvant chemotherapy.[8,25]

The demonstration that BCSCs exist in a dynamic equilibrium of E- and M-like states[14] suggests that CSC epithelial −mesenchymal plasticity may contribute to therapeutic resistance and metastatic relapse. The plasticity of CSCs allowing them to transition from a proliferating epithelial state to an invasive, mesenchymal state facilitates these cells escaping from traditional therapies and disseminating into the circulation and distant organs. Conversely, the plasticity allowing them to transition from an invasive, mesenchymal state to a proliferating epithelial state facilitates metastatic colonization. This suggests that CSCs are not a fixed population but rather a dynamic, metaplastic phenotypic state that is regulated by the tumor microenvironment (i.e., growth factor and/or inflammatory signaling, stromal−tumor interactions and metabolic reprograming). In agreement with this notion, published studies have shown that HER2 overexpression drives the self-renewal of $ALDH^+$ E-BCSCs that are sensitive to the HER2 antibody trastuzumab.[27] Conversely, resistance to HER2 blockade is associated with an increase in $CD24^-CD44^+$ M-BCSCs resulting from the activation of an IL6 driven inflammatory loop.[28] Moreover, in trastuzumab-resistant $HER2^+$ breast cancer, a combinatory approach targeting IL6 receptor by tocilizumab and HER2 by trastuzumab synergistically abrogated tumor growth and metastases by eliminating both M- and E-BCSCs,[28] providing a proof-of-concept that simultaneously targeting distinct CSC states may eradicate metastatic and/or drug-resistant breast cancer by eliminating the refractory CSC population. These studies also imply that therapeutic approaches targeting either state alone may not be sufficient to eliminate CSCs, since the targeted cell population could be rapidly regenerated by cells in alternative states. Thus, multiple CSC therapies attacking distinct forms of CSCs may be required to effectively eliminate these lethal seeds of cancer, leading to more effective therapies.

Cell Redox as a Key Regulator of CSC Plasticity

Cancer cells are characterized by increased levels of oxidative stress, generated by increased metabolic demands and oncogenic signaling.[29,30] Many chemotherapeutic agents or ionizing radiation, by inducing oxidative stress, are able to elicit reactive oxygen species (ROS)-mediated cytotoxicity in bulk tumor cells which are more susceptible to second oxidative insults compared to their normal counterparts.[31,32] However, ROS levels in the quiescent CSCs are significantly different as compared to the bulk of tumor cells.[7] Recently, we demonstrated that modulation of redox potential through co-inhibition of glycolysis and nuclear factor erythroid 2-related factor 2 (NRF2)-mediated antioxidant responses is able to disrupt the state equilibrium of BCSCs, leading to terminal differentiation and apoptosis of both M- and E-BCSCs.[18] This provided a novel framework using pro-oxidant-based therapeutics targeting metabolism of distinct CSC states to effectively eliminate the refractory CSC populations.

As is the case for their normal tissue counterparts, CSCs obtain energy principally through two different metabolic pathways: mitochondrial respiration, which depends on oxygen; and fermentation of glucose through aerobic glycolysis, which is oxygen-independent. CSCs appear to be capable of deriving energy from both sources. In the quiescent M state, they primarily rely on glycolysis; in the proliferative E state, they utilize oxygen to fuel mitochondrial oxidative phosphorylation (OXPHOS). Such dynamic utilization of distinct metabolic pathways depending on the tumor microenvironment is critical for cells in these CSC states to meet redox homeostasis and bioenergetics needs. Thus, the ROS generated from the mitochondrial OXPHOS is not merely a by-product of cancer metabolism, but rather serves as a key mediator regulating CSC plasticity.

ROS as a Secondary Messenger in Cell Signaling

Metabolic reactions utilizing molecular oxygen (O_2) produce ROS including superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$). Although ROS are generated in various cellular compartments such as the peroxisomes and endoplasmic reticulum, it is well recognized that mitochondrial OXPHOS serves the major source of ROS production, as ~2% of the oxygen consumed by the mitochondria is estimated to be reduced to form superoxide anion.[29,33,34] Mitochondrial ROS are not stable and rapidly dismutated by the manganese superoxide dismutase (MnSOD, SOD2) at the mitochondria or Cu/Zn-containing SOD (SOD1) in the cytosol into H_2O_2, which is permeable to the cell and/or nuclear membranes and further converted to HO.[35]

At high levels, ROS induce damage to various cellular components including DNA, proteins and lipids, leading to

cell senescence, death, or oncogenic transformation.[29] The extensive production of ROS during normal metabolism requires the activation of adequate antioxidant defenses to maintain redox homeostasis. Recent evidence has suggested that, although high levels of ROS are toxic, low-to-intermediate levels of ROS are crucial for cell development and homeostasis.[36] ROS act as important secondary massager to modulate a wide variety of signaling molecules including kinases, phosphatases, and transcription factors including JAK-STAT,[37,38] p38 MAPK/ERK/JNK,[39-41] PI3K-AKT,[42,43] NF-κB,[44-46] PKA,[47] PKC,[48,49] NRF2/Keap1,[50] Hippo-FOXO,[51] etc., which in turn stimulate diverse cellular responses including cell survival, proliferation and differentiation,[52,53] and cell migration, adhesion and invasion.[54,55]

CSC Radio-Resistance is Associated With Low Reactive Oxygen Species

The antitumor effects of both radiation and chemotherapy are mediated, at least partially, by the generation of ROS, which results in DNA damage and subsequent single-stranded and double-stranded breaks.[56-58] In neural stem cells and hematopoietic stem cells (HSCs), protection from oxidative stress is critical for the maintenance of their self-renewal,[59-61] and mice deficient in ATM kinase or *FoxO1*, *FoxO3*, and *FoxO4* transcription factors exhibit elevated ROS levels in the HSC compartment, leading to rapid extinction of HSCs.[60,62,63] In parallel studies, Diehn et al. documented that human and mouse BCSCs, similar to their normal tissue counterparts, are characterized by low levels of ROS associated with increased expression of free radical scavenging systems that account for the radio-resistance of these cells.[7] This study suggests that strategies that block these antioxidant defenses may successfully target CSCs. Indeed, pharmacological depletion of ROS scavengers in BCSCs markedly decreases their clonogenicity resulting in radiosensitization.[7] Accordingly, treatment of human acute myeloid leukemia (AML) with parthenolide, a naturally occurring compound that induces ROS, effectively targets AML stem and progenitor cells for apoptosis.[64]

E- and M-Like CSCs Intrinsically Differ in Their Redox States

As summarized above, there is compelling evidence that low levels of intracellular ROS are required for maintaining CSCs including HSCs[60-62] and mammary stem cells (MaSCs).[7] Like their normal counterparts, the CD44 + /CD24- mesenchymal BCSCs are characterized by low ROS.[7] Epigenetic silencing of the gluconeogenic enzyme fructose-1,6- biphosphatase FBP1 by Snail facilitates increased glycolysis while suppressing OXPHOS to maintain low ROS levels in M-like BCSCs, promoting their self-renewal and an epithelial-to-mesenchymal transition-like CSC phenotype in triple negative breast cancer (TNBC).[65]

The mammary epithelium is comprised of an outer layer of basal and an inner layer of luminal epithelial cells maintained by distinct basal and luminal stem cells that give rise to cells restricted to the basal and luminal lineages respectively during mammary gland development.[66] Interestingly, MaSCs located in the basal compartment ($EpCAM^{-/low}MUC1^{-}CD49f^{+}CD90^{+}$) are characterized by lower ROS levels while the luminal epithelial cells including the primitive luminal stem and/or progenitor cells ($EpCAM^{+}MUC1^{+}CD49f^{+}CD90^{-}$) are characterized by higher levels of ROS.[67] Moreover, this study also demonstrated that the primitive luminal stem cells and/or progenitor cells display increased mitochondria mass and greater rates of oxygen consumption, and that glutathione-dependent and -independent antioxidant defense mechanisms are active in the basal and luminal mammary epithelial compartments respectively.[67] These distinct luminal and basal stem cell populations identified in mammary epithelium are reflective of the $ALDH^{+}$ and $CD24^{-}CD44^{+}$CSC states isolated in different subtypes of breast cancers.[14]

The finding that basal and luminal MaSCs maintain different ROS levels and lineage-specific mechanisms of ROS control suggest that the E- and M-like BCSCs reflective of their respective luminal and basal stem cell lineages may inherit distinct ROS levels and metabolic pathways to control their respective redox states. Indeed, our recent studies in TNBC revealed that E-BCSCs and M-BCSCs display significantly different ROS levels and divergent responses to metabolic and/or oxidant stressors.[18] We further demonstrated that metabolic and/or oxidative stress generated by 2-deoxyglucose (2DG), H_2O_2, or hypoxia promotes the transition of ROS^{lo} M-BCSCs to a ROS^{hi} E-state, and this transition was reversed by *N*-acetylcysteine and mediated by activation of the AMP-activated protein kinase (AMPK)-hypoxia-inducible factor 1α (HIF1α) axis.[18] These differential responses of E- and M-BCSCs to metabolic and/or oxidative stressors are linked to their distinct bioenergetics and redox metabolism, which is discussed in the following section.

Metabolic Pathways of CSCs

For most slowly proliferating normal tissue cells, mitochondria serve as the main source of energy production through the tricarboxylic acid (TCA) cycle coupled with OXPHOS in the mitochondria membrane, which generates 36 ATP per molecule of glucose. Cancer cells, however, are characterized by high rate of proliferation and thus need to adapt their cellular metabolism to support increased proliferation by providing rapid ATP generation as well as biosynthesis of nucleotides, proteins, and lipids to support tumor growth. Almost 100 years ago, Otto Warburg reported that cancer cells preferentially utilize glycolysis (which generates 2 ATP per unit of glucose) to generate copious amounts of lactate, regardless of the presence of oxygen.[68] Such an inefficient way of energy production, however, is necessary for generation of various metabolic intermediates important for maintaining tumor redox homeostasis and anabolic metabolism (macromolecular biosynthesis). Despite a glycolytic phenotype in the bulk of tumor cells, the metabolic pathways utilized by the small subset of CSCs are less well

characterized. Currently there is no consensus on the metabolism of CSCs and a growing body of evidence in different cancer types indicates that the metabolism of CSCs is context-dependent and reliant on glycolysis and/or OXPHOS.

Metabolic Heterogeneity and Plasticity of CSCs

It was originally hypothesized that CSCs display a glycolytic phenotype with similarities to the quiescent adult stem cells.[69] This hypothesis is supported by the findings that transition from somatic oxidative metabolism into pluripotency-dependent glycolysis facilitates nuclear reprogramming and formation of induced pluripotent stem cells.[70,71] In contrast, bioenergetics transition from glycolysis to OXPHOS occurs during differentiation of human embryonic stem cells.[72] Thus, metabolic reprograming is not merely associated with stemness, but also acts as a regulator of cell differentiation.

A number of studies in breast cancer supported the above notion by demonstrating that BCSCs enriched by sphere formation,[73] $CD24^{-}CD44^{+}$[65,73] or $CD49f^{high}EpCAM^{low}$[74] preferentially utilize glycolysis over mitochondrial OXPHOS compared to the differentiated cells from the same tumor. This glycolytic phenotype is also found in the $CD133^{+}$ hepatocellular CSCs[75] and the radio-resistant sphere-forming nasopharyngeal carcinoma cells.[76] Despite the studies suggesting a dependence of CSCs on glycolysis, other studies demonstrate that CSCs might also rely on oxidative metabolism. For example, quantitative proteomics studies of breast cancer cells revealed that key mitochondrial-related proteins involved in fatty acid β-oxidation and ketone metabolism as well as mitochondrial biogenesis were significantly upregulated in the mammospheres relative to the epithelial monolayers, suggesting that clonal expansion of BCSCs requires mitochondrial OXPHOS.[77] Indeed, XCT790, a well-established inhibitor of estrogen-related receptor α, which functions as an essential cofactor of PGC-1α required for mitochondrial biogenesis,[78,79] suppresses BCSC activity by blocking several independent signaling pathways normally required for the self-renewal of CSCs, including Sonic hedgehog, TGFβ-SMAD, STAT3, and Wnt signaling.[80] This PGC-1α-mediated mitochondrial biogenesis and OXPHOS is essential for the functional motility of breast cancer cells and metastasis.[81] The requirement of mitochondrial biogenesis for the self-renewal of CSCs is also supported by the findings that a number of antibiotics (i.e., Azithromycin and Tetracycline) targeting mitochondrial ribosomes effectively eradicate CSCs across multiple tumor types.[82] This functional link of mitochondria to the CSC phenotype was subsequently confirmed by the findings that proliferative $ALDH^{+}$ BCSCs display increased mitochondrial mass and activity, and that high mitochondrial mass identifies a subpopulation of BCSCs that are chemoresistant.[83,84] A recent study further demonstrated that MYC and MCL1, two genes frequently amplified in TNBC, cooperatively promote mitochondrial OXPHOS, leading to ROS-mediated HIF1α stabilization and enrichment of $ALDH^{+}$BCSCs.[85]

The roles of mitochondrial oxidative metabolism in conferring therapeutic resistance by promoting the maintenance of CSCs have also been described in other malignancies. For example, the oncofetal insulin-like growth factor 2 mRNA-binding protein IMP2 in glioblastoma has been shown to control OXPHOS, which is crucial for preserving $CD133^{+}$ glioblastoma CSCs.[86] In AML, the quiescent leukemia stem cells (LSCs) express high levels of BCL-2 and have a selective dependency on oxidative respiration.[87] Furthermore, the BCL-2 inhibitors ABT-737 and ABT-263 selectively inhibit the quiescent LSCs by targeting OXPHOS.[87] Similarly, in chronic myeloid leukemia (CML), the primitive $CD34^{+}$ LSCs rely on oxidative metabolism for their survival. Treatment with the combination of imatinib and tigecycline, an antibiotic inhibiting mitochondrial protein translation, selectively eradicates CML LSCs in vitro and in a xenograft model of human CML.[88] The antidiabetic drug metformin, which blocks mitochondrial function via inhibition of the ETC complex I, inhibits cellular transformation and selectively kills BCSCs by depleting the TCA cycle and glycolytic intermediates and suppressing nucleotide biosynthesis.[89] In pancreatic ductal adenocarcinoma, metformin specifically targets $CD133^{+}$ CSCs, which display elevated expression of PGC-1α essential for mitochondrial biogenesis, OXPHOS functionality, and the self-renewal and tumorigenic potential of pancreatic CSCs.[90] Interestingly, metformin-resistant CSC clones in pancreatic ductal adenocarcinoma emerge due to an intermediate glycolytic and/or OXPHOS phenotype driven by increased expression of MYC, and genetic and/or pharmacological suppression of MYC restores metformin sensitivity in metformin-resistant pancreatic CSCs.[90] Thus, the balance of MYC/PGC-1α expression appears to determine the dynamic metabolic states (glycolysis versus OXPHOS) of pancreatic CSCs. This metabolic heterogeneity and plasticity of CSCs identified in various tumor types highlight that CSCs display metabolic plasticity enabling them to adapt to changing tumor microenvironments and nutrient availability.

CSCs in Distinct E and M States Depend Upon Different Metabolic Pathways

As discussed in the previous section, the Warburg effect characterized by increased aerobic glycolysis in the bulk of tumor cells represents a striking metabolic difference between cancer and its normal tissue cells. However, despite the dependence of cancers on glycolysis, glycolytic inhibitors such as 2DG and Lonidamine exhibit little effect on solid tumor growth.[91,92] Although the mechanisms accounting for the lack of sensitivity of cancer cells to glycolytic inhibition remain to be fully characterized, a recent study suggested that mTORC1-dependent metabolic rewiring underlies the escape of cancer cells to glycolytic addiction.[93]

The phenotypic plasticity of BCSCs enabling them to transition between a quiescent mesenchymal state and proliferative epithelial state facilitates the capacity of BCSCs to initiate and grow primary tumors, to invade the basement membrane and tissue vasculature, and ultimately colonize

distant organs to form clinically significant metastases.[17,19] Despite the functional significance of this CSC plasticity in tumor development, treatment resistance and metastatic relapse, the cellular and molecular mechanisms regulating BCSC plasticity by the complex tumor microenvironment remain largely elusive. Currently, little is known about the metabolic pathways active in distinct BCSC states and how these CSC states evolve under various metabolic stressors such as glycolytic inhibition, nutrient deprivation, hypoxia, and xenobiotic and/or oxidant stress. These metabolic differences of M- and E-like CSCs may confer resistance to glycolytic inhibition and play critical roles for tumor cell survival and progression under stress. As CSCs exhibit a dynamic equilibrium of proliferative E- and quiescent M-like states coordinately driving tumor growth, metastasis and treatment resistance, elucidation of these metabolic differences will help to define and exploit metabolic vulnerabilities of each CSC state, providing a conceptual framework to effectively target this critical tumor cell population.

To address these questions, we investigated how metabolic and/or oxidative stress modulates the state dynamics of BCSCs and identified markedly different responses of M- and E-BCSCs to oxidant stress that are closely linked to their distinct redox potentials. Specifically, metabolic and/or oxidative stress generated by 2DG, H_2O_2 or hypoxia, by activating AMPK-dependent HIF1α stabilization and NRF2-mediatted antioxidant responses, promotes the transition of BCSCs from a quiescent, ROS^{low} M-like state to a ROS^{high} proliferative E-state.[18] Interestingly, these divergent responses of M- and E-BCSCs to oxidative stress are closely associated with their different metabolic pathways. As depicted in Figure 1, the $CD24^{-}CD44^{+}$M-BCSCs and $ALDH^{+}$E-BCSCs significantly differ in many aspects of cellular metabolism. Although both CSC states exhibit elevated glycolysis regulatory genes, the relatively quiescent M-BCSCs exhibit higher glycolytic rate in glucose-rich culturing conditions. In contrast, the proliferative E-BCSCs are endowed with robust expression of mitochondrial OXPHOS regulatory genes and exhibit highest metabolic plasticity for OXPHOS under glycolytic inhibited conditions. These proliferative E-BCSCs are also endowed with robust NRF2-mediated antioxidant responses, exemplified by highly elevated expression of a wide variety of antioxidant genes involved in drug transport and detoxification, NADPH (Nicotinamide adenine dinucleotide phosphate, reduced form) production, thioredoxin (TXN), and glutathione (GSH) antioxidant pathways, etc. These metabolic differences of M- and E-BCSCs suggest that while glycolysis plays an important role in supporting the low redox states of M-BCSCs, both mitochondrial respiration and NRF2-mediated antioxidant defenses are important for E-BCSCs to maintain their proliferation and to prevent the accumulation of oxidative stress generated from mitochondrial OXPHOS.

	E-BCSCs	M-BCSCs	Bulk tumor cells
ROS level	++	+/-	+++
Glycolysis	+	++	+/-
OXPHOS	++	+	+/-
NRF2 activity	high	low	low
2DG sensitivity	low	high	high
Sensitivity to NRF2 pathway inhibition	high	low	high

Figure 1 Metabolic differences of E- and M-BCSCs versus the bulk tumor cells. The $CD24^{-}CD44^{+}$M-BCSCs and $ALDH^{+}$E-BCSCs significant differ in many aspects of cellular metabolism as compared to the bulk tumor cells identified as $ALDH^{-}CD24^{+}CD44^{-}$.

As metabolic and/or oxidative stress produced by 2DG-mediated glycolytic inhibition facilitates the transition of quiescent, ROS^{low} M-BCSCs to a proliferative, ROS^{high} E-state susceptible to the blockade of NRF2 or its downstream TXN and GSH antioxidant pathways, we validated a combinatory treatment approach utilizing 2DG together with inhibitors of TXN (by AUR) and GSH (by BSO) antioxidant pathways. This combination treatment approach significantly suppressed tumor growth, tumor-initiating potential and metastasis by abrogating both M- and E-BCSCs in patient-derived xenograft and systemic metastasis models of TNBC.[18] As 2DG is not an FDA-approved cancer therapeutic, our preclinical studies suggest a pro-oxidant-based therapeutic approach utilizing standard-of-care radio-chemotherapy and antiangiogenic therapies (all of which generate oxidative stress) in combination with inhibitors of NRF2-mediated antioxidant responses to treat metastatic cancer (i.e., TNBC). As illustrated in Figure 2, this combinatory approach, by elevating oxidant stress in the ROS^{low} M-like CSCs, promotes the transition of quiescent M-BCSCs to a ROS^{high} E-state that is more susceptible to inhibition of NRF-mediated antioxidant pathways (i.e., NRF2 inhibitor Trig or inhibitors of TXN and/or GSH pathways such as AUR and/or BSO), leading to terminal differentiation and subsequent apoptosis of the both M- and E-like BCSCs. Thus, elucidating metabolic differences of distinct CSC states may provide a conceptual framework to effectively target CSCs in various tumor types, leading to enhanced treatment responses for traditional cancer therapies.

Redox-Regulated Signaling Pathways Modulating CSC Phenotypic/Metabolic Plasticity and Therapeutic Responses

Although a plethora of ROS-dependent signaling pathways including PTEN/PI3K/AKT/mTOR,[94,95] JAK/STAT,[37,96] Notch,[97-99] Hippo,[51] ATM/p53,[100-102] Wnt,[65,103,104] and NF-kB[46,105-107] cascades have been implicated in regulating CSCs through modulation of cancer metabolism and redox homeostasis, our recent studies demonstrate a prominent role of ROS-induced HIF1α and NRF2, two redox-sensitive transcription factors, in regulation of BCSC plasticity during metabolic stress.[18] In this section, we focus on the roles of HIF1α and NRF2 in regulating

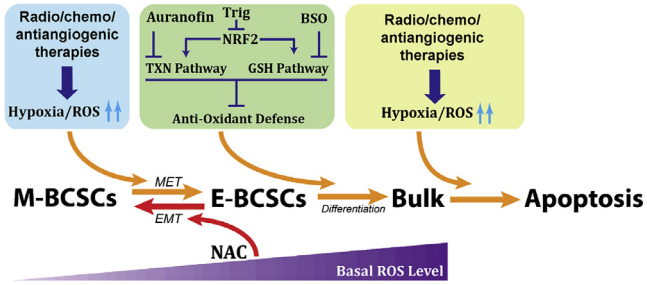

Figure 2 A pro-oxidant-based therapeutic approach with potential to increase treatment responses for traditional cancer therapies. Combination strategies utilizing traditional cancer therapies (i.e., standard radio-chemotherapies or anti-angiogenic therapies) in combination with inhibitors of NRF2-mediated antioxidant responses (i.e., NRF2 inhibitor Trig or inhibitors of TXN/GSH antioxidant pathways including AUR/BSO) enhance treatment responses by simultaneously targeting CSCs of distinct states.

CSC metabolism and phenotypic plasticity as well as the clinical implications of targeting these redox-sensitive signaling pathways to overcome therapeutic resistance.

Roles of HIF1α in Regulation of Redox and Intermediary Metabolism

Advanced cancers often display intratumor hypoxia, which promotes tumor metastasis and therapeutic resistance largely mediated by induction and/or stabilization of HIFs.[108,109] HIFs including HIF1 and HIF2 are transcription factors mediating hypoxic responses and HIF1 functions as a master regulator of glycolysis.[110] HIF1 has two subunits: HIF1α and HIF1β. HIF1 protein levels increase under hypoxic stress and decrease under normoxic conditions due to oxygen-dependent prolyl hydroxylation of HIF1α, which targets HIF1α for proteasomal degradation via the von Hippel-Lindau ubiquitin ligase complex.[111] HIF1 promotes glycolysis through transcriptional activation of a number of glycolytic genes including glucose transporters (i.e., GLUT1 and GLUT3), hexokinases (i.e., HK1 and HK2), pyruvate kinase and lactate dehydrogenase. HIF1α also promotes the transcription of the *PDK1* gene encoding pyruvate dehydrogenase kinase I, which phosphorylates and inactivates pyruvate dehydrogenase, the first enzymatic entry into the TCA cycle.[112,113] This HIF1α-mediated metabolic rewiring by increased influx to the glycolytic pathway and reduced influx to the TCA cycle helps to overcome elevated oxidant stress associated with hypoxia by blunting the production of mitochondrial ROS.

In addition to modifying metabolic influx to glycolytic pathway vs TCA cycle, HIF1α also mediates increased metabolic influx to the serine biosynthetic pathway and mitochondrial one carbon (folate cycle) metabolism. This facilitates NADPH and glutathione synthesis to reduce mitochondrial oxidant stress to promote BCSC maintenance and lung metastasis.[114,115] This role of HIF1α in maintaining ROS homeostasis including redox balance at the mitochondria is critical for the maintenance of CSCs under metabolic stressful conditions (i.e., chemotherapy or antiangiogenic therapy). Indeed, when TNBCs were treated with paclitaxel or gemcitabine, induction of HIF activity including HIF1α and HIF2α led to increased ALDH$^+$ BCSCs and chemotherapy resistance, associated with increased secretion of IL-6 and IL-8,[116] two prominent inflammatory cytokines promoting BCSC activity.[117,118] Subsequent studies identified a redox-mediated mechanism of HIF1α promoting a chemotherapy-induced BCSC phenotype.[119]

The development of antiangiogenic agents was thought to be a major advance in cancer treatment. Several therapeutic agents targeting tumor neovascularization have been developed, including antibodies (i.e., bevacizumab) targeting vascular endothelial growth factor (VEGF) and receptor tyrosine kinase inhibitors (i.e., Sunitinib and Nintedanib) that block signaling mediated by the VEGF receptor. Despite the encouraging responses of some tumor types including colorectal,[120] renal cell,[121] and pancreatic neuroendocrine[122] carcinomas to antiangiogenic therapies, numerous other tumor types, particularly breast cancer,[123-125] are poorly responsive to antiangiogenic regimens. Previous studies in our laboratory showed that that tumor hypoxia generated by antiangiogenic agents enriches ALDH$^+$ E-BCSCs within the tumor hypoxic zones in a HIF1α-dependent manner,[126] suggesting that the induction of ALDH$^+$ E-BCSCs by standard antiangiogenic therapy may contribute to antiangiogenic resistance in breast cancer. Thus, targeting HIF1α-dependent induction of ALDH$^+$BCSCs by chemo or antiangiogenic therapy may overcome chemotherapy or antiangiogenic resistance. In support of this idea, co-administration of HIF inhibitors such as digoxin together with standard chemotherapeutic (paclitaxel and gemcitabine) overcome chemoresistance and prevented tumor relapse in TNBC xenograft mice.[116] Similarly, concurrent administration of a novel HIF1α inhibitor CRLX101 (an investigational nanoparticle-drug conjugated with camptothecin) and bevacizumab led to decreased induction of both HIF1α and ALDH$^+$CSCs in breast tumors leading to tumor regression and delayed tumor recurrence in preclinical mouse models.[127]

Roles of NRF2 in Regulation of Redox and Intermediary Metabolism

The NRF2 is one of the most important transcription factors orchestrating intrinsic resistance to oxidative stress and adaptive antioxidant responses to various environmental stressors.[50,128] NRF2 has been traditionally regarded as a tumor suppressor due to its roles as the main defense mechanism of the cell and a major regulator of cell survival. Yet, recent studies demonstrate that hyperactivation of the NRF2 pathway creates an environment that not only supports the survival of the normal cells, but also the growth and progression of malignant cells, protecting them against oxidative and/or xenobiotic stress, chemotherapeutic agents, and radiotherapy.[129] Accumulating evidence is emerging to demonstrate that NRF2 profoundly influences the metabolism of glucose, lipids, amino acids, and nucleotides.[128,130] Through regulation of redox and intermediary metabolism of tumor

cells, NRF2 pathway serves as a driver of cancer progression, metastasis, and resistance to therapy.

Regulation of NRF2 Activity

NRF2 belongs to the cap "n" collar subfamily of basic leucine zipper transcription factors equipped with 7 modular NRF2-ECH homology domains (Neh1-7), each of which preforming distinct functions to regulate NRF2 activity.[128] In unstressed conditions, NRF2 protein levels remain low in the cytoplasm, where it forms a complex through dimerization of its Neh2 domain with the Kelch-like ECH-associated protein 1 (Keap1), an NRF2 negative regulator that targets NRF2 for ubiquitination and proteasomal degradation.[131,132] Electrophiles and oxidants inhibit Keap1-mediated proteasomal degradation of NRF2, thereby enabling NRF2 accumulation and nuclear translocation to initiate the transcription of a wide variety of antioxidant genes that facilitate cellular adaptation to stress.[128] Once activated, NRF2 hetero-dimerizes with the small MAF proteins to control the basal and inducible expression of over 200 genes that contain antioxidant response elements (AREs) in their regulatory regions.[133] Although NRF2 is principally regulated by Keap1, the Neh6 domain of NRF2 confers additional mechanism of stability control by recruiting β-transducin repeat-containing protein (β-TrCP), a substrate adaptor for the S-phase kinase-associated protein 1 (Skp1)−Cul1−Rbx1 core E3 complex. This negative regulation of NRF2 by β-TrCP is enhanced by glycogen synthase kinase 3 (GSK3) activities.[134-136]

In addition to ubiquitination, NRF2 protein expression is subject to other forms of post-translational modifications including phosphorylation and acetylation, which allow modulation of NRF2 activity by fine-tuning its subcellular localization in the cytoplasm and/or nucleus. For example, activation of the AMPK, a master regulator of metabolism to maintain cellular bioenergetics during metabolic stress, increases NRF2 activity through phosphorylation of GSK3β, leading to the inhibition of GSK3β function in promoting NRF2 nuclear exclusion and degradation.[137,138] AMPK is also capable of directly phosphorylating NRF2 at Ser550 to facilitate its nuclear localization.[139] In addition to the phosphorylation of NRF2, multiple lysine residues in the Neh1 domain of NRF2 were shown to be acetylated by p300/CBP, which augments promoter-specific DNA binding of NRF2 to ARE sequences and induction of antioxidant responsive gene expression during stress.[140]

NRF2 activity is also controlled by transcriptional regulation. For example, functional AREs in mouse NRF2 promoter region have been identified, which mediate the autoregulation of NRF2 through transcriptional activation, providing a positive feedback mechanism.[141] NRF2 activity can also be regulated by various polymorphisms in its promoter and protein coding regions and a single nucleotide polymorphism in the ARE-like sequences of human NRF2 promoter is associated with diminished NRF2 expression a single nucleotide polymorphism in the ARE-like sequences of human NRF2 promoter is associated with diminished NRF2 expression and increased susceptibility to lung cancer.[142] The NRF2 promoter also contains a NF-kB binding site, thereby enabling its transcriptional activation by inflammatory stimuli such as lipopolysaccharide.[143] This constitutively high NRF2 expression driven by NF-kB in human acute myeloid leukemia underlies its chemoresistance.[144]

NRF2 in Metabolic Reprograming of Cancer

Beyond its function as a master regulator of antioxidant responses, NRF2 has recently been recognized as a key transcription factor mediating metabolic reprogramming in cancer cells.[128,130,145] Through direct regulation of key metabolic genes or indirectly through crosstalk with other transcription factors, NRF2 serves as a major hub to modulate numerous enzymes in major metabolic pathways of carbohydrates, nucleic acids, lipids, and amino acids.

NRF2 and Glucose Metabolism

Metabolic reprogramming refers to a process that cancer cells utilize in order to rewire their metabolic pathways and energy production network to support their proliferative and/or invasive characteristics. This phenomenon was first demonstrated in cancers by the Warburg effect of aerobic glycolysis observed in the 1920s.[68] Although glycolysis represents one of the remarkable features of cancer metabolic alternations, the underlying mechanisms driving this metabolic alteration and its influences on cancer development and progression are still not fully understood. Activation of NRF2 increases glucose uptake and diverts it to the pentose phosphate pathway (PPP) by promoting enzyme expression in the oxidative (including glucose-6-phosphate dehydrogenase, G6PD and 6-phosphogluconate dehydrogenase) and nonoxidative (including transketolase, TKT and transaldolase, TALDO1) arms of PPP.[130,146,147] In addition to G6PD and phosphogluconate dehydrogenase, NRF2 also promotes the expression of two other NADPH synthetic enzymes ME1 and IDH1,[146] suggesting a role of NRF2 in replenishing NADPH, the main reducing equivalent required for redox hemostasis and biosynthesis of lipids and nucleotides. This role of NRF2 in promoting nucleotide synthesis is supported by the findings that the expression of de novo purine synthesis enzymes, phosphoribosyl pyrophosphate amidotransferase and methylenetetrahydrofolate dehydrogenase 2, was significantly decreased following knockdown of NRF2 in A549 cells (which is deficient of Keap1).[146] The role of NRF2 in redirecting glycolytic intermediates into anabolic pathways to support tumor growth was further demonstrated in a recent study by showing that activation of NRF2 upregulates the expression of key serine biosynthesis enzymes including PHGDH, PSAT1, and SHMT and diverts glycolytic intermediate 3-phosphoglycerate to serine and/or glycine synthetic pathway, which augments glutathione and nucleotide production and confers poor prognosis in human nonsmall cell lung cancer.[148]

The folate cycle, through transfer of one carbon unit between tetrahydrofolate and its derivatives in the cytoplasm or mitochondria, produces metabolites essential for cell growth, including nucleotides, methionine, and NADPH.[149] Interestingly, methylenetetrahydrofolate dehydrogenase1L, an enzyme critical for folate cycle maintenance,[150] is

transcriptionally activated by NRF2, which confers metabolic advantages in hepatocellular carcinoma.[151] Together with the study showing that HIF1α induced by chemotherapeutic agents promotes BCSCs and lung metastasis by redirecting glycolytic intermediates to serine synthesis and mitochondrial one carbon metabolism,[115] these studies provide evidence for a role of NRF2 and HIF1α to confer metabolic advantages to cancer cells including CSCs through metabolic reprograming.

NRF2 and Glutamine Metabolism

In addition to glycolysis, many tumors fuel their cellular bioenergetics by degradation of glutamine through glutaminolysis, which is catalyzed by a NRF2 target gene glutaminase, thereby providing cancer cells with nitrogen for the biosynthesis of nucleotides and nonessential amino acids.[128,146] The glutamate generated by gene glutaminase can be deaminated into α-ketoglutarate to fuel the TCA cycle and this step is enhanced by the NRF2 target gene *ME1*.[152] Alternatively, glutamate can be used to fuel glutathione biosynthesis, which is catalyzed by glutamate-cysteine ligase (with catalytic GCLC and regulatory GCLM subunits) and glutathione synthetase, two enzymes also regulated NRF2.[152] Upregulated expression of GCLC and GCLM and ME1 by NRF2 has been shown to direct increased carbon flux from glutamine toward GSH biosynthesis and TCA cycle,[146] illustrating a role of NRF2 in rewiring glutamine metabolism in cancer. Of note, the ability of NRF2 to promote cell proliferation through metabolic reprogramming (i.e., PPP shunting, NADPH production, nucleotide synthesis and glutamine metabolism) is augmented in the presence of active PI3K-Akt signaling,[146] suggesting a positive feedback mechanism between oncogenic pathways and NRF2 in driving the malignant phenotype.

NRF2 and Lipid Metabolism

As summarized in two recent reviews,[128,130] NRF2 negatively regulates a variety of genes involved in fatty acid biosynthesis, desaturation, and transport. In contrast, a number of lipases involved in the degradation of triglycerides and/or phospholipids and enzymes involved in mitochondrial fatty acid β-oxidation (FAO) are positively regulated by NRF2. The FAO products including NADH and FADH2 counteract ROS accumulation to reduce oxidative stress and increase ATP production by serving as the electron carriers of the ETC complex.[153] Several studies have reported a function of FAO in the maintenance of HSCs and CD8 + memory T cells, which display some stem like characteristics.[154-156]

Recent studies also implicate a role of FAO in driving a CSC-like phenotype and chemoresistance. For instances, the promyelocytic leukemia gene expressed in breast cancer acts as both a negative regulator of PGC-1α acetylation and a potent activator of PPAR signaling and fatty acid oxidation, which promotes ATP production and inhibits anoikis, a property associated with CSCs and metastasis.[157] The expression of the oncogenic transcription factor MYC is elevated in many TNBCs, which drives dysregulated FAO activity.[158] Pharmacologic inhibition of FAO catastrophically decreased energy metabolism in MYC-overexpressing TNBC cells and blocked tumor growth in a MYC-driven transgenic TNBC model and a MYC-overexpressing TNBC patient-derived xenograft model.[158] The activation of JAK/STAT3 promotes M-like BCSC phenotype and tumor growth.[159] Inhibiting JAK/STAT3 blocks the self-renewal of M-like BCSCs and the expression of diverse lipid metabolic genes, including carnitine palmitoyltransferase 1B, a rate-limiting enzyme of FAO pathway; and blocking FAO re-sensitize breast cancer cells to chemotherapy while reducing cancer stemness in vivo.[160]

Roles of NRF2 and HIF1α in Promoting CSC Plasticity

Our recent studies demonstrated that NRF2 and HIF1α are critical for promoting an E-BCSC phenotype during metabolic and/or oxidative stress and suppression of NRF2 or HIF1α impedes the maintenance and clonal expansion of E-BCSCs.[18] As shown in Figure 3, three different mechanisms by which metabolic and/or oxidant stress induces an enhanced E-BCSC phenotype are identified: (1) conversion of M- to E-BCSCs, which is mediated by AMPK-dependent HIF1α stabilization, (2) activation of HIF1α-NOTCH self-renewal pathway, (3) activation of NRF2-mediated ALDH1A1/3 expression, which is independent of HIF1α.

Although our studies identify ROS-AMPK-HIF1α and NRF2 pathways in regulation of BCSC state dynamics, additional studies are necessary to determine how activation of AMPK regulates HIF1α stability during metabolic and/or oxidative stress. Interestingly, previous studies showed that NRF2 knockdown reduces HIF1α protein levels and, consequently, the expression of VEGF, PDGF, and angiopoietin in glioblastoma,[161] suggesting that NRF2 mediates HIF1α stabilization induced by hypoxic stress. Therefore, it is likely that that enhanced NRF2 expression not only promotes the

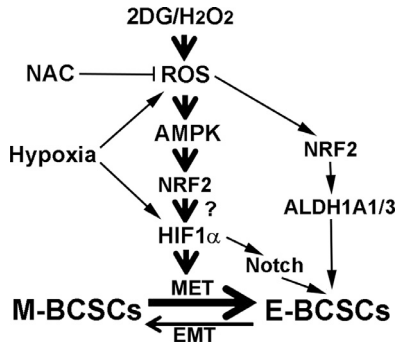

Figure 3 NRF2 acts as a central hub to facilitate the transition of BCSCs from the M to E state as well as the maintenance/proliferation of E-BCSCs under metabolic/oxidant stress. The activation of NRF2 during metabolic/oxidant stress promotes CSC plasticity by mediating AMPK-dependent HIF1α stabilization, which is required for the conversion of M- to E-BCSCs. NRF2 activation also mediates the activation of HIF1α-NOTCH self-renewal pathway and the expression of NRF2 target genes ALDH1A1/3 to facilitate the propagation of E-BCSCs.

self-renewal and/or proliferation of E-BCSCs, but also mediates AMPK-dependent HIF1α stabilization required for facilitating M to E state transition of BCSCs under metabolic and/or oxidative stress. If so, NRF2 would serve as an important hub in facilitating BCSC M to E state transition as well as maintenance and proliferation of E-BCSCs under metabolic and/or oxidant stress (Fig. 3). In addition, as the tumor microenvironment including nutrient and/or oxygen availability and tumor stroma constantly impacts tumor behavior, future studies to assess how these environmental factors affect BCSC metabolism and phenotypic plasticity in vivo will be critical.

One plausible mechanism of NRF2 and HIF1α mediating treatment resistance is through metabolic reprograming, which confers metabolic advantages in CSCs, especially E-like CSCs that express elevated levels of NRF2 and HIF1α. This idea is supported by our recent gene profiling analyses of E- and M-BCSCs isolated from two patient-derived xenograft models of TNBC, which identified differential expression patterns of glycolysis and PPP pathway genes in M- and E-BCSCs.[18] Specifically, a wide variety of glycolytic enzymes ranging from HK1 to LDHD were highly elevated in M- but less robustly in E-BCSCs. In contrast, various PPP enzyme genes, including G6PD, were highly upregulated in E- but less robustly in M-BCSCs.[18] This robust elevation of PPP genes in E- but not M-BCSCs suggests that elevated expression of NRF2 and/or HIF1α in E-BCSCs redirects glycolytic intermediates to PPP pathway, promoting NADPH production and nucleotide biosynthesis to support the propagation of E-BCSCs.

Concluding Remarks

Recent findings that E- and M-like CSCs significantly differ in their redox states, metabolic pathways and sensitivities to metabolic and/or oxidant stress have identified a novel framework exploiting metabolic vulnerabilities of distinct CSC states.[18] As increased oxidant stress, through activation of HIF1α and NRF2 signaling, promotes the transition of quiescent M-CSCs to an E-like state that is more susceptible to the inhibition of redox metabolism especially NRF2-mediated antioxidant pathways,[18] future pro-oxidant-based therapeutic strategies utilizing conventional cancer therapies (i.e., standard chemotherapeutic and/or antiangiogenic agents, radiation therapy) together with agents targeting NRF2-mediated antioxidant responses may prove to be efficient in overcoming resistance associated with traditional cancer therapies by targeting both M- and E-like CSCs.

In addition to traditional cancer therapies that are associated with toxicity to normal cells, pharmacological ascorbate (high doses of Vitamin C given via intravenous injection) has re-emerged as a nontoxic and easily implementable pro-oxidant which has the potential to increase treatment efficacy when combined with standard-of-care radio-chemotherapy.[162-164] Recent studies have demonstrated that oxidant-mediated disruption of iron metabolism causes the selective susceptibility of nonsmall cell lung cancer and GBM cancer cells to pharmacological ascorbate,[165] supporting a generalized mechanism for the use of pharmacological ascorbate in cancer therapy. As iron-dependent accumulation of lipid hydroperoxides can trigger ferroptosis, a regulated cell death nexus linking metabolism, redox biology and diseases,[166] future studies are necessary to determine if pharmacological ascorbate disrupts NRF2-regulated iron metabolism in CSCs to induce ferroptosis.

Considering the central roles of NRF2 in regulating antioxidant defenses and metabolism, future metabolic tracing studies dissecting how activation of NRF2 rewires the metabolism of CSCs to promote their phenotypic and/or metabolic plasticity and proliferation under metabolic and/or oxidative stress are also required, which will not only validate NRF2 as a critical target to abrogate CSC plasticity and proliferation, but also identify potential new targets downstream of NRF2 in driving CSC self-renewal and/or proliferation and treatment resistance. These mechanistic studies may reveal novel therapeutic strategies to target the metabolic vulnerabilities of CSCs, thereby improving treatment efficacy.

References

1. Wicha MS, Liu S, Dontu G. Cancer stem cells: An old idea—a paradigm shift. Cancer Res 66:1883-1890, 2006. discussion 1895-1886
2. Liu S, Wicha MS. Targeting breast cancer stem cells. J Clin Oncol 28:4006-4012, 2010
3. Charafe-Jauffret E, Monville F, Ginestier C, et al: Cancer stem cells in breast: Current opinion and future challenges. Pathobiology 75:75-84, 2008
4. Balic M, Lin H, Young L, et al: Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. Clin Cancer Res 12:5615-5621, 2006
5. Creighton CJ, Li X, Landis M, et al: Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci USA 106:13820-13825, 2009
6. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 5:275-284, 2005
7. Diehn M, Cho RW, Lobo NA, et al: Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 458:780-783, 2009
8. Li X, Lewis MT, Huang J, et al: Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst 100:672-679, 2008
9. Phillips TM, McBride WH, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. J Natl Cancer Inst 98:1777-1785, 2006
10. Zhang M, Atkinson RL, Rosen JM. Selective targeting of radiation-resistant tumor-initiating cells. Proc Natl Acad Sci USA 107:3522-3527, 2010
11. O'Brien-Ball C, Biddle A. Reprogramming to developmental plasticity in cancer stem cells. Dev Biol 430:266-274, 2017
12. Wainwright EN, Scaffidi P. Epigenetics and cancer stem cells: Unleashing, hijacking, and restricting cellular plasticity. Trends Cancer 3:372-386, 2017
13. Brooks MD, Burness ML, Wicha MS. Therapeutic implications of cellular heterogeneity and plasticity in breast cancer. Cell Stem Cell 17:260-271, 2015
14. Liu S, Cong Y, Wang D, et al: Breast cancer stem cells transition between epithelial and mesenchymal states reflective of their normal counterparts. Stem Cell Rep 2:78-91, 2014
15. Mani SA, Guo W, Liao MJ, et al: The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133:704-715, 2008

16. Zhu Y, Luo M, Brooks M, et al: Biological and clinical significance of cancer stem cell plasticity. Clin Transl Med 3:32, 2014
17. Luo M, Brooks M, Wicha MS. Epithelial-mesenchymal plasticity of breast cancer stem cells: Implications for metastasis and therapeutic resistance. Curr Pharm Des 21:1301-1310, 2015
18. Luo M, Shang L, Brooks MD, et al: Targeting breast cancer stem cell state equilibrium through modulation of redox signaling. Cell Metab 28:69-86. e66, 2018
19. Luo M, Clouthier SG, Deol Y, et al: Breast cancer stem cells: Current advances and clinical implications. Methods Mol Biol 1293:1-49, 2015
20. Lagadec C, Vlashi E, Della Donna L, et al: Survival and self-renewing capacity of breast cancer initiating cells during fractionated radiation treatment. Breast Cancer Res.: BCR 12:R13, 2010
21. Karimi-Busheri F, Rasouli-Nia A, Mackey JR, et al: Senescence evasion by MCF-7 human breast tumor-initiating cells. Breast Cancer Res.: BCR 12:R31, 2010
22. Fillmore CM, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. Breast Cancer Res.: BCR 10:R25, 2008
23. Shafee N, Smith CR, Wei S, et al: Cancer stem cells contribute to cisplatin resistance in Brca1/p53-mediated mouse mammary tumors. Cancer Res 68:3243-3250, 2008
24. Woodward WA, Chen MS, Behbod F, et al: WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. Proc Natl Acad Sci USA 104:618-623, 2007
25. Yu F, Yao H, Zhu P, et al: let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell 131:1109-1123, 2007
26. Zielske SP, Spalding AC, Wicha MS, et al: Ablation of breast cancer stem cells with radiation. Transl Oncol 4:227-233, 2011
27. Ithimakin S, Day KC, Malik F, et al: HER2 drives luminal breast cancer stem cells in the absence of HER2 amplification: Implications for efficacy of adjuvant trastuzumab. Cancer Res 73:1635-1646, 2013
28. Korkaya H, Kim GI, Davis A, et al: Activation of an IL6 inflammatory loop mediates trastuzumab resistance in HER2+ breast cancer by expanding the cancer stem cell population. Mol Cell 47:570-584, 2012
29. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. Nature Rev Drug Discovery 12:931-947, 2013
30. Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res 51:794-798, 1991
31. Raj L, Ide T, Gurkar AU, et al: Selective killing of cancer cells by a small molecule targeting the stress response to ROS. Nature 475:231-234, 2011
32. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? Nat Rev Drug Discovery 8:579-591, 2009
33. Finkel T. Signal transduction by mitochondrial oxidants. J Biol Chem 287:4434-4440, 2012
34. Handy DE, Loscalzo J. Redox regulation of mitochondrial function. Antioxid Redox Signaling 16:1323-1367, 2012
35. Hancock JT, Desikan R, Neill SJ. Role of reactive oxygen species in cell signalling pathways. Biochem Soc Trans 29:345-350, 2001
36. Raman D, Foo CH, Clement MV, et al: Breast cancer: A molecular and redox snapshot. Antioxid Redox Signaling 25:337-370, 2016
37. Li L, Cheung SH, Evans EL, et al: Modulation of gene expression and tumor cell growth by redox modification of STAT3. Cancer Res 70:8222-8232, 2010
38. Simon AR, Rai U, Fanburg BL, et al: Activation of the JAK-STAT pathway by reactive oxygen species. Am J Physiol 275:C1640-C1652, 1998
39. Son Y, Kim S, Chung HT, et al: Reactive oxygen species in the activation of MAP kinases. Methods Enzymol 528:27-48, 2013
40. Martindale JL, Holbrook NJ. Cellular response to oxidative stress: Signaling for suicide and survival. J Cellular Physiol 192:1-15, 2002
41. Gutierrez-Uzquiza A, Arechederra M, Bragado P, et al: p38alpha mediates cell survival in response to oxidative stress via induction of antioxidant genes: Effect on the p70S6K pathway. J Biol Chem 287:2632-2642, 2012
42. Leslie NR, Bennett D, Lindsay YE, et al: Redox regulation of PI 3-kinase signalling via inactivation of PTEN. EMBO J 22:5501-5510, 2003
43. Luo L, Kaur Kumar J, Clement MV. Redox control of cytosolic Akt phosphorylation in PTEN null cells. Free Radical Biol Med 53:1697-1707, 2012
44. Cichon MA, Radisky DC. ROS-induced epithelial-mesenchymal transition in mammary epithelial cells is mediated by NF-kB-dependent activation of Snail. Oncotarget 5:2827-2838, 2014
45. Gloire G, Legrand-Poels S, Piette J. NF-kappaB activation by reactive oxygen species: Fifteen years later. Biochem Pharmacol 72:1493-1505, 2006
46. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-kappaB signaling. Cell Res 21:103-115, 2011
47. Brennan JP, Bardswell SC, Burgoyne JR, et al: Oxidant-induced activation of type I protein kinase A is mediated by RI subunit interprotein disulfide bond formation. J Biol Chem 281:21827-21836, 2006
48. Giorgi C, Agnoletto C, Baldini C, et al: Redox control of protein kinase C: Cell- and disease-specific aspects. Antioxid Redox Signaling 13:1051-1085, 2010
49. Perez LM, Milkiewicz P, Ahmed-Choudhury J, et al: Oxidative stress induces actin-cytoskeletal and tight-junctional alterations in hepatocytes by a Ca2+ -dependent, PKC-mediated mechanism: Protective effect of PKA. Free Radical Biol Med 40:2005-2017, 2006
50. Tonelli C, Chio IIC, Tuveson DA. Transcriptional regulation by Nrf2. Antioxid Redox Signal. 29(17):1727-1745, 2018. https://doi.org/10.1089/ars.2017.7342
51. Ashraf A, Pervaiz S. Hippo circuitry and the redox modulation of hippo components in cancer cell fate decisions. Int J Biochem Cell Biol 69:20-28, 2015
52. Janssen-Heininger YM, Mossman BT, Heintz NH, et al: Redox-based regulation of signal transduction: Principles, pitfalls, and promises. Free Radical Biol Med 45:1-17, 2008
53. Rhee SG. Cell signaling. H2O2, a necessary evil for cell signaling. Science 312:1882-1883, 2006
54. Hurd TR, DeGennaro M, Lehmann R. Redox regulation of cell migration and adhesion. Trends Cell Biol 22:107-115, 2012
55. Tochhawng L, Deng S, Pervaiz S, et al: Redox regulation of cancer cell migration and invasion. Mitochondrion 13:246-253, 2013
56. Ward JF. Biochemistry of DNA lesions. Radiat Res Suppl 8:S103-S111, 1985
57. Powell S, McMillan TJ. DNA damage and repair following treatment with ionizing radiation. Radiother Oncol 19:95-108, 1990
58. Kryston TB, Georgiev AB, Pissis P, et al: Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res 711:193-201, 2011
59. Smith J, Ladi E, Mayer-Proschel M, et al: Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. Proc Natl Acad Sci USA 97:10032-10037, 2000
60. Ito K, Hirao A, Arai F, et al: Regulation of oxidative stress by ATM is required for self-renewal of haematopoietic stem cells. Nature 431:997-1002, 2004
61. Ito K, Hirao A, Arai F, et al: Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med 12:446-451, 2006
62. Tothova Z, Kollipara R, Huntly BJ, et al: FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 128:325-339, 2007
63. Miyamoto K, Araki KY, Naka K, et al: Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 1:101-112, 2007
64. Guzman ML, Rossi RM, Karnischky L, et al: The sesquiterpene lactone parthenolide induces apoptosis of human acute myelogenous leukemia stem and progenitor cells. Blood 105:4163-4169, 2005
65. Dong C, Yuan T, Wu Y, et al: Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell 23:316-331, 2013
66. Van Keymeulen A, Rocha AS, Ousset M, et al: Distinct stem cells contribute to mammary gland development and maintenance. Nature 479:189-193, 2011
67. Kannan N, Nguyen LV, Makarem M, et al: Glutathione-dependent and -independent oxidative stress-control mechanisms distinguish normal

human mammary epithelial cell subsets. Proc Natl Acad Sci USA 111:7789-7794, 2014
68. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol 8:519-530, 1927
69. Folmes CD, Dzeja PP, Nelson TJ, et al: Metabolic plasticity in stem cell homeostasis and differentiation. Cell Stem Cell 11:596-606, 2012
70. Folmes CD, Nelson TJ, Martinez-Fernandez A, et al: Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. Cell Metab 14:264-271, 2011
71. Panopoulos AD, Yanes O, Ruiz S, et al: The metabolome of induced pluripotent stem cells reveals metabolic changes occurring in somatic cell reprogramming. Cell Res 22:168-177, 2012
72. Cho YM, Kwon S, Pak YK, et al: Dynamic changes in mitochondrial biogenesis and antioxidant enzymes during the spontaneous differentiation of human embryonic stem cells. Biochem Biophys Res Commun 348:1472-1478, 2006
73. Ciavardelli D, Rossi C, Barcaroli D, et al: Breast cancer stem cells rely on fermentative glycolysis and are sensitive to 2-deoxyglucose treatment. Cell Death Dis 5:e1336, 2014
74. Feng W, Gentles A, Nair RV, et al: Targeting unique metabolic properties of breast tumor initiating cells. Stem Cells 32:1734-1745, 2014
75. Song K, Kwon H, Han C, et al: Active glycolytic metabolism in CD133 (+) hepatocellular cancer stem cells: Regulation by MIR-122. Oncotarget 6:40822-40835, 2015
76. Shen YA, Wang CY, Hsieh YT, et al: Metabolic reprogramming orchestrates cancer stem cell properties in nasopharyngeal carcinoma. Cell Cycle 14:86-98, 2015
77. Lamb R, Harrison H, Hulit J, et al: Mitochondria as new therapeutic targets for eradicating cancer stem cells: Quantitative proteomics and functional validation via MCT1/2 inhibition. Oncotarget 5:11029-11037, 2014
78. Deblois G, Giguere V. Functional and physiological genomics of estrogen-related receptors (ERRs) in health and disease. Biochim Biophys Acta 1812:1032-1040, 2011
79. Deblois G, St-Pierre J, Giguere V. The PGC-1/ERR signaling axis in cancer. Oncogene 32:3483-3490, 2013
80. De Luca A, Fiorillo M, Peiris-Pages M, et al: Mitochondrial biogenesis is required for the anchorage-independent survival and propagation of stem-like cancer cells. Oncotarget 6:14777-14795, 2015
81. LeBleu VS, O'Connell JT, Gonzalez Herrera KN, et al: PGC-1alpha mediates mitochondrial biogenesis and oxidative phosphorylation in cancer cells to promote metastasis. Nat Cell Biol 16:992-1003, 2014. 1001-1015
82. Lamb R, Ozsvari B, Lisanti CL, et al: Antibiotics that target mitochondria effectively eradicate cancer stem cells, across multiple tumor types: Treating cancer like an infectious disease. Oncotarget 6:4569-4584, 2015
83. Lamb R, Bonuccelli G, Ozsvari B, et al: Mitochondrial mass, a new metabolic biomarker for stem-like cancer cells: understanding WNT/FGF-driven anabolic signaling. Oncotarget 6:30453-30471, 2015
84. Farnie G, Sotgia F, Lisanti MP. High mitochondrial mass identifies a sub-population of stem-like cancer cells that are chemo-resistant. Oncotarget 6:30472-30486, 2015
85. Lee KM, Giltnane JM, Balko JM, et al: MYC and MCL1 cooperatively promote chemotherapy-resistant breast cancer stem cells via regulation of mitochondrial oxidative phosphorylation. Cell Metab 26:633-647. e637, 2017
86. Janiszewska M, Suva ML, Riggi N, et al: Imp2 controls oxidative phosphorylation and is crucial for preserving glioblastoma cancer stem cells. Genes Dev 26:1926-1944, 2012
87. Lagadinou ED, Sach A, Callahan K, et al: BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell 12:329-341, 2013
88. Kuntz EM, Baquero P, Michie AM, et al: Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. Nat Med 23:1234-1240, 2017
89. Janzer A, German NJ, Gonzalez-Herrera KN, et al: Metformin and phenformin deplete tricarboxylic acid cycle and glycolytic intermediates during cell transformation and NTPs in cancer stem cells. Proc Natl Acad Sci USA 111:10574-10579, 2014
90. Sancho P, Burgos-Ramos E, Tavera A, et al: MYC/PGC-1 alpha balance determines the metabolic phenotype and plasticity of pancreatic cancer stem cells. Cell Metab 22:590-605, 2015
91. Papaldo P, Lopez M, Cortesi E, et al: Addition of either lonidamine or granulocyte colony-stimulating factor does not improve survival in early breast cancer patients treated with high-dose epirubicin and cyclophosphamide. J Clin Oncol 21:3462-3468, 2003
92. Prasanna VK, Venkataramana NK, Dwarakanath BS, et al: Differential responses of tumors and normal brain to the combined treatment of 2-DG and radiation in glioablastoma. J Cancer Res Ther 5(Suppl 1):S44-S47, 2009
93. Pusapati RV, Daemen A, Wilson C, et al: mTORC1-dependent metabolic reprogramming underlies escape from glycolysis addiction in cancer cells. Cancer Cell 29:548-562, 2016
94. Elstrom RL, Bauer DE, Buzzai M, et al: Akt stimulates aerobic glycolysis in cancer cells. Cancer Res 64:3892-3899, 2004
95. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer Cell 12:9-22, 2007
96. Zhou J, Wulfkuhle J, Zhang H, et al: Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proc Natl Acad Sci USA 104:16158-16163, 2007
97. Charles N, Ozawa T, Squatrito M, et al: Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell 6:141-152, 2010
98. McAuliffe SM, Morgan SL, Wyant GA, et al: Targeting Notch, a key pathway for ovarian cancer stem cells, sensitizes tumors to platinum therapy. Proc Natl Acad Sci USA 109:E2939-E2948, 2012
99. Dontu G, Jackson KW, McNicholas E, et al: Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res.: BCR 6:R605-R615, 2004
100. Cosentino C, Grieco D, Costanzo V. ATM activates the pentose phosphate pathway promoting anti-oxidant defence and DNA repair. EMBO J 30:546-555, 2011
101. Sablina AA, Budanov AV, Ilyinskaya GV, et al: The antioxidant function of the p53 tumor suppressor. Nat Med 11:1306-1313, 2005
102. Gandhi UH, Kaushal N, Hegde S, et al: Selenium suppresses leukemia through the action of endogenous eicosanoids. Cancer Res 74:3890-3901, 2014
103. Funato Y, Michiue T, Asashima M, et al: The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt-beta-catenin signalling through dishevelled. Nat Cell Biol 8:501-508, 2006
104. Korswagen HC. Regulation of the Wnt/beta-catenin pathway by redox signaling. Dev Cell 10:687-688, 2006
105. Myant KB, Cammareri P, McGhee EJ, et al: ROS production and NF-kappaB activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. Cell Stem Cell 12:761-773, 2013
106. Jin Y, Lu Z, Ding K, et al: Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: Inactivation of the NF-kappaB pathway and generation of reactive oxygen species. Cancer Res 70:2516-2527, 2010
107. Bubici C, Papa S, Dean K, et al: Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: Molecular basis and biological significance. Oncogene 25:6731-6748, 2006
108. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 11:393-410, 2011
109. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Investig 123:3664-3671, 2013
110. Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer 8:705-713, 2008
111. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 148:399-408, 2012
112. Kim JW, Tchernyshyov I, Semenza GL, et al: HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3:177-185, 2006

113. Papandreou I, Cairns RA, Fontana L, et al: HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. Cell Metab 3:187-197, 2006
114. Semenza GL. Hypoxia-inducible factors: Coupling glucose metabolism and redox regulation with induction of the breast cancer stem cell phenotype. EMBO J 36:252-259, 2017
115. Samanta D, Park Y, Andrabi SA, et al: PHGDH expression is required for mitochondrial redox homeostasis, breast cancer stem cell maintenance, and lung metastasis. Cancer Res 76:4430-4442, 2016
116. Samanta D, Gilkes DM, Chaturvedi P, et al: Hypoxia-inducible factors are required for chemotherapy resistance of breast cancer stem cells. Proc Natl Acad Sci USA 111:E5429-E5438, 2014
117. Liu S, Ginestier C, Ou SJ, et al: Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res 71:614-624, 2011
118. Ginestier C, Liu S, Diebel ME, et al: CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts. J Clin Investig 120:485-497, 2010
119. Lu H, Samanta D, Xiang L, et al: Chemotherapy triggers HIF-1-dependent glutathione synthesis and copper chelation that induces the breast cancer stem cell phenotype. Proc Natl Acad Sci USA 112:E4600-E4609, 2015
120. Hurwitz H, Fehrenbacher L, Novotny W, et al: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350:2335-2342, 2004
121. Motzer RJ, Hutson TE, Tomczak P, et al: Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 356:115-124, 2007
122. Raymond E, Dahan L, Raoul JL, et al: Sunitinib malate for the treatment of pancreatic neuroendocrine tumors. N Engl J Med 364:501-513, 2011
123. Kerbel RS. Issues regarding improving the impact of antiangiogenic drugs for the treatment of breast cancer. Breast 18(Suppl 3):S41-S47, 2009
124. Miller K, Wang M, Gralow J, et al: Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 357:2666-2676, 2007
125. Rose S. FDA pulls approval for avastin in breast cancer. Cancer Discovery 1. OF1-2, 2011
126. Conley SJ, Gheordunescu E, Kakarala P, et al: Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proc Natl Acad Sci USA 109:2784-2789, 2012
127. Conley SJ, Baker TL, Burnett JP, et al: CRLX101, an investigational camptothecin-containing nanoparticle-drug conjugate, targets cancer stem cells and impedes resistance to antiangiogenic therapy in mouse models of breast cancer. Breast Cancer Res Treatment 150:559-567, 2015
128. Hayes JD, Dinkova-Kostova AT. The Nrf2 regulatory network provides an interface between redox and intermediary metabolism. Trends Biochem Sci 39:199-218, 2014
129. Menegon S, Columbano A, Giordano S. The dual roles of NRF2 in cancer. Trends Mol Med 22:578-593, 2016
130. Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the hallmarks of cancer. Cancer Cell. 34(1):21-43, 2018. https://doi.org/10.1016/j.ccell.2018.03.022
131. McMahon M, Thomas N, Itoh K, et al: Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: A two-site interaction model for the Nrf2-Keap1 complex. J Biol Chem 281:24756-24768, 2006
132. Tong KI, Katoh Y, Kusunoki H, et al: Keap1 recruits Neh2 through binding to ETGE and DLG motifs: Characterization of the two-site molecular recognition model. Mol Cellular Biol 26:2887-2900, 2006
133. Zhu M, Fahl WE. Functional characterization of transcription regulators that interact with the electrophile response element. Biochem Biophys Res Commun 289:212-219, 2001
134. Chowdhry S, Zhang Y, McMahon M, et al: Nrf2 is controlled by two distinct beta-TrCP recognition motifs in its Neh6 domain, one of which can be modulated by GSK-3 activity. Oncogene 32:3765-3781, 2013
135. Rada P, Rojo AI, Chowdhry S, et al: SCF/{beta}-TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. Mol Cellular Biol 31:1121-1133, 2011
136. Rada P, Rojo AI, Evrard-Todeschi N, et al: Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/beta-TrCP axis. Mol Cellular Biol 32:3486-3499, 2012
137. Jain AK, Jaiswal AK. GSK-3beta acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. J Biol Chem 282:16502-16510, 2007
138. Salazar M, Rojo AI, Velasco D, et al: Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. J Biol Chem 281:14841-14851, 2006
139. Joo MS, Kim WD, Lee KY, et al: AMPK facilitates nuclear accumulation of Nrf2 by phosphorylating at serine 550. Mol Cellular Biol 36:1931-1942, 2016
140. Sun Z, Chin YE, Zhang DD. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Mol Cellular Biol 29:2658-2672, 2009
141. Kwak MK, Itoh K, Yamamoto M, et al: Enhanced expression of the transcription factor Nrf2 by cancer chemopreventive agents: Role of antioxidant response element-like sequences in the nrf2 promoter. Mol Cellular Biol 22:2883-2892, 2002
142. Okano Y, Nezu U, Enokida Y, et al: SNP (-617C > A) in ARE-like loci of the NRF2 gene: A new biomarker for prognosis of lung adenocarcinoma in Japanese non-smoking women. PloS One 8:e73794, 2013
143. Rushworth SA, MacEwan DJ, O'Connell MA. Lipopolysaccharide-induced expression of NAD(P)H: Quinone oxidoreductase 1 and heme oxygenase-1 protects against excessive inflammatory responses in human monocytes. J Immunol 181:6730-6737, 2008
144. Rushworth SA, Zaitseva L, Murray MY, et al: The high Nrf2 expression in human acute myeloid leukemia is driven by NF-kappaB and underlies its chemo-resistance. Blood 120:5188-5198, 2012
145. Tebay LE, Robertson H, Durant ST, et al: Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radical Biol Med 88:108-146, 2015
146. Mitsuishi Y, Taguchi K, Kawatani Y, et al: Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. Cancer Cell 22:66-79, 2012
147. Heiss EH, Schachner D, Zimmermann K, et al: Glucose availability is a decisive factor for Nrf2-mediated gene expression. Redox Biol 1:359-365, 2013
148. DeNicola GM, Chen PH, Mullarky E, et al: NRF2 regulates serine biosynthesis in non-small cell lung cancer. Nat Genet 47:1475-1481, 2015
149. Fan J, Ye J, Kamphorst JJ, et al: Quantitative flux analysis reveals folate-dependent NADPH production. Nature 510:298-302, 2014
150. Tibbetts AS, Appling DR. Compartmentalization of Mammalian folate-mediated one-carbon metabolism. Ann Rev Nutr 30:57-81, 2010
151. Lee D, Xu IM, Chiu DK, et al: Folate cycle enzyme MTHFD1L confers metabolic advantages in hepatocellular carcinoma. J Clin Investig 127:1856-1872, 2017
152. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: Glutamine metabolism to cancer therapy. Nat Rev Cancer 16:619-634, 2016
153. Carracedo A, Cantley LC, Pandolfi PP. Cancer metabolism: Fatty acid oxidation in the limelight. Nat Rev Cancer 13:227-232, 2013
154. Ito K, Carracedo A, Weiss D, et al: A PML-PPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. Nat Med 18:1350-1358, 2012
155. Gattinoni L, Lugli E, Ji Y, et al: A human memory T cell subset with stem cell-like properties. Nat Med 17:1290-1297, 2011
156. Lugli E, Dominguez MH, Gattinoni L, et al: Superior T memory stem cell persistence supports long-lived T cell memory. J Clin Investig 123:594-599, 2013
157. Carracedo A, Weiss D, Leliaert AK, et al: A metabolic prosurvival role for PML in breast cancer. J Clin Investig 122:3088-3100, 2012

158. Camarda R, Zhou AY, Kohnz RA, et al: Inhibition of fatty acid oxidation as a therapy for MYC-overexpressing triple-negative breast cancer. Nat Med 22:427-432, 2016
159. Marotta LL, Almendro V, Marusyk A, et al: The JAK2/STAT3 signaling pathway is required for growth of CD44(+)CD24(-) stem cell-like breast cancer cells in human tumors. J Clin Investig 121:2723-2735, 2011
160. Wang T, Fahrmann JF, Lee H, et al: JAK/STAT3-regulated fatty acid beta-oxidation is critical for breast cancer stem cell self-renewal and chemoresistance. Cell Metab 27:136-150. e135, 2018
161. Ji X, Wang H, Zhu J, et al: Knockdown of Nrf2 suppresses glioblastoma angiogenesis by inhibiting hypoxia-induced activation of HIF-1alpha. Int J Cancer 135:574-584, 2014
162. Ma Y, Chapman J, Levine M, et al: High-dose parenteral ascorbate enhanced chemosensitivity of ovarian cancer and reduced toxicity of chemotherapy. Sci Transl Med 6:222ra218, 2014
163. Monti DA, Mitchell E, Bazzan AJ, et al: Phase I evaluation of intravenous ascorbic acid in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. PloS One 7: e29794, 2012
164. Welsh JL, Wagner BA, van't Erve TJ, et al: Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): Results from a phase I clinical trial. Cancer Chemother Pharmacol 71:765-775, 2013
165. Schoenfeld JD, Sibenaller ZA, Mapuskar KA, et al: O2(-) and H2O2-Mediated Disruption of Fe metabolism causes the differential susceptibility of NSCLC and GBM cancer cells to pharmacological ascorbate. Cancer Cell 31:487-500. e488, 2017
166. Stockwell BR, Friedmann Angeli JP, Bayir H, et al: Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. Cell 171:273-285, 2017