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REVIEW

Reactive oxygen species-dependent signaling regulates cancer

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Abstract Historically, it has been assumed that oxidative stress contributes to tumor initiation and progression solely by inducing genomic instability. Recent studies indicate that reactive oxygen species are upregulated in tumors and can lead to aberrant induction of signaling networks that cause tumorigenesis and metastasis. Here we review the role of redox-dependent signaling pathways and transcription factors that regulate tumorigenesis.

Keywords Mitochondria · HIF · ROS · Metastasis · Tumorigenesis · NADPH oxidase

Introduction

Oxidative stress is a hallmark of many tumors and is caused by an imbalance between the generation of reactive oxygen species (ROS) and the cell's ability to clear oxidants. Uncontrolled increases in ROS can lead to direct damage of proteins, lipids, and DNA [1]. However, studies show that lower levels of ROS can activate intracellular signaling pathways [2] that lead to cellular proliferation and gene transcription [3]. ROS can potentially be carcinogenic and promote tumor progression [4]. Historically, it has been assumed that oxidative stress contributes to tumor initiation and progression by causing genomic instability [5]. However, in the past two decades studies have suggested that, in addition to causing genomic instability, ROS can also increase tumorigenesis by activating signaling pathways that regulate cellular proliferation, angiogenesis, and metastasis [6] (Fig. 1). Here we focus on ROS regulation of signaling pathways and transcription factors for tumor initiation and progression.

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Sources of reactive oxygen species

ROS are generated when oxygen is reduced resulting in the production of reactive species, including hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), and superoxide (·O_2). Superoxide can be generated both enzymatically and nonenzymatically. Superoxide dismutases (SOD1, SOD2, SOD3) convert superoxide to hydrogen peroxide. Glutathione peroxidase or catalase can convert hydrogen peroxide to water. Hydrogen peroxide and superoxide are implicated in the activation of signaling pathways [1–3]. By contrast, hydrogen peroxide can react with iron to generate hydroxyl radicals that cause DNA damage [7, 8].

NADPH oxidase is one of the best described enzymatic sources of superoxide [9, 10]. NADPH oxidase catalyzes the production of superoxide from oxygen and NADPH. NADPH oxidase was first found to be present in phagocytes [11]. Recent studies indicate that NADPH oxidase family members are found in a wide array of tissues [10] and include the Nox family members NOX1–5, and DUOX1–2. Five Nox isoforms (Nox1–5) form the basis of distinct NADPH oxidases [10]. The prototypic NADPH oxidase is composed of two membrane-bound components consisting of a catalytic Nox2 subunit (also known as

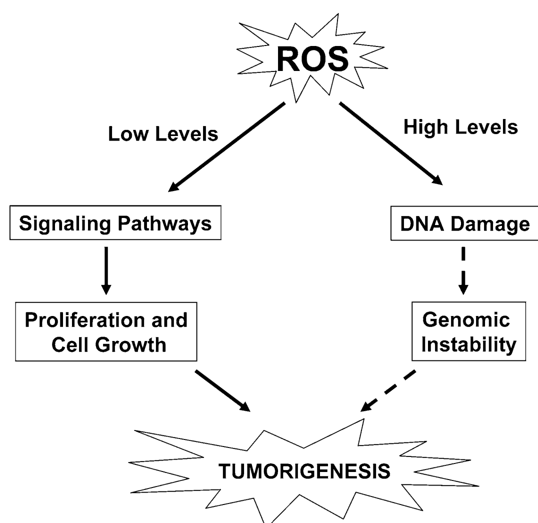

Fig. 1 ROS levels determine cellular outcomes. Induction of ROS at lower levels leads to activation of signaling pathways responsible for regulating cellular proliferation and growth. Conversely, high levels of ROS generation can lead to DNA damage, resulting in genomic instability

gp91^{phox}) and a p22^{phox} subunit. The activation of this catalytic core relies on association with several cytosolic proteins, p67^{PHOX}, p47^{PHOX}, p40^{PHOX}, and rac 1 or 2 [12]. Activation of the oxidase occurs when the cytosolic components migrate to the cell membrane so that the complete oxidase can assemble [10]. The notable exception is Nox4, which does not require p47^{PHOX}, p67^{PHOX}, or Rac for activation [13, 14]. Fibroblasts overexpressing Nox1 displayed increased levels of superoxide and exhibited a transformed phenotype in xenograft models [15]. Nox1 signals angiogenic and tumorigenic effects through hydrogen peroxide [16]. Classically, NADPH oxidases have been thought to generate superoxide at the plasma membrane and release it into the extracellular space where it is converted into hydrogen peroxide. Subsequently, the extracellular hydrogen peroxide can traverse back through plasma membranes into the cytosol. Recent evidence suggests that NADPH oxidase-generated superoxide can also occur in endosomes and endoplasmic reticulum [17, 18]. The superoxide generated in these intracellular compartments can be converted into hydrogen peroxide, which traverses the membranes of these intracellular compartments into the cytosol. NADPH oxidase signaling localized to the plasma membrane, such as the PI3 kinase pathway, likely regulates signaling associated with plasma membrane [19]. By contrast, NADPH oxidase-dependent superoxide generation in intracellular compartments regulates NF-κB-dependent transcription [17].

The electron transport chain in the mitochondria is the major site of non-enzymatic superoxide formation. The mitochondrial electron transport chain (ETC) is made up of four multi-protein complexes (I–IV) embedded in the inner membrane. Complex I and II oxidize NADH and $FADH_2$ and transfer the resulting electrons to ubiquinol, which carries electrons to complex III. Complex III shunts the electrons across the intermembrane space to cytochrome *c*, which brings electrons to complex IV. Complex IV then uses the electrons to reduce oxygen to water. Complexes I, II, and III generate superoxide as a result of electron flux through these complexes [20]. Complexes I and II generate ROS within the mitochondrial matrix while complex III generates superoxide and releases it into either the intermembrane space or the matrix [21–26]. Superoxide generated in the intermembrane space can escape into the cytoplasm through voltage-dependent anion channels [27], and these ROS can initiate intracellular signaling [28].

Oncogenes induce ROS

Many cancer cells show increased levels of ROS [29]. NIH 3T3 fibroblasts stably transformed with a constitutively active H-RasV12 produced large amounts of the ROS superoxide through Rac, a member of the Ras superfamily [30]. The mitogenic activity of NIH 3T3 fibroblasts expressing H-RasV12 was inhibited by treatment with the chemical antioxidant *N*-acetyl-L-cysteine (NAC) indicating that Ras can stimulate mitogenic signaling through production of ROS. Additionally, oncogenic K-Ras generation of hydrogen peroxide contributes to the tumorigenicity [31]. Rac can also induce ROS resulting in cell spreading and migration [32]. Collectively, these studies provide evidence that the Ras oncogene can regulate cell proliferation and cell migration.

Deregulated expression of Myc also increases production of ROS. Normal human fibroblasts expressing Myc have increased levels of ROS [33]. The increase in ROS levels in these cells correlated with an induction of DNA damage without activation of apoptosis [33]. These data suggest that Myc can deregulate the cell's DNA damage response leading to tumor progression through genetic instability. Mitochondrial-targeted vitamin E analog (a potent antioxidant) protected cells from Myc-elicited oxidative DNA damage [34]. These results suggest that Myc-induced cellular transformation is dependent on mitochondrial ROS. By contrast, c-Myc overexpression in NIH3T3 MEFs leads to ROS accumulation, which activates apoptosis [35]. Recent studies indicate that there is a specific “threshold” level of Myc expression that correlates with Myc's biological activities [36, 37]. Low levels of deregulated Myc are conducive to cell proliferation and oncogenesis, while high levels of Myc overexpression correspond to activation of apoptosis [36]. We speculate that the level of ROS induced by c-Myc could be responsible for Myc's biological output. High Myc

expression induces high levels of ROS leading to DNA damage or apoptosis, while lower Myc expression induces lower levels of ROS resulting in cellular proliferation and tumor growth.

ROS activate signaling pathways

Low or transient levels of ROS can activate cellular proliferation or survival signaling pathways, while high levels of ROS can initiate damage or cell death. ROS can activate kinases and/or inhibit phosphatases resulting in stimulation of signaling pathways [38, 39]. ROS can also regulate proteinases and matrix metalloproteins [40]. Many phospho-proteins and proteinases contain cysteine residues which, upon oxidation, result in formation of disulfide bonds leading to activation of signal transduction pathways [41, 42]. This reaction is reversible since the disulfide reductases thioredoxin (Trx) or glutaredoxin (Grx) catalyze the reversible reduction of disulfides to a dithiol within the target protein. Trx and Grx contain a common dithiol/disulfide active site motif (Cys-X-X-Cys) [43]. The reduction of disulfide results in the oxidation of Trx forming a disulfide within the Trx, which is then reduced by thioredoxin reductase using electrons from NADPH. An example of this redox-based signaling is the activation of the apoptosis signal-regulating kinase 1 (ASK1). ASK1 is bound to a reduced Trx in its inactive form. ROS oxidize Trx causing disassociation from ASK1 to initiate activation through auto-phosphorylation of ASK1 [44]. ROS also are regulators of signaling pathways such as the extracellular signal-regulated kinase (ERK) MAPK pathway, which is important for cell proliferation [45, 46], and the PI3K/Akt pathway, which is important for cell growth and survival [47, 48] and transcription factors such as hypoxia inducible factors (HIFs) [28]. In the next sections we focus on the role ROS play in the regulation of ERK, PI3K, and HIFs (Fig. 2).

Extracellular signal-regulated kinase

ERK1/2 are mitogen-activated protein kinases (MAPK) that are important in cell proliferation, differentiation, invasion, and apoptosis [49–52]. Dysregulation of the MAPK pathway can lead to uncontrolled cellular proliferation or permanent cell cycle arrest [53]. The Ras oncogene can activate the ERK MAPK pathway. Ras activates Raf, which stimulates MEK1/2, followed by activation of ERK1/2 MAPK to promote cell proliferation through the induction of cyclin D1. Multiple studies indicate that activated Ras induces an accumulation of cyclin D1 [54–56]. Ras activation of ERK1/2 is necessary and sufficient for transcriptional induction of the cyclin D1 gene [57, 58]. ERK1/2-sustained activation of cyclin D1 expression is

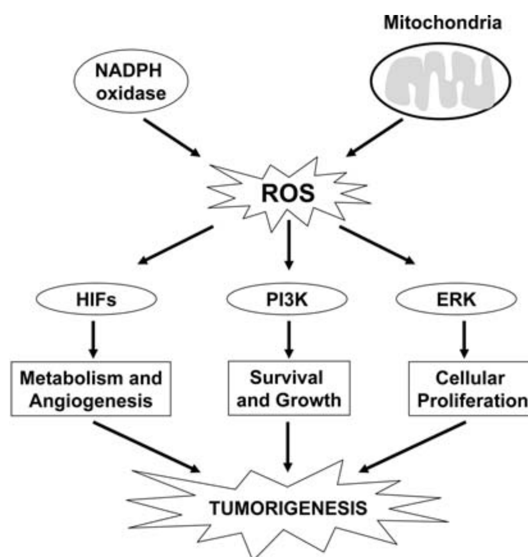

Fig. 2 ROS can activate pathways that regulate tumorigenesis. ROS generated from mitochondria or NADPH oxidases can activate PI3K or ERK MAPK signaling pathways and the transcription factors HIFs to regulate metabolism, angiogenesis, survival, cell growth, and proliferation

required throughout G1 in order to proceed to the S phase [59, 60]. Conversely, high levels of ERK1/2 activation over long periods of time can lead to cell cycle arrest [61–63]. Thus, the duration and the intensity of the ERK1/2 signal determine the outcome of the cellular response.

There is integration between the levels of ROS in a cell and the levels of MAPK signaling that are necessary for cell cycle arrest or progression. MEFs deficient in manganese superoxide dismutase (MnSOD) have increased levels of superoxide corresponding to a sustained expression of cyclin D1 and an inability to exit the proliferative phase of the cell cycle [64]. In contrast, MEFs that over-expressed MnSOD underwent quiescence and did not display elevated levels of superoxide or cyclin D1 expression [64]. Additionally, ROS can inactivate phosphatases responsible for dephosphorylating ERK1/2, resulting in sustained ERK1/2 activation [65]. The best-characterized example of ROS-mediated inactivation of a phosphatase is PTP1B, which is inhibited by the reversible ROS-mediated oxidation of an active site cysteine residue [66]. ROS levels increase throughout the cell cycle, and ablation of ROS using the antioxidant Tempol leads to late G1 cell cycle arrest [67]. Although, there is substantial evidence that ROS regulate ERK activation, the direct targets of ROS responsible for ERK activation still need to be elucidated.

Phosphoinositide 3 kinase signaling pathway

The phosphoinositide 3-kinase (PI3K) signaling pathway is required for a number of cellular processes including cell

growth, survival, proliferation, and motility [68]. Upon pathway activation, growth factor receptors activate the catalytic subunit, p110, of PI3K through recruitment of the regulatory subunit, p85, or through direct Ras activation of p110. p110 then phosphorylates phosphoinositides (PI) to generate PI (3,4,5) P3 (PIP3). Binding of PIP3 to the pleckstrin homology (PH) domain of Akt allows for translocation of Akt to the plasma membrane, where Akt is phosphorylated and activated by phosphoinositide-dependent kinase 1 (PDK1) and mTORC2 [69, 70]. Akt is negatively regulated by the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome ten), a phospholipid phosphatase that inhibits the activity of PI3K by dephosphorylating PIP3 [71]. Overexpression of Akt is associated with resistance to apoptosis, increased cell growth, and cellular proliferation [72]. Mammalian cells express three Akt genes, Akt1, Akt2, and Akt 3. Furthermore, inactivating mutations or deletions of PTEN lead to uncontrolled Akt activity and are frequently seen in human malignancies [71]. Amplification and overexpression of the gene encoding p110 is also observed in many human cancers [73]. Additionally, oncogenic Ras mutations can activate Akt in epithelial tumors [74].

The PI3K pathway can be affected by the redox state of the cell [48, 75]. Exogenous hydrogen peroxide treatment of cells results in activation of Akt [76]. The best known direct target of oxidants in the PI3K/Akt pathway is PTEN. ROS can oxidize cysteine residues of protein tyrosine phosphatases (PTPs) resulting in their inactivation [38]. PTEN is inactivated by hydrogen peroxide through the formation of a disulfide between the active site cysteine (Cys^{124}) and a vicinal cysteine (Cys^{71}) [77, 78]. Mitochondrial-generated ROS can also inhibit PTEN resulting in endothelial cell sprouting in a three-dimensional in vitro angiogenesis assay [79]. Other phosphatases such as protein phosphatase 2A (PP2A) are also redox sensitive and can regulate the PI3K/Akt pathway [80]. PP2A can dephosphorylate Akt on both threonine 308 and serine 493, thereby inactivating Akt activity [81]. The consequence of inhibiting these important phosphatases is a dysregulation of Akt signaling leading to uncontrolled cellular proliferation, enhanced survival, and growth.

ROS activate hypoxia inducible factors

Hypoxia activates a family of transcription factors called hypoxia inducible factors (HIFs). HIFs are a heterodimer consisting of a constitutively stable subunit, HIFβ, and an oxygen sensitive subunit, HIFα [82]. There are three HIFα isoforms, HIF-1α, HIF-2α, and HIF-3α. Under normal oxygen conditions (21% O_2), HIFα is hydroxylated at proline residues by prolyl hydroxylases (PHDs) [83, 84]. Hydroxylated proline residues of HIFα protein are recognized by the E3 ubiquitin ligase von Hippel-Landau protein (pVHL), which targets HIFα to the proteosome [85–87]. Oxygen tension also regulates the interaction of HIF-1α with the transcriptional co-activators p300 and CBP. Asparagine hydroxylation of residue 803 of HIF-1α by the enzyme FIH-1 (factor inhibiting HIF-1) blocks the binding of p300 and CBP to HIF-1α, thus inhibiting HIF-1-mediated gene transcription [88–90]. Under hypoxia, HIFα is not hydroxylated by PHDs and FIH, which prevents pVHL targeting to the proteosome, allowing HIFα to translocate to the nucleus and dimerize with HIFβ. Moreover, p300 and CBP can then be recruited to the HIF complex allowing transcriptional activation of HIF target genes. HIF-1α and HIF-2α have been implicated in regulating tumorigenesis by controlling genes involved in metabolism, angiogenesis, and metastasis [91–93].

Hypoxia increases mitochondrial ROS to stabilize HIFα [94]. Early studies demonstrated that cells depleted of mitochondrial DNA by treatment with ethidium bromide (ρ cells) failed to increase ROS and stabilize HIF-1α during hypoxia [95]. ρ Cells are respiratory incompetent and do not generate ROS [96]. Antioxidants also prevented hypoxic stabilization of HIF-1α [97]. ETC inhibitors such as rotenone, myxothiazol, and stigmatellin can block hypoxic stabilization of HIF-1α [95, 97, 98]. Additionally, cells depleted of cytochrome *c* or the Rieske–Fe–S protein (RISP), a complex III subunit, fail to increase ROS production during hypoxia and do not stabilize HIF-1α protein under hypoxic conditions [99–101].

Recently, we demonstrated that complex III is the site of ROS generation during hypoxia responsible for stabilizing HIF-1α protein [102]. Complex III is made up of 11 subunits, 3 of which are responsible for electron transport (Rieske–Fe–S protein, cytochrome *b*, and cytochrome c_1). Two electrons travel from ubiquinol to cytochrome *c* through the ubiquinone (Q) cycle within complex III [103]. One electron from ubiquinol is transferred to the Rieske–Fe–S/cytochrome c_1 subunits, resulting in the transient formation of the radical ubisemiquinone, which ultimately passes the second electron to cytochrome *b*. Ubisemiquinone can pass an electron to oxygen to produce superoxide at the Qo site of complex III. Thus, cells depleted of RISP cannot initiate the Q-cycle and generate ROS, while cells depleted of cytochrome *b* can still generate ROS because ubisemiquinone can be generated. Indeed, we found that cells deficient in cytochrome *b* can generate ROS during hypoxia and stabilize HIF-1α protein [102] suggesting that ROS generation during hypoxia is dependent on oxygen-induced changes in the redox state of the electron transport chain. In contrast, depletion of RISP does not allow ROS generation or stabilization of HIF-1α protein in cells deficient in cytochrome *b* during hypoxia. Although there is strong genetic evidence to indicate that complex III

participates in ROS generation during hypoxia to stabilize HIF-1α protein, the mechanism by which hypoxia stimulates ROS is not known.

ROS regulate tumorigenesis

A long-standing dogma in cancer is that the elevated ROS levels observed in tumors increase genomic instability resulting in progression of cancer [5, 104]. While the high levels of ROS can cause direct damage to the genome, lower levels of ROS might induce genomic instability through regulating signaling pathways. It is also possible that ROS may not regulate genomic instability at all in certain tumors but may regulate induction of genes that are required for tumorigenicity. In support of this premise, Chi Dang and colleagues recently demonstrated that in various MYC-driven cancers, the ability of commonly utilized antioxidants vitamin C and *N*-acetylcysteine to prevent tumorigenesis was due to diminishing HIF activity [105]. These investigators did not detect any genomic instability in Myc-driven cancers [105]. These results are consistent with the observation that overexpression of MnSOD prevents tumorigenesis of human melanoma cells and breast cancer cells in nude mice [106, 107] and MnSOD decreases hypoxic activation of HIF-1 [108]. These results are reinforced by the observation that NAC prevents tumorigenesis in a mouse model of constitutively active Rac1-driven Kaposi's sarcoma by inhibiting AKT activation, HIF-1α protein induction, VEGF expression, and angiogenesis [109]. Thus, ROS activation of HIFs is likely another mechanism to induce tumorigenicity.

The confusion regarding ROS regulation of tumorigenesis comes from the observations that ROS can induce senescence or apoptosis. Tumor suppressors such as p53 engage senescence or apoptosis to limit tumor growth [110]. This raises the question as to why ROS do not trigger senescence or apoptosis in tumor cells. A plausible explanation is that tumor cells have mechanisms that evade ROS induction of senescence or apoptosis. Activation of Akt is sufficient to induce $p19^{ARF}$ and p16, p53 phosphorylation, and premature senescence [111]. In contrast, cells deficient in AKT were more resistant to oncogene-induced senescence [111]. These results suggest that the ROS are likely to be beneficial to cells with an activated oncogene only if the pathways of senescence are impaired. Indeed the loss of PTEN (increasing AKT activation) promotes senescence in a mouse model of prostate cancer, and invasive prostate cancer arises only when p53 is lost [112]. ROS induction of apoptosis would also need to be impaired for tumorigenesis. One mechanism that tumor cells utilize to evade ROS-induced apoptosis is to disengage ROS activation of p38α MAPK pathway [113]. ROS-stimulated p38 MAPK activity acts to induce apoptosis in wild-type MEFs. In contrast, MEFs deficient in p38α were resistant to ROS-induced apoptosis [113]. Interestingly, human cancer cell lines with high levels of ROS have impaired p38α activation [113]. Collectively, these studies indicate that tumor cells impair the p38α MAPK pathway and p53 pathway to evade ROS-dependent apoptosis and senescence, respectively, in order for tumorigenesis to proceed (Fig. 3).

Although the increase in ROS levels observed in cancer cells is causal for tumorigenesis, it might also provide a therapeutic potential to kill tumor cells. β-Phenylethyl isothiocyanate (PEITC), a natural compound known to increase intracellular ROS levels [114], preferentially killed ovarian epithelial cells transformed with the H-Ras(V12) oncogene or expression of Bcr-Abl oncogene in hematopoietic cells but not normal cell lines [115]. PEITC in vivo prolonged survival of mice injected with Ras-transformed ovarian epithelial cells suggesting that modulating ROS levels provides a potential therapeutic target for cancer. These findings were corroborated by the observation that mice injected with an ovarian cancer cell line expressing Akt activation showed reduction in tumor size after treatment with rapamycin and PEITC [111]. Rapamycin analogs are currently being used in clinical trials. One concern in using rapamycin analogs for cancer therapy is that they cause hyperactivation of Akt, which promotes resistance to therapeutic agents that induce apoptosis. The hyperactivation of AKT by rapamycin is unable to prevent oxidant-induced cell death by PEITC in cancer cells. Thus, PEITC or other compounds that promote ROS generation could be exploited for eradication of tumors with elevated AKT.

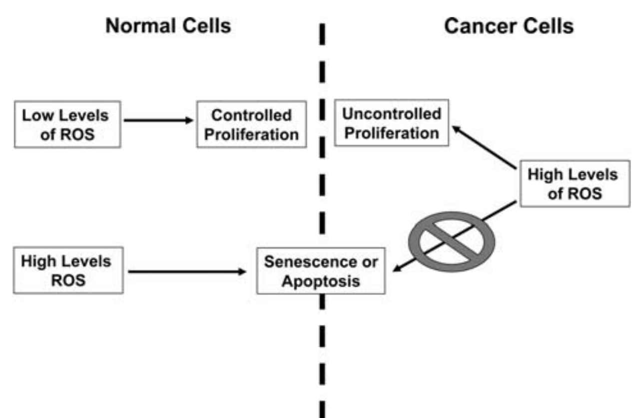

Fig. 3 ROS signaling in normal versus cancer cells. In normal cells, low levels of ROS can regulate controlled proliferation while high levels of ROS lead to cell death or senescence. Cancer cells with mutations in tumor suppressors can evade ROS induction of cell death or senescence. Thus, cancer cells exhibit high levels of ROS leading to uncontrolled cellular proliferation

ROS regulate metastasis

Metastasis involves the displacement of tumor cells from their primary residence to the lymphatic and blood vessels where they circulate and eventually repopulate within normal tissue elsewhere in the body [116]. Epithelial tumor cells undergo a mesenchymal-like transition (EMT) during initiation of metastasis, which is characterized by loss of cell adhesion, repression of E-cadherin, and increased cell mobility [117]. Matrix metalloproteinases (MMPs) help detach primary tumor cells from extracellular matrix. Matrix metalloproteinases such as MMP3 can also cause EMT in cultured cells [118, 119]. ROS can both activate and induce gene expression of MMPs [120, 121]. Conversely, MMP3 induces expression of an alternately spliced form of Rac1b, which causes an increase in ROS production in mouse mammary epithelial cells [122]. The increase in ROS stimulates expression of the transcription factor Snail, which is sufficient to induce EMT [122]. Thus, ROS activation of EMT likely contributes to metastasis (Fig. 4).

The other major regulator of metastasis is the hypoxic microenvironment [123]. The degree of hypoxia positively correlates with metastasis [124, 125]. Hypoxia stimulates EMT through multiple mechanisms including upregulation of HIF-1α, hepatocyte growth factor, Snail, and Twist [126]. Hypoxia in multiple cancer cell lines induces EMT by inhibiting the activity of GSK3β through generation of mitochondrial ROS [124]. This results in the upregulation of the EMT-inducing transcription factor Snail [127].

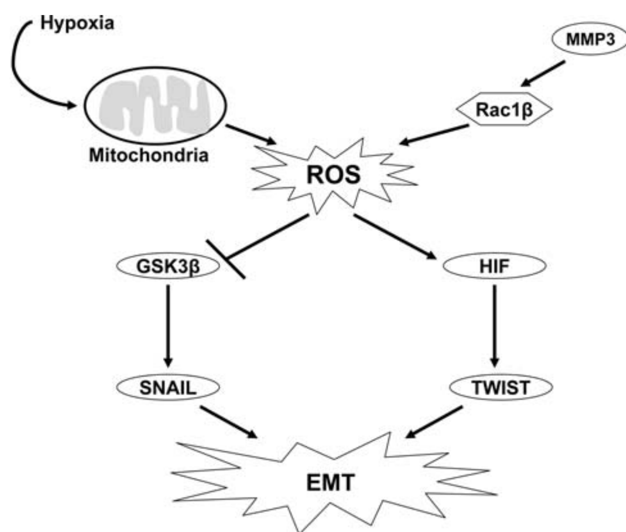

Fig. 4 ROS can induce tumor metastasis. ROS generated from the mitochondria under hypoxic conditions can activate HIF-1α, which can lead to subsequent activation of the transcription factor, TWIST, resulting in EMT. Mitochondrial ROS during hypoxia can inhibit GSK3β, resulting in upregulation of SNAIL. ROS are also generated from an alternate spliced variant of Rac1β, which leads to activation of the transcription factor, SNAIL, resulting in EMT

Additionally, ROS can modulate β-catenin signaling, which may provide a further mechanism in ROS regulation of EMT [128, 129]. To the extent that the mitochondrial ROS are required for activation of HIFs and inhibition of GSK3β, one can speculate that mitochondrial ROS are likely to be important regulators of metastasis.

Mutations in mitochondrial proteins increase tumorigenicity

Somatic mitochondrial DNA (mtDNA) mutations have been observed in primary human cancers, but whether they are causal in tumorigenesis is not firmly established [130, 131]. Prostate cancers display a variety of mutations within the mitochondrial genome [132]. To test whether these mutations can increase tumorigenicity, Wallace and colleagues introduced mutations of ATP6, a component of complex V encoded by mitochondrial DNA, into the PC3 prostate cancer cell line. PC3 cells harboring the mutant ATP6 T8993G generated tumors seven times larger than PC3 cells harboring wild-type ATP6 [132]. Interestingly, this mutation increased ROS. However, the investigators did not test whether the increase in ROS due to the mutation was responsible for tumorigenesis [132]. The best evidence that mitochondrial mutations enhance tumorigenicity due to an increase in ROS comes from a case in which mtDNA from a mouse tumor cell line with poorly metastatic potential was replaced with mtDNA from a highly metastatic tumor cell line. The recipient tumor cells acquired the highly metastatic potential of the transferred mtDNA. The highly metastatic mtDNA exhibited complex I deficiency due to a mutation in NADH dehydrogenase subunit 6 (ND6), a subunit of complex I, which increased ROS production [133]. Furthermore, NAC inhibited the metastatic potential of these highly metastatic tumor cells indicating that mitochondrial mutations can increase metastasis through ROS [133].

Nuclear mutations of mitochondrial proteins in addition to mutations in mitochondrial DNA are associated with increase tumorigenicity [134]. Paraganglioma tumor cells harbor germline mutations in genes that encode the succinate dehydrogenase (SDH) proteins. SDH is the only membrane-bound enzyme of the TCA cycle and is also a functional member (complex II) of the electron transport chain (ETC). SDH is a complex of four different polypeptides (SDHA, SDHB, SDHC, and SDHD) and several prosthetic groups that include FAD, non-haem iron (iron–sulphur centers), ubiquinone, and haem$_b$ [135]. Mutations in *SDHB*, *SDHC*, or *SDHD* genes have been associated with paraganglioma [136–138]. Loss of SDHD elevates HIF-1 under normoxia [139]. Interestingly, based on the structure and mechanism of complex II, it is

predicted that mutations in *SDHB*, *SDHC*, or *SDHD* would increase ROS generation while mutations in *SDHA* would not [132]. Indeed, loss of SDHA protein by RNAi does not increase ROS, HIF activation, or tumorigenicity [140]. In contrast, loss of SDHB increases ROS production under normoxia triggering HIF-dependent tumorigenicity [140]. It is likely that the increase in ROS coupled with an increase in succinate levels cooperate to activate HIFs under normoxia [141]. The build-up of succinate would prevent the forward HIFα hydroxylation reaction. Recent evidence suggests mutations in the TCA cycle fumarate hydratase (FH) that are associated with hereditary leiomyomatosis and renal cell cancer (HLRCC) also exhibit a ROS-dependent activation of HIF [142]. As more metabolic mutations are uncovered, we predict that loss of function mutations of mitochondrial proteins that increase ROS will increase tumorigenicity.

Conclusions

It is well documented that ROS are upregulated in tumors and can lead to aberrant induction of signaling networks that cause tumorigenesis and metastasis. Recent studies show that ROS have specific signaling pathways and transcription factors that play an important role in the initiation and progression of cancer. Antioxidants prevent tumorigenicity in part by preventing the activation of the transcription factor HIFs. The elevated ROS observed in tumor cells also represent a paradoxical therapeutic potential. Drugs that cause mild oxidative stress in normal cells are likely to further enhance ROS levels in cancer cells resulting in their death. The future is to design better drugs that work as antioxidants and prooxidants for cancer therapy as well elucidate the biological targets of ROS.

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