The role of ROS in tumour development and progression

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Abstract | Eukaryotic cells have developed complex systems to regulate the production and response to reactive oxygen species (ROS). Different ROS control diverse aspects of cell behaviour from signalling to death, and deregulation of ROS production and ROS limitation pathways are common features of cancer cells. ROS also function to modulate the tumour environment, affecting the various stromal cells that provide metabolic support, a blood supply and immune responses to the tumour. Although it is clear that ROS play important roles during tumorigenesis, it has been difficult to reliably predict the effect of ROS modulating therapies. We now understand that the responses to ROS are highly complex and dependent on multiple factors, including the types, levels, localization and persistence of ROS, as well as the origin, environment and stage of the tumours themselves. This increasing understanding of the complexity of ROS in malignancies will be key to unlocking the potential of ROS-targeting therapies for cancer treatment.

Reactive oxygen species
(ROS). Unstable and reactive molecules that originate from oxygen during cellular metabolism.

NADPH
A reduced form of $NADP^+$, the reducing agent for many anabolic reactions and regenerating antioxidants. Also used for generating reactive oxygen species (ROS) via NADPH oxidase (NOX).

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Aerobic respiration is a highly efficient means of energy production that has supported the development of all eukaryotes. However, a by-product of aerobic respiration is the generation of reactive oxygen species (ROS) that can be toxic to DNA, proteins and lipids. Numerous mechanisms that limit ROS have developed to protect cells from oxidative damage — but ROS have also adapted to serve as signalling molecules to support many aspects of cell behaviour. ROS can therefore be both essential and lethal, and the various responses to ROS are important to normal physiology and in the development of many diseases, including cancer. ROS impact the behaviour of both cancer cells and the stromal components of the tumour to modulate cancer development and survival. In this Review, we examine the different characteristics of ROS in cancer biology and highlight some of the diverse and complex roles of ROS in tumour and stromal cells at different stages of cancer development.

ROS production and control

Cells can produce ROS through numerous mechanisms, which are summarized in BOX 1 and described in more detail in other reviews[1,2]. Importantly, although there is a tendency to think of ROS as a single entity, different ROS can have very different targets and activities. For example, hydrogen peroxide (H_2O_2) plays a key role in signalling through its ability to selectively modify and regulate the function of many proteins, whereas other forms of ROS are more likely to lead to damage and toxicity. These include the ability of superoxide ($O_2^{\cdot -}$) to damage iron cluster proteins, and highly reactive hydroxyl radicals ($^{\cdot}OH$), which irreversibly damage proteins, DNA and lipids, leading to cell death[3,4]. Peroxynitrite ($ONOO^-$) also causes lipid and DNA damage as well as nitration of various amino acids to alter protein function[5]. We discuss the consequences of these functions of ROS on tumorigenesis more fully below.

ROS-regulating systems

To control ROS, cells possess various antioxidant systems such as superoxide dismutases (SODs), which convert $O_2^{\cdot -}$ to H_2O_2, and multiple enzymes that convert H_2O_2 to water, including catalase (CAT), peroxiredoxins (PRDXs) and glutathione peroxidases (GPXs)[2] (FIG. 1). Cofactors for the PRDX and GPX-catalysed reactions are reduced thioredoxin (TRX) and reduced glutathione (GSH), respectively, and glutathione-*S*-transferases (GSTs) also use GSH to detoxify reactive compounds produced by oxidative stress[6,7]. GSH (the most abundant endogenous antioxidant) and TRX are regenerated by reductases using the cofactor NADPH as an electron donor. NADPH is therefore essential for the activity of these antioxidant defence mechanisms and several pathways in mammalian cells allow for the regeneration of NADPH from $NADP^+$ (REFS[8–10]) (FIG. 2). The production of NADPH can be further supplemented by the de novo synthesis of $NADP^+$ by NAD^+ kinase (NADK)[11–14] and limited by the NADPH phosphatases such as the cytosolic MESH1 and the mitochondrial Nocturnin[15,16].

Although detoxification of ROS is often conceptualized as a linear path, the interplay between various

Box 1 | **Intracellular ROS generation**

Cells are exposed to numerous species of reactive oxygen species (ROS), including superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH)[269,270]. Although ROS can be generated through several mechanisms, the main sources of endogenous ROS are mitochondrial metabolism, peroxisomes and the activity of the family of transmembrane NADPH oxidases (NOXs)[26,51,271] (FIG. 1). In the mitochondria, several sites in the respiratory chain — including complex 1, complex 3 and oxoglutarate dehydrogenase — donate electrons to molecular oxygen and form $O_2^{\cdot-}$ radicals[51,269]. $O_2^{\cdot-}$ released into the mitochondrial matrix or the cytoplasm is rapidly converted to H_2O_2 but can also react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$). Through the iron-dependent Fenton reaction, H_2O_2 also produces ·OH. Similar to mitochondria, peroxisomes are high consumers of oxygen and whereas they do not generate ATP, they produce ROS, principally as H_2O_2. The NADPH oxidases (NADPH oxidase (NOX)/dual oxidase (DUOX) family) produce $O_2^{\cdot-}$ that — depending on membrane localization — is released into the intracellular or extracellular space, where it can be converted to H_2O_2. One member of this family — NOX4 — produces H_2O_2 directly[272]. The endoplasmic reticulum also generates ROS during oxidative protein folding through the activity of endoplasmic reticulum oxidoreductase 1 (ERO1) and protein disulfide isomerase (PDI) to catalyse protein disulphide formation[273], and also during prolonged endoplasmic reticulum stress as a response to the accumulation of misfolded proteins[274].

antioxidant pathways plays an important role in the ultimate outcome following redox stress. For example, although the SODs are considered part of an antioxidant response, without additional activity of GPX or other enzymes that catalyse the conversion of H_2O_2, the activity of SODs alone in response to a rising level of oxidative radicals can increase H_2O_2 levels. This could lead to an increase in H_2O_2-mediated signalling or lead to the sensitization of cells to further oxidative stress[17,18]. Furthermore, NADPH is required for many cellular processes beyond ROS detoxification, including anabolic reactions that drive fatty acid, proline and nucleotide synthesis[8,19–21], and NADPH is also used by NADPH oxidases (NOXs) to generate ROS (BOX 1). Consequently, pathways maintaining NADPH homeostasis are critical to balance the production and use of NADPH to meet these disparate demands[10,22].

Finally, mitochondrial ROS (mtROS) can be limited by the process of mitophagy, which removes damaged ROS-producing mitochondria through targeted autophagy[23]. Of note, however, in some situations the induction of mitophagy has been shown to increase ROS production[24].

Spatial control of ROS

Another way in which ROS can be regulated is to control their localization within the cell, and several mechanisms exist to compartmentalize ROS-producing and ROS-degrading systems. The NOXs are present in multiple subcellular locations, including the plasma membrane, endoplasmic reticulum, peroxisomes and mitochondria. Isoforms of the antioxidants PRDX, GPX and GST also show distinct subcellular localization, and organelles such as mitochondria and peroxisomes harbour numerous other ROS regulating enzymes (FIG. 1). NADPH is produced in specific subcellular locations (FIG. 2) — by the oxidative pentose phosphate pathway (oxPPP) in the cytosol, by different isoforms of malic enzymes, isocitrate dehydrogenases (IDHs) and enzymes of the one-carbon cycle that are localized to both the cytosol and mitochondria, and by mitochondrial enzymes such as nicotinamide nucleotide transhydrogenase (NNT) and glutamate dehydrogenase 1 (GLUD1) and GLUD2 (REFS[10,25]). Compartmentalized regulation of NADPH may also be achieved by subcellular localization of NADKs and NADPH phosphatases[26,27]. Although NADPH itself is unable to cross the inner mitochondrial membrane, IDH-dependent shuttling allows the exchange of cytosolic and mitochondrial NADPH reducing equivalents[22].

Further mechanisms to localize ROS and allow for a restricted response include the control of the location of mitochondria. The trafficking of mitochondria to different subcellular locations can affect signalling output[28] and selective fragmentation of mitochondria at sites of damage allows ROS-dependent signalling for repair[29]. Additionally, ROS produced in one location within the cell can signal ROS production in another compartment, with reciprocal crosstalk between mtROS and membrane ROS[30]. ROS regulating systems can also be relocated in response to certain stimuli to allow localized and selective responses. For example, NOX proteins are targeted to lamellipodia and membrane ruffles to provide the localized ROS necessary for directional migration[31,32]. Similarly, mitochondria can be redistributed to these regions of the cell to provide energy for migration and invasion[33].

ROS and the development of cancer

The powerful and potentially dangerous functions of ROS play critical roles under many conditions that drive abnormal cell behaviour, such as cancer. As discussed below, many tumour promoting events — including activation of oncogenes, loss of tumour suppressor function, changes in mitochondrial activity, increased hypoxia and altered stromal interactions — can promote ROS production. Indeed, cancer cells have been shown to carry more ROS than their normal counterparts[34]. However, the consequences of these increases in ROS can be very different, with evidence that they both support and inhibit malignant behaviour.

Tumour-promoting functions of ROS

There are several ways in which increased ROS contribute to tumour development and the enhanced ROS driven by oncogenic perturbations can be required for tumorigenicity[35]. Whereas the damaging effects of ROS can be detrimental to cell survival, the acquisition of DNA damage and genomic instability can drive the accumulation of oncogenic alterations that promote cancer development[36,37]. More directly, the H_2O_2 derived from membrane and mitochondrial sources can reversibly oxidize cysteine residues in proteins, thereby controlling their activity in a manner analogous to other post-translational modifications such as phosphorylation[38]. Improved detection has identified reversible oxidation of thousands of proteins[38–40], including those involved in signalling pathways that are well-established mediators of cancer cell survival, proliferation, metabolism, invasion and metastasis. Responses to ROS that modulate epigenetic regulation of gene expression by modifying the activity of DNA

Fenton reaction
The formation of hydroxyl radicals (· OH) and hydroperoxyl radicals from hydrogen peroxide (H_2O_2) and Fe^{2+} ions.

Mitophagy
The selective removal of dysfunctional mitochondria. Mitophagy is a type of autophagy, which is a cell intrinsic mechanism that removes and recycles unnecessary or dysfunctional cellular components and promotes short-term survival during starvation or repair during stress.

Peroxisomes
Small membrane-bound organelles that contain several reactive oxygen species (ROS)-producing and ROS-degrading enzymes for various oxidation and lipid biosynthesis reactions.

Oxidative pentose phosphate pathway
(oxPPP). An arm of the metabolic pathway that branches from glycolysis, generating NADPH and nucleotides.

Genomic instability
A high frequency of DNA mutations, chromosomal rearrangements or aneuploidy frequently seen during tumorigenesis.

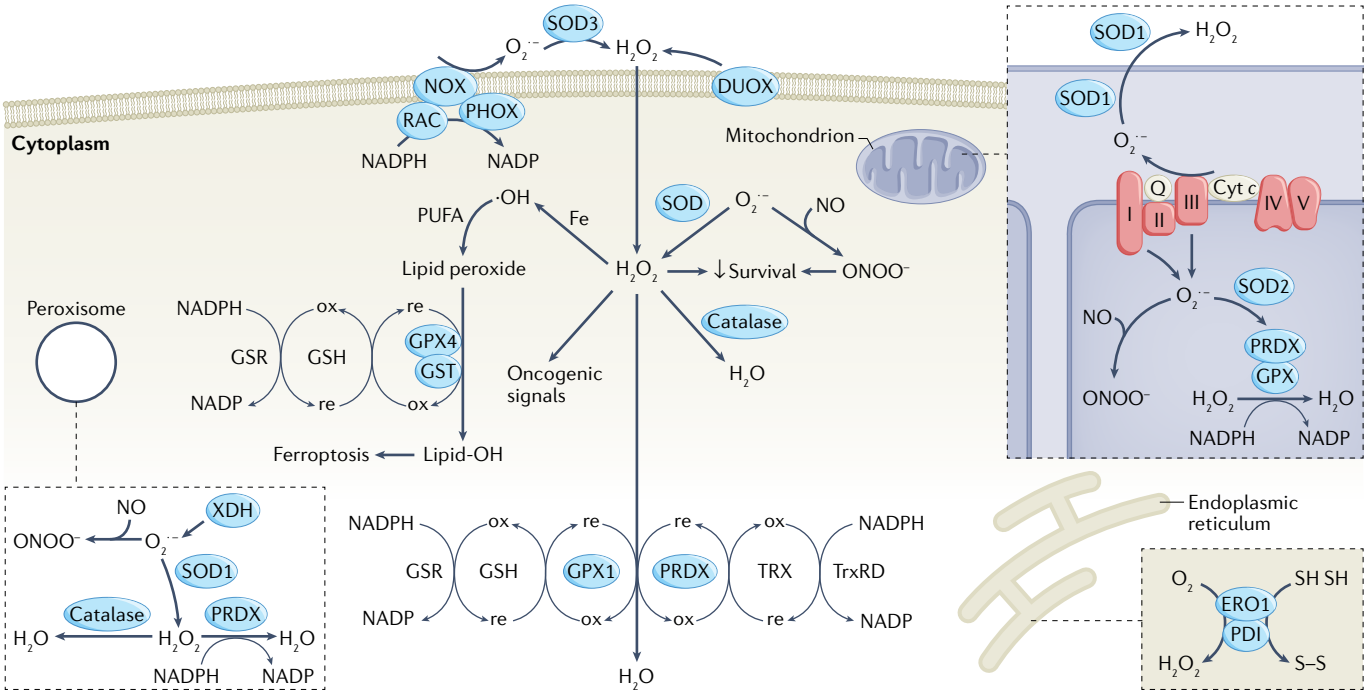

Fig. 1 | **Generation and metabolism of ROS.** Membrane reactive oxygen species (ROS) are generated by the NADPH oxidase (NOX)/dual oxidase (DUOX) system (which includes factors such as RAC and phagocytic oxidase (PHOX)) on the plasma membrane. These systems generate superoxide ($O_2^{\cdot-}$) and, subsequently, hydrogen peroxide (H_2O_2) through the activity of superoxide dismutase 3 (SOD3). Mitochondrial complex I and III generate $O_2^{\cdot-}$, which is metabolized to H_2O_2 by SOD2 in the matrix or SOD1 in the intermembrane space or outer mitochondrial membrane. The iron (Fe)-dependent Fenton pathway generates hydroxyl radicals (·OH) from H_2O_2. Highly active ·OH can form lipid peroxide, especially from polyunsaturated fatty acids (PUFAs). Antioxidant pathways include the glutathione peroxidase (GPX)/glutathione reductase (GSR) systems in the cytosol and mitochondria, which use reduced glutathione (GSH) (regenerated using NADPH) to convert H_2O_2 (GPX1) or lipid peroxide (GPX4); the peroxiredoxin (PRDX)/thioredoxin reductase (TrxRD) systems in the cytosol or mitochondria, which convert H_2O_2, using thioredoxin (TRX) and NADPH; catalase (CAT), which can also hydrolyse H_2O_2 to water; and glutathione-S-transferases (GSTs), which detoxify reactive compounds by conjugating GSH to them. Many components of the membrane and mitochondrial ROS generating and scavenging systems are altered in cancers, leading to both increased and decreased ROS. These result in a wide range of pro-tumour or antitumour responses, depending on the context. Cyt *c*, cytochrome *c*; ERO1, endoplasmic reticulum oxidoreductase 1; NO, nitric oxide; $ONOO^-$, peroxynitrite; ox, oxidation; PDI, protein disulfide isomerase; re, reduction; XDH, xanthine dehydrogenase/oxidase.

methyltransferases (DNMTs) or histone deacetylases (HDACs), or hypomethylation due to oxidized DNA damage, may also promote oncogenic transformation[41].

One well-established ROS regulator with clear oncogenic activity is RAC1, a small GTPase that functions to drive cell proliferation, survival and motility — in part by contributing to the activation of NOX at the plasma membrane[42–44]. In a mouse model of intestinal adenocarcinoma, RAC1 is activated after loss of the tumour suppressor adenomatous polyposis coli (APC) and is required for tumorigenesis — a response that depends on the production of ROS through NOX1 (REF.[45]). RAC1B — a constitutively active form of RAC1 (REF.[46]) — and constitutively active mutants of RAC1 have been implicated in the development of melanoma and lung cancer[47–49]. mtROS also contribute to cancer development and, intriguingly, an early study suggested that RAC1B expression drives malignant transformation by increasing mtROS[36]. ROS produced by the mitochondria can also regulate many signalling pathways that promote the acquisition of oncogenic phenotypes[37,50]. Unexpectedly, a recent report showed that H_2O_2 does not diffuse out of the mitochondria, suggesting that the production and export of mitochondrial $O_2^{\cdot-}$ is key to the control of cytosolic signalling pathways[51]. Of course, mitochondrial H_2O_2 may play a direct role in the modification and regulation of mitochondrial proteins. mtROS are required for lung cancer development[35] and an increase in mitochondrial $O_2^{\cdot-}$ levels resulting from SIRT3 (deacetylase in mitochondria) deficiency enables cells to become more susceptible to transformation and, subsequently, promotes the development of mammary tumours in mice[52]. Interestingly, the ability of mtROS to promote the survival and proliferation of cancer cells is specifically supported by mitochondrial complex I activity[53].

Failure of the mechanisms that limit ROS can also increase tumorigenesis. Mice deficient in PRDX1 or either SOD1 or SOD2 show an increase in several types of malignancies[54–58], whereas high expression of MnSOD (mitochondrial) limits tumour development in a mouse model of T cell lymphoma[59]. Deletion of one or more Gpx genes (*Gpx1*, *Gpx2* and *Gpx3*) also enhances the susceptibility of mice to cancer development[60], and GPX3 downregulation is often seen in human cancer, consistent with tumour suppressor functions[61]. A role for increased expression of the GSTs in cancer development and drug resistance has also been described[7,62].

Tumour-suppressing functions of ROS

In contrast to the tumour-promoting effects of ROS discussed above, increased oxidative damage and enhanced ROS-dependent death signalling can also effectively prevent some steps in tumorigenesis. Indeed, it has become clear that the increased oxidative stress burden associated with malignant progression leads to a dependence of tumour cells on the induction of antioxidant

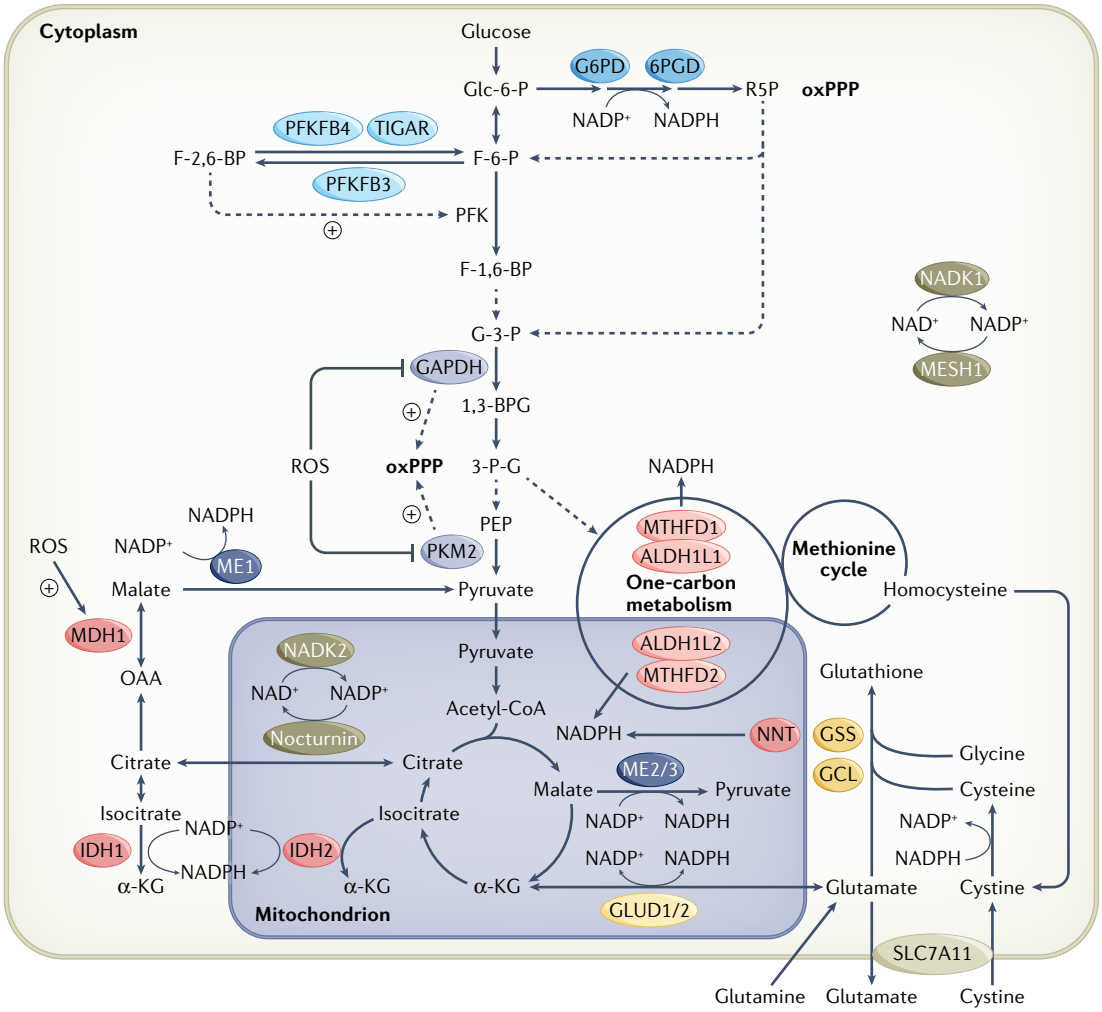

Fig. 2 | **NADPH and glutathione metabolism.** Principal pathways for generating NADPH, the reduced form of NADP+ used as a reducing agent for antioxidant reactions. The oxidative pentose phosphate pathway (oxPPP) diverts glucose-6-phosphate (Glc-6-P) from the glycolytic pathway, using the enzymes glucose-6-phosphate 1-dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) to generate NADPH. Enzymes such as 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3), PFKFB4 and TIGAR regulate the activity of phosphofructokinase (PFK) by controlling the level of fructose-2,6-bisphosphate (F-2,6-BP), an allosteric activator (indicated by +) of PFK. Decreased activity of PFK leads to accumulation of fructose 6-phosphate (F-6-P), allowing increased flux into the oxPPP. Similarly, reactive oxygen species (ROS)-induced inhibition of the glycolytic enzymes pyruvate kinase isoform M2 (PKM2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) limits flux through glycolysis, and thereby increases flow through the oxPPP. Malic enzymes located in the cytosol (ME1) and mitochondria (ME2 and ME3) generate NADPH from the conversion of malate to pyruvate. Isocitrate dehydrogenases (IDHs) located in the cytosol (IDH1) or mitochondria (IDH2) generate NADPH from the conversion of isocitrate to α-ketoglutarate (α-KG). Folate-mediated one-carbon metabolism generates NADPH in either the cytosol through methylenetetrahydrofolate dehydrogenase (MTHFD1) and aldehyde dehydrogenase family 1 member L1 (ALDH1L1) activity or the mitochondria through MTHFD2 and ALDH1L2 activity. Glutathione is synthesized from glycine, glutamate and cysteine, by the enzyme glutamate-cysteine ligase (GCL), composed of GCL catalytic subunit (GCLC) and GCL modifier subunit (GCLM) and glutathione synthase (GSS). Cysteine is derived from cystine, which can either be imported by solute carrier family 7 member 11 (SLC7A11; also known as xCT) or produced by the methionine cycle, which is coupled to one-carbon metabolism. Production of cysteine from cystine consumes NADPH. Import of cystine requires export of glutamate, which in turn impacts glutamine metabolism and reductive carboxylation. These enzymes have been shown to have important roles in modulating cancer development as discussed in the main text. 1,3-BPG, 1,3-bisphosphoglyceric acid; 3-P-G, 3-phosphoglyceric acid; acetyl-CoA, acetyl-coenzyme A; G-3-P, glyceraldehyde 3-phosphate; GLUD, glutamate dehydrogenase; MDH1, malate dehydrogenase 1; NADK, NAD+ kinase; NNT, NADP transhydrogenase; OAA, oxaloacetate; PEP, phosphoenolpyruvic acid; R5P, ribose 5-phosphate.

Ferroptosis
A type of cell death that is dependent on iron and reactive oxygen species (ROS), promoting a toxic accumulation of oxidized lipids.

Lipid peroxidation
A chain reaction leading to the oxidation of lipids by oxidants that reacts with carbon–carbon double bonds, resulting in damaged membranes and cell death.

Cystine
An amino acid produced by the oxidation of two cysteine molecules, which are connected through a disulfide bond, and the predominant form of cysteine in cell culture media and the extracellular space.

defence mechanisms, which allows them to tolerate the deleterious effects of increased ROS[1,63,64]. ROS accumulation can provoke senescence and several forms of cell death, with recent interest focusing on ferroptosis, a form of cell death that is induced by iron-dependent lipid peroxidation resulting from both membrane ROS and mtROS[65]. Ferroptosis is limited by GPX4, which uses GSH to reduce lipid hydroperoxides, and various cancers show increased sensitivity to GPX4 inhibitors compared with normal cells or tissue[66,67]. GST may also help to detoxify peroxidized lipids and so could limit ferroptosis[68]. Importantly, the acquisition of resistance to therapy is associated with increased ROS and a higher sensitivity to ferroptosis, making these cancer cells more susceptible to loss of GPX4 function[69]. Although ferroptosis generally leads to the elimination of cancer cells and so reduces malignant progression, a recent study has suggested that the induction of ferroptosis and subsequent macrophage infiltration can also help to promote the progression of pancreatic cancer in mice[70].

Antioxidant defence mechanisms

Taken together, there is considerable evidence to show that increased ROS can both promote the acquisition of oncogenic phenotypes and also limit tumour development by provoking an enhanced sensitivity to cell death. Not unexpectedly, therefore, several mechanisms that control ROS have been shown to play a role in tumorigenesis. Notably, numerous studies have shown cancer development to be dependent on the central ROS regulating systems involving GSH and NADPH.

GSH generation. GSH is an abundant component of the antioxidant capacity of the cell, and regulation of GSH levels — through both de novo glutathione synthesis and NADPH-dependent regeneration of GSH from oxidized glutathione (GSSG) — can be key to enabling tumour cell survival[71]. Consequently, GSH depletion led to ROS accumulation and cell death selectively in RAS transformed cells[72], and inhibition of glutathione biosynthesis by buthionine sulfoximine (BSO) reduced tumour growth in breast cancer xenograft models in nude mice[73]. Interestingly, in a spontaneous mouse mammary tumour model, loss of glutamate-cysteine ligase modifier subunit (GCLM; a regulator of glutathione synthesis) — which results in a 10–25% reduction of GSH levels — impaired tumour initiation without limiting the later stages of tumour progression[74]. Although these results suggest that alternative antioxidant pathways may compensate for decreased GSH in more advanced cancers, they could also indicate a role for increased ROS in later stages of tumour progression — a point we will return to later.

The biosynthesis of glutathione requires glutamate, glycine and cysteine, and the ability of cancer cells to synthesize or acquire these amino acids can be critical to maintain GSH levels and cell survival[75–77]. Under oxidative stress, the import of cysteine (through the alanine–serine–cysteine (ASC) transporters) or cystine (through the solute carrier family 7 member 11 (SLC7A11; also known as xCT)) is necessary for tumour cell proliferation and survival[76,78]. Oncogenic events such as KRAS activation (which increases ROS production) can induce expression of SLC7A11 to help limit oxidative stress[79] — with one clear consequence being protection from ferroptosis[80]. Consequently, RAS transformation sensitizes cells to erastin, an activator of ferroptosis that functions by inhibiting SLC7A11 (REF.[81]). However, one outcome of cystine import is the depletion of intracellular glutamate, which is exchanged for cystine through SLC7A11. In addition to glutathione synthesis, glutamate is required for the transamination reactions that allow the cell to synthesize other non-essential amino acids (NEAAs). As a result, antioxidant defence pathways that lead to increased cystine import also deplete intracellular glutamate and so render cells more dependent on a supply of exogenous NEAAs. These cells are then sensitized to the restriction of exogenous NEAA availability, a vulnerability that can be targeted for therapy[82].

Regulation of NADPH production. As discussed above, NADPH is not only a required cofactor for both PRDX and GPX-dependent antioxidant pathways, but also a critical component of anabolic pathways that are important for cancer proliferation. Not surprisingly, and as we discuss below, many of the NADPH producing pathways are enhanced in cancer cells[8,9].

The pentose phosphate pathway. A major source of cytosolic NADPH is the oxPPP. Several cancer types overexpress glucose-6-phosphate 1-dehydrogenase (G6PD) — the first and rate-limiting enzyme of the oxPPP — which is correlated with poor disease prognosis[83]. Alterations in some of the indirect regulators of the PPP have also been implicated in the development of various types of cancer[84]. A key response to oxidative stress is the diversion of glycolytic intermediates into the oxPPP, mediated by ROS-induced modification and inhibition of glycolytic enzymes such as pyruvate kinase isoform M2 (PKM2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)[85,86] (FIG. 2). This modification of PKM2 is required to limit ROS and allow the efficient growth of human lung cancer cells in a mouse recipient[86]. Similarly, several members of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB) family function to partition glucose flux through either glycolysis or the PPP[84,87–91]. TIGAR — a fructose bisphosphatase that can regulate fructose-2,6-bisphosphate (F-2,6-BP) levels and dampen glycolysis to support PPP flux — shows antioxidant and tumour regulating activities[92]. In several tumour models, deletion of TIGAR results in increased ROS and reduced tumorigenesis, consistent with the concept that unrestrained ROS accumulation is deleterious to cancer cells[93–96]. Similarly, depletion of PFKFB4 also increases F-2,6-BP levels and decreases oxPPP activity, leading to tumour cell death[90]. B cell tumours depend on the oxPPP for survival, and in these cells depletion of serine/threonine-protein phosphatase 2A (PP2A) — which leads to an accumulation of F-2,6-BP and decreased oxPPP flux — results in cell death that can be rescued by TIGAR[97]. Of note, several regulators of glycolysis, including PFKFB4 and fructose-1,6-bisphosphatase 1 (FBP1), possess

additional functions that regulate transcription factors such as steroid receptor coactivator 3 (SRC3; also known as NCOA3) or hypoxia-inducible factor 1α (HIF1α) and HIF2α. The moonlighting activities of these glycolytic enzymes therefore also help to control the expression of genes that regulate glycolysis and the PPP[88,98]. In renal cell cancers — which almost all show loss of FBP1 — the resultant loss of control of HIF function results in an increase in PPP activity, that at least partially explains the oncogenic response to the deletion of this enzyme[98].

Taken together, these results are consistent with a role for increased oxPPP activity in cancer development. However, deletion of G6PD in mutant KRAS-driven lung, breast and colon cancer does not impact proliferation in vivo, or spontaneous metastasis from mammary to lung[99]. Instead, mutant KRAS promotes the activation of the non-oxidative branch of the PPP, which provides ribose for nucleotide synthesis[100] but does not regenerate NADPH, and it seems likely that in these tumours an antioxidant capacity is provided through KRAS-induced increased flux of other NADPH generating pathways — such as malate dehydrogenase 1 (MDH1), malic enzyme 1 (ME1), IDH1 (REFS[8,100,101]) and serine metabolism[102] — as well as increasing overall synthesis of glutathione[79].

In a clear example of the complex knock-on effects of metabolic perturbations that are needed for ROS regulation in cancer development, NADPH generation through the oxPPP has also been shown to be required to support GSH production. As mentioned above, cancer cells depend on GSH to protect against ferroptosis and frequently upregulate SLC7A11 to import cystine. However, acquiring the cysteine needed to make glutathione by importing cystine not only depletes cells of glutamate but is further complicated by the need to reduce cystine to cysteine — a process that uses NAPDH. As a result, SLC7A11-high cancer cells depend on the NADPH producing ability of the oxPPP. Human cancer cell lines with high levels of SLC7A11 show increased expression of PPP genes and the glucose transporter GLUT1, and are more sensitive to glucose deprivation and PPP inhibitors[103]. This complex pattern of induced dependencies resulting from the need to cope with an increased susceptibility to ferroptosis provides several interesting targets for therapeutic intervention.

Malic enzymes. The cytosolic (ME1) and mitochondrial (ME2 and ME3) malic enzymes also produce NADPH to support antioxidant function. Whereas ME1 is overexpressed in some cancer types[104,105], in pancreatic cancer cells *ME2* is found to be co-deleted with *SMAD4*, a tumour suppressor gene located close to the *ME2* locus[106]. This can lead to a selective dependency of these tumour cells on ME3 activity to limit excessive mtROS accumulation[106]. In other cancer types, such as gastric tumours, co-deletion of *ME2* with *SMAD4* leads to a compensatory overexpression of ME1, which is required to limit ROS under conditions of glucose deprivation or matrix detachment[107]. ME1 activity is also important to generate NADPH for antioxidant activity in pancreatic cancer cells[100].

Folate metabolism. Several steps in the mitochondrial folate cycle, fuelled by serine catabolism, generate NADPH that limits mtROS[9] and supports cancer cells. An increase in this pathway driven by an induction of serine hydroxymethyltransferase 2 (SHMT2) can protect MYC-driven cancer cells from increased ROS and cell death under hypoxia[108], and overexpression of methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) and aldehyde dehydrogenase 1 family member L2 (ALDH1L2) — two NADPH-producing mitochondrial folate cycle enzymes — is associated with more aggressive cancers[109–111].

IDH1 and IDH2. Much attention has focused on the function of mutant IDH1 and IDH2, which are found in several types of human cancer — including glioma and lymphoma — and produce the oncometabolite 2-hydroxyglutarate (2-HG), consuming NADPH in the process[112]. However, the reaction catalysed by wild-type IDH1 and IDH2 produces NADPH, and although their role in the development of cancer is less well explored, both enzymes have been shown to be overexpressed in numerous different cancer types[113], including non-small-cell lung cancer, glioblastoma and breast cancers[114–116]. In glioblastoma cells, inhibition of IDH1 increased ROS and reduced tumour growth, whereas IDH2 overexpression in breast cancer cells promoted tumour growth and lowered sensitivity of the cells to ROS[117]. These studies support a contribution of IDH1 and IDH2-generated NADPH to cancer development.

NADPH synthesis. The systems for NADPH synthesis can also be perturbed to support cancer development. For example, oncogenic KRAS activates NADK, which supports the growth of pancreatic and colon cancers by maintaining NADPH levels[11,12,118,119]. By contrast, downregulation of NADPH phosphatase would be predicted to sustain antioxidant capacity in cancer cells. Depletion of MESH1 has been shown to protect various human cancer cell lines from ferroptosis[15], and it will be interesting to determine whether MESH1 or Nocturnin contribute to cancer development in humans.

Oncogenic mutations that regulate ROS

As discussed above, malignant conversion is accompanied by increased oxidative stress and to avoid reaching a potentially damaging level of ROS, many cancers accumulate genetic alterations that support antioxidant protection and are important for overall cancer cell survival in a stressed environment.

A key ROS regulator in cancer cells is the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2), which induces the expression of many antioxidant genes, including the glutathione biosynthesis genes GCLM and glutamate-cysteine ligase catalytic subunit (GCLC), the cystine transporter SLC7A11 and NADPH generating enzymes such as ME1, IDH1 and G6PD[120–122]. NRF2 function is controlled by kelch-like ECH-associated protein 1 (KEAP1), a ubiquitin ligase that targets NRF2 for degradation, and increased ROS levels induce a protective antioxidant response by disrupting the degradation of NRF2 by KEAP1 (REF.[123]). Although NRF2

has been associated with suppression of early stages of malignancy in some mouse models of liver, forestomach and urinary bladder cancer[124–126], it is required for tumorigenesis in several other model systems, including human cancer cell xenografts and mouse lung and pancreatic cancers[121,127,128]. NRF2 is also found to be activated in various types of human cancers through mechanisms such as mutations in *KEAP1* (REFS[128,129]). Intriguingly, oncogenes such as KRAS, BRAF and MYC, which lead to increased ROS generation, also drive compensatory antioxidant responses[100,127,130,131] that can be mediated by increasing cystine transport (as described above) and by inducing the expression of NRF2 (REF.[127]). Control of ROS by NRF2 has also been shown to be critical for the recurrence of dormant breast cancers[132].

Another critical but complex ROS regulator in cancer development is the tumour suppressor protein p53, which shows both antioxidant and pro-oxidant functions[92,133–135]. p53-induced ROS can drive cell death through apoptosis and ferroptosis[136–139], whereas the antioxidant activities of p53 are likely to contribute to tumour suppression by preventing the accumulation of damage[134,140,141]. However — paradoxically — these ROS-limiting functions of p53 have also been suggested to play a role in supporting tumour development, by preventing excessive oxidative stress[133]. Interestingly, some of the p53 point mutations that are commonly expressed in cancers selectively retain this ability to protect cells from ROS-induced elimination[142].

ROS and metastasis

As tumours progress, they start to invade nearby tissues and metastasize to distant organs — the cause of death of patients with many types of cancer. Compared with cell proliferation and survival at the primary site, successful metastasis imposes additional requirements, such as the ability to migrate and invade, survival following loss of normal cell contact and in the circulation, and the ability to enter and re-establish growth in the alien environment of a distant organ[143]. Just as in primary tumour development, ROS can be involved in promoting or limiting each of these steps (FIG. 3).

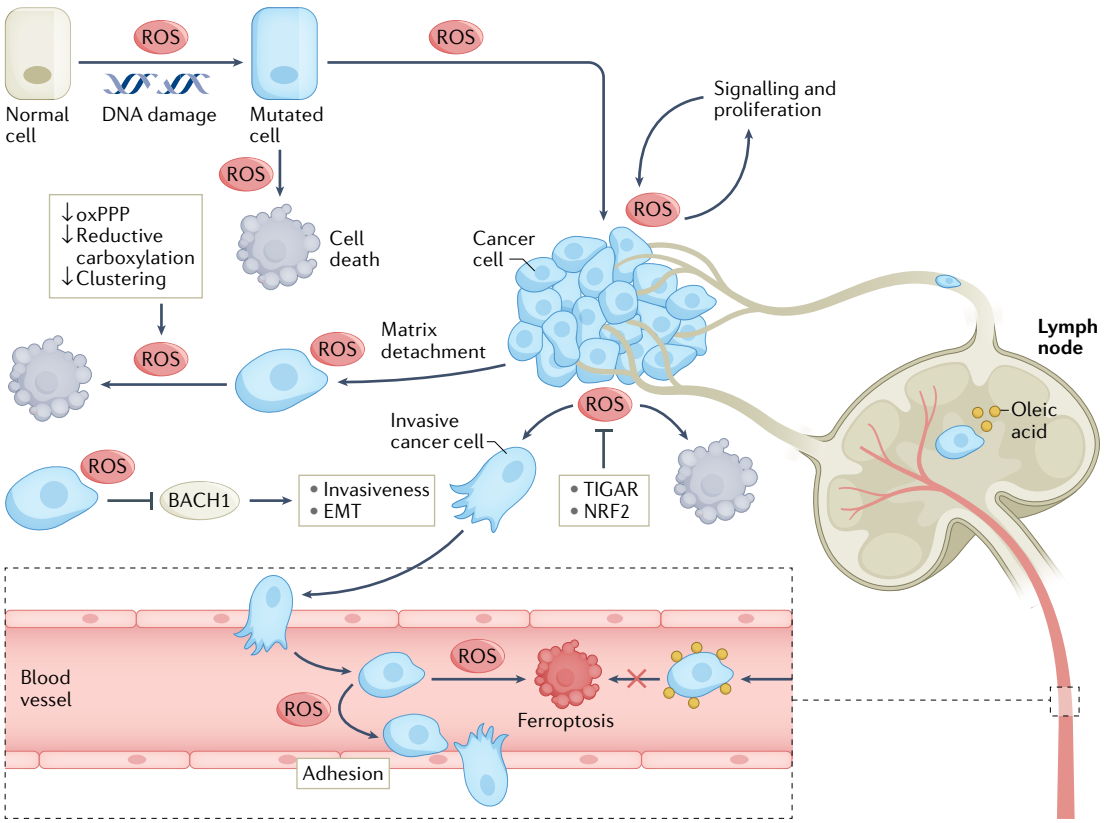

Fig. 3 | **ROS and tumour progression: tumour cell survival and invasiveness.** Reactive oxygen species (ROS) can promote DNA damage, leading to the acquisition of potentially oncogenic mutations. ROS also function as signalling molecules to drive proliferation and responses that contribute to metastasis, including epithelial-to-mesenchymal transition (EMT), migration and invasion and adhesion to endothelial cells. However, accumulation of excess ROS, resulting from oncogene activation, matrix detachment or the high ROS environment in circulation, can also lead to cell death. Tumour cells are therefore sensitized to loss of antioxidant capacity that results from decreased NADPH production — such as lower oxidative pentose phosphate pathway (oxPPP) activity or decreased reductive carboxylation — and increased mitochondrial ROS (mtROS) production resulting from the inhibition of tumour cell clustering. Antioxidants such as nuclear factor erythroid 2-related factor 2 (NRF2) and TIGAR inhibit ROS-mediated cell death and promote tumour development, but under some conditions the increased ROS signalling resulting from NRF2 and TIGAR loss enhances metastasis. However, ROS can also inhibit BACH1, a transcriptional regulator that promotes invasiveness — a response that limits metastasis. Cancer cells that travel through the lymphatic system acquire oleic acid, providing resistance to ferroptosis and supporting metastatic dissemination.

Invadopodia
Protrusions from the cell membrane that induce degradation of the extracellular matrix and contribute to the ability of cancers to invade and metastasize.

Epithelial-to-mesenchymal transition
(EMT). A cellular process that involves losing epithelial characteristics such as intercellular contacts and cell polarity and gaining mesenchymal characteristics such as migratory and invasive properties. Essential in normal development, wound healing and fibrosis. EMT also contributes to tumour metastasis.

N-acetyl-L-cysteine
(NAC). An exogenous antioxidant, providing cysteine for glutathione synthesis and reducing free radicals via the generation of persulfides.

Cancer stem cells
(CSCs). Cancer cells that acquire some stem-like cell properties to enable them to self-renew to increase the capacity for metastasis, relapse and drug resistance.

Reductive carboxylation
A metabolic pathway that uses glutamine to produce citrate from α-ketoglutarate (α-KG) via the enzymes isocitrate dehydrogenase 1 (IDH1) and IDH2, rather than the normal tricarboxylic acid (TCA) cycle in which citrate is generated from acetyl-coenzyme A (acetyl-CoA) and oxaloacetate.

Circulating tumour cells
(CTCs). Cancer cells released into the lymphatic system or vasculature and carried around the body in blood circulation. These cells act as a precursor or seed for distant metastasis.

Invasion

Cancer dissemination begins with the ability of cancer cells to migrate and invade their surrounding stroma. Rearrangements of the actin cytoskeleton to form protrusions such as invadopodia or pseudopodia aid migration and invasion of cancer cells, and the formation of these structures is dependent on ROS signalling[31,32,144]. NOX1-generated ROS drives signalling pathways, such as p38 MAPK and RHOA–RHO-associated protein kinase (ROCK), that control the extent and direction of invasion[145,146]. The import of H_2O_2 from the extracellular space via aquaporin 3 (AQP3) also promotes migration and invasion of breast cancer cells[147,148], and aquaporin expression is associated with poor survival in several cancer types[149]. An increase in both membrane ROS and mtROS has been shown to be important for activating the different types of matrix metalloproteinases (MMPs)[150] that degrade the extracellular matrix[151,152] to allow cancer cells to invade into neighbouring tissue. Increased MMP activity in SOD2-overexpressing cells led to enhanced migration and metastasis that was limited by the expression of GPX, implicating a role for H_2O_2 in this response in breast and ovarian cancers[153–155]. Interestingly, SOD2 expression is higher in the cells located at the leading edge of the tumour in human breast cancers, as well as being higher in metastatic gastric tumours[156,157]. ROS produced by cells with extra centrosomes can also act in a cell non-autonomous manner to promote invasiveness in neighbouring cells in mammary organoid and zebrafish models[158]. By contrast to these invasion-promoting functions of ROS, two recent studies in mouse models of lung cancer have shown that limiting ROS supports cancer dissemination through the stabilization of BACH1, a transcriptional repressor that is normally degraded in response to ROS[159,160]. Antioxidant treatment leads to increased BACH1, and the subsequent activation of genes involved in migration and metastasis. In this context, increased ROS limit, rather than support, invasion.

Epithelial-to-mesenchymal transition

Another feature shown by many invasive cancers of epithelial origin is an epithelial-to-mesenchymal transition (EMT)[161]. Various EMT transcription factors drive this change, which supports tumour progression[162]. However, cancers tend to show a rather mixed and incomplete switch to the mesenchymal phenotype, allowing for a reversed mesenchymal-to-epithelial transition (MET)[163,164] — a process that becomes important for cells to successfully grow at the secondary metastatic site. Many studies have shown that increases in both membrane ROS and mtROS — induced by disparate cancer-associated signals — drive EMT[165–168]; and treatment of cancer cells with the antioxidant *N*-acetyl-L-cysteine (NAC) can induce a reversion to an epithelial phenotype[169]. Furthermore, EMT has been associated with the induction of cells with a more progenitor, self-renewing phenotype, termed cancer stem cells (CSCs)[170]. CSCs are thought to mediate many important clinical aspects of cancer including dormancy, drug resistance and recurrence. Although ROS signalling, by driving EMT, can induce the cells to acquire some stem-like properties, some human and mouse CSCs have been shown to maintain lower ROS levels than their normal counterparts due to enhanced antioxidant defence, making them less susceptible to elimination in response to further increases in ROS[171]. Furthermore, a recent study of cancer cells that persist following drug treatment has shown that the retention of proliferative capacity is underpinned by the ability of this population of cells to limit ROS[172].

Detachment

Although primary tumours frequently show elevated ROS, the processes involved in cancer dissemination can drive further increases of ROS that become detrimental to cell survival. One of these ROS-inducing events is loss of matrix attachment, which in normal breast cells causes death due to an increase of ROS — a response that can be rescued by oncogenic overexpression of ERBB2. The detachment-induced ROS response results from lower oxPPP flux[173], and the activation of G6PD in immortalized fibroblasts or epithelial cells promotes antioxidant activity and allows anchorage-independent growth[174]. Detached cancer cells can also change their metabolism of glutamine to increase reductive carboxylation and support mitochondrial NADPH production by IDH2, so limiting mtROS and allowing anchorage-independent survival[22]. The importance of reductive carboxylation to provide mitochondrial antioxidant support is further illustrated by the requirement of detached cells for fatty acid synthase (FASN), loss of which impairs IDH1-dependent reductive carboxylation[175]. Detached cancer cells can also enhance their survival by clustering — which promotes hypoxia and mitophagy to remove damaged mitochondria and lower ROS. Cancer cells that are unable to cluster suffer from increased mtROS and subsequent cell death[176]. By contrast, detachment-induced mitophagy in untransformed mammary cells impairs the generation of mitochondrial NADPH, contributing to the increased ROS and cell death[24]. The basis for these different responses to mitophagy is unclear, but they illustrate the benefit of elimination of damaged mitochondria in cancer cells compared with the disadvantage of losing functional mitochondria in normal cells.

Circulation

Following detachment from the primary tumour, cancer cells spread through the circulation as circulating tumour cells (CTCs). Blood is an oxidizing environment that poses additional oxidative challenges to these disseminating tumour cells. CTCs from melanoma have higher ROS levels compared with primary tumours and whereas antioxidant treatment does not significantly change the growth of subcutaneous tumours derived from melanoma cells, it increases both the number of CTCs in circulation and the frequency of metastasis to the lung[177–179]. The ROS defence in circulating melanoma cells depends on lactate uptake through monocarboxylate transporter 1 (MCT1), which supports the oxPPP and NADPH production[180]. Single CTCs from breast, prostate and lung cancers also show increased expression of β-globin (HBB), which is induced by the increased ROS level when cells are in suspension and can drive

Electron transport chain
(Also known as the respiratory chain). Mitochondrial protein complexes that transfer electrons to create a proton gradient across the membrane, which is coupled to generate ATP via oxidative phosphorylation through ATP synthase.

Mitohormesis
Mild mitochondrial stress that increases antioxidative defences against later, higher reactive oxygen species (ROS) insults.

Angiogenesis
The growth of new blood vessels; in cancer, this refers to the development of abnormal vascularization during tumorigenesis.

Endothelial cells
Cells in a single layer that line the blood vessel.

an antioxidant response. Here again, the ROS increase in response to deletion of HBB does not affect primary tumour growth or invasive potential but reduces CTC-derived lung metastasis in vivo[181]. Interestingly, the route of dissemination also influences ROS and the survival of cancer cells. Melanoma CTCs in the blood show higher levels of lipid oxidation and are more sensitive to ferroptosis than cells circulating in the lymph. This is because lymph contains less free iron and has higher levels of GSH and the monounsaturated fatty acid oleic acid — both potent inhibitors of ferroptosis. As a result, cells circulating in the lymph metastasize more successfully than those circulating in the blood[182].

Do ROS promote or prevent metastasis?

Taken together, the data suggest that the various steps in metastatic progression of cancer can be both enhanced and decreased by ROS, leading to the generation of apparently conflicting studies that variously link either ROS limitation or ROS generation with an increase in metastasis. For example, reports of ROS-mediated promotion of metastatic capacity include the observation that the antioxidant mitochondrial catalase can decrease lung metastasis in a mouse breast cancer model[183], and scavenging ROS using catalase after peritoneal surgery (which leads to an acute inflammatory reaction and ROS production after surgical trauma in a rat model) also reduced tumour recurrence[184–186]. Enhancing mtROS by partial inhibition of the electron transport chain can also lead to an increase in metastasis, which is inhibited by mitochondrially targeted antioxidant treatment in vivo in lung cancer and melanoma models in the mouse[187,188]. ROS accumulation and the induction of HIF1α in a mammary cancer mouse model led to the expression of numerous target genes that drive increased metastasis to the lungs without affecting primary tumour latency. Again, this metastatic response was blunted by feeding mice with the antioxidant butylated hydroxyanisole (BHA)[189]. However, these studies are balanced by more recent work, discussed above, showing that increased ROS can inhibit several steps in the metastatic process — along with a role for antioxidants in supporting the ability of tumour cells to successfully colonize distant sites[160,177–179,182]. The distinction between pro-oxidant and antioxidant responses is further complicated by the ability of mtROS to activate a response called mitohormesis, which induces a mitochondrial unfolded protein response that subsequently lowers mtROS and promotes metastasis[190]. Overall, there is good evidence that ROS can both promote and inhibit metastasis. The key question now is to understand how the ultimate response is determined. Although details remain to be clarified, the outcome appears to depend on multiple factors that are discussed more fully below.

ROS and stromal compartments

Although the cell autonomous responses to ROS discussed above play a critical role in determining the behaviour of cancers, the situation is further complicated in vivo by the ROS-dependent interplay between tumour and non-transformed cells in the tumour microenvironment (TME) (FIG. 4). An important component of malignant tumours are stromal elements such as immune cells, fibroblasts, adipocytes, nerves and blood vessels, and diverse ROS functions in these stromal compartments make key contributions to shaping the trajectory of cancer development. Whereas some stromal cell types can limit tumour development, there is good evidence that many of them not only promote the survival and proliferation of the cancer cells in the primary tumour but may also dictate the metastatic potential[191]. ROS are produced both within and outside the cell and help to mediate the communication between cancer cells and the stroma. Recent studies have started to reveal the importance of ROS regulation in stromal cells and how their responses to changes in ROS levels can affect tumour cell behaviour.

ROS in endothelial cells

One key role for enhanced ROS in tumour development is to promote pathological angiogenesis. Increased NOX1-derived ROS in cancer cells can induce HIF1α, a master regulator of the hypoxic response that drives several pro-tumorigenic responses, including the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF)[192,193]. Inhibition of NOX-driven ROS production by overexpressing catalase or GPX can reduce angiogenesis and tumour growth of transformed mouse fibroblasts and human ovarian cancer cells grown on a chicken chorioallantoic membrane (CAM)[193,194]. NOX1 also mediates oncogenic RAS-induced upregulation of VEGF and angiogenesis via ERK phosphorylation in colon cancer cells[195]. In addition, mtROS are required for HIF1α stabilization in liver cancer cells[196], as well as increasing angiogenesis by oxidizing PTEN, which activates PI3K signalling and the expression of VEGF. Consequently, the expression of catalase at either the cytosolic or mitochondrial compartment can reverse the effects of ROS on angiogenesis (as assessed in the CAM assay) in human fibrosarcoma cells[197].

ROS can also directly influence angiogenesis by acting in endothelial cells themselves. For example, angiopoietin 1 (ANG1) promotes angiogenesis by increasing the production of ROS, leading to increased endothelial cell survival and migration, a response that is blunted when catalase is introduced to remove H_2O_2. Consistently, remodelling of tracheal vessels by ANG1 is more prominent in catalase-deficient mice[198]. Macrophage-generated oxidized lipids are recognized by Toll-like receptor 2 (TLR2) expressed on endothelial cells and promote angiogenesis, so that neutralization of oxidized lipids decreases vascularization and tumour growth in a mouse melanoma model[199]. This is an interesting contrast to the effect of limiting ROS and lipid oxidation in the melanoma cells themselves — which leads to increased metastasis[182] — illustrating the potentially counteractive effects of ROS in cancer and stromal cells.

Apart from the direct impact of ROS on angiogenesis, ROS also act to induce tumour cell adhesion to endothelial cells[200] in a step that could promote distant metastasis. However, the arrest of melanoma cells in the microvasculature can also create a localized increase of endothelial ROS to levels that induce tumour cell death[201,202]. The survival and metastasis of melanoma cells in the endothelial

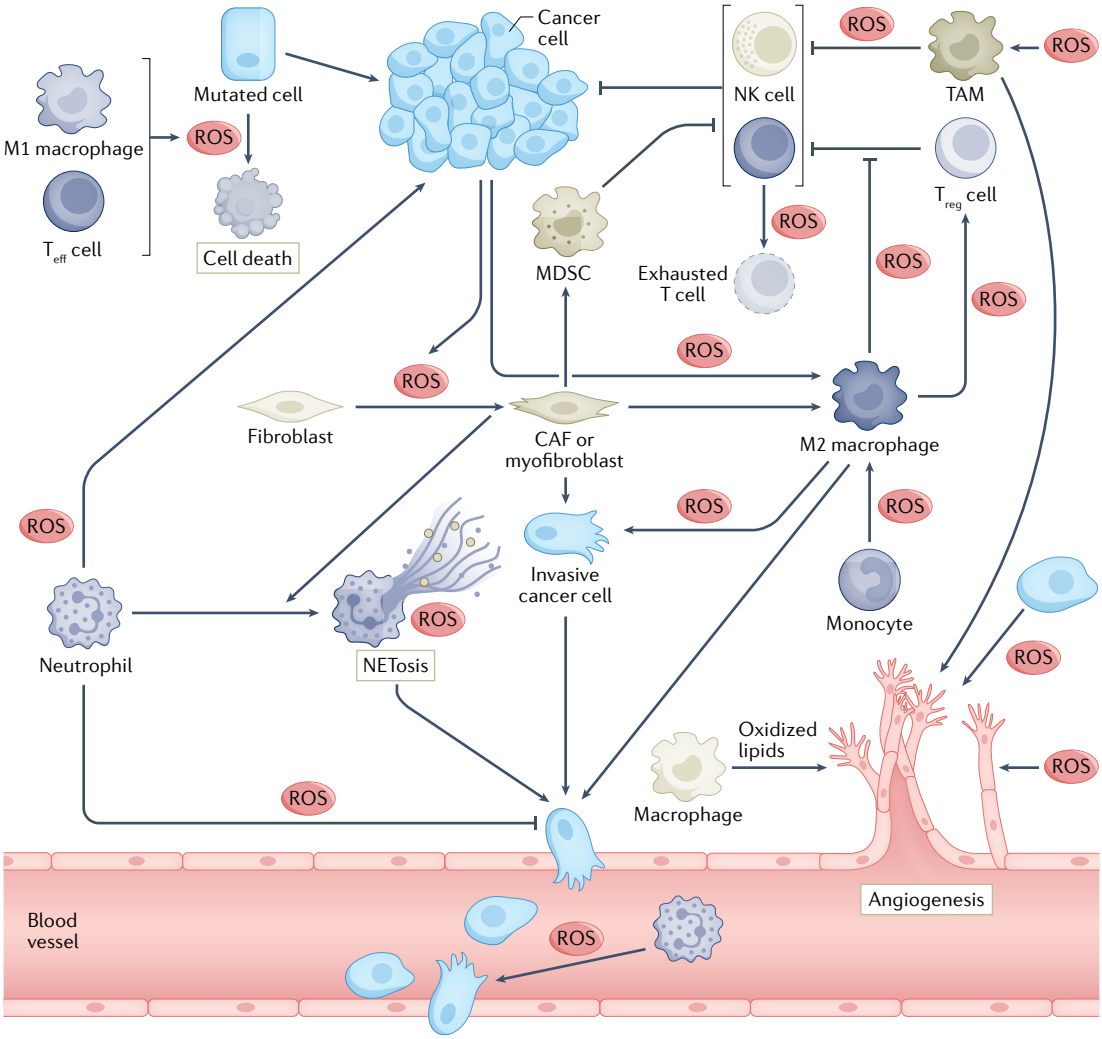

Fig. 4 | **ROS and tumour progression: interactions with stromal compartments.** Reactive oxygen species (ROS) affect tumour and stromal cells to either impede or promote cancer progression, depending on the integration of the response of all cells in the tumour microenvironment (TME). ROS produced by cancer cells are likely to influence all ROS-dependent responses illustrated. ROS promote the formation of cancer-associated fibroblasts (CAFs) and myofibroblasts, which facilitate cancer growth and invasiveness. CAFs also promote a pro-tumour stromal environment by increasing numbers of tumour-supporting myeloid cells. CAF-induced myeloid-derived suppressor cells (MDSCs) inhibit cytotoxic T cells and CAF and ROS-induced M2 macrophages also exhibit immunosuppressive functions, including inhibition of cytotoxic T cells and natural killer (NK) cells and induction of regulatory T (T_{reg}) cells. M2 macrophages also induce invasive behaviour in cancer cells. Tumour-associated macrophages (TAMs) can be induced in response to ROS to adopt immunosuppressive and angiogenic capacity. TAMs secrete ROS and lower the activity of effector T (T_{eff}) cells and NK cells. Whereas the T cell-dependent antitumour response is dependent on ROS, an excess of ROS in the tumour stroma can lead to T cell dysfunction and T cell exhaustion, reducing the ability of these cells to suppress tumour progression. CAFs can induce formation of neutrophil extracellular traps (NETs) through induction of ROS in neutrophils, a response that promotes metastasis. Neutrophils can also generate ROS that induce DNA damage and promote tumour metastasis by impairing endothelial cell junctions to facilitate extravasation. However, in some tumours, neutrophils can also inhibit invasion, in part by killing cancer cells through increased ROS. Finally, increased ROS in either tumour or endothelial cells can drive angiogenesis to promote tumour progression.

Extravasation
The movement of cells from the blood vessels to the surrounding tissues.

Cancer-associated fibroblasts
(CAFs). Cells derived from resident normal fibroblasts or from other sites such as the endothelium or bone marrow (mesenchymal stem cells). These cells can remodel extracellular matrix or secrete factors that affect the cancer and other cells in the tumour microenvironment.

compartment therefore depend on the balance between ROS-induced responses of both tumour and endothelial cells that result in cell death and cancer cell adhesion to the blood vessels. The integrity of the endothelial wall can be compromised by RAC1-mediated ROS production[203] and in an in vivo model of breast cancer, obesity leads to increased ROS production in neutrophils and impairs endothelial junctions, thereby promoting metastasis by facilitating tumour cell extravasation[204,205].

ROS in fibroblasts

Many studies have shown a tumour supporting effect of cancer-associated fibroblasts (CAFs), including myofibroblasts, that depends to some extent on increased ROS in the tumour cells. For example, co-culture of prostate cancer cells and CAFs can increase mitochondrial activity and mtROS in the cancer cells, leading to the induction of metastatic features. This effect is inhibited by treatment with the mtROS scavenger MitoTempo[206]. ROS in

Monocytes
A type of white blood cell that gives rise to macrophages and dendritic cells.

Myeloid-derived suppressor cells
(MDSCs). Immature myeloid cells that are immunosuppressive during chronic infection and in the tumour microenvironment.

CD8⁺ T cell
A cytotoxic T cell that is a key component of the adaptive immune response, recognizing peptides through the T cell receptor (TCR), and able to clear infected, damaged cells and cancer cells.

Neutrophil extracellular traps
(NETs). Extracellular fibres consisting of DNA–histone complexes and proteins such as proteases and myeloperoxidase produced by neutrophils that bind pathogens extracellularly.

Tumour-associated macrophages
(TAMs). Monocyte-derived or tissue-resident macrophages (important components of the innate immune response) present in the tumour microenvironment that modulate immune suppression and angiogenesis.

CD4⁺ T cells
T helper cells that support the activity of other immune cells by releasing cytokines and through cell–cell interactions.

Chimeric antigen receptor (CAR) T cells
Genetically engineered T cells that produce designed T cell receptors (TCRs) against tumour cells for immunotherapy.

fibroblasts themselves can also promote their conversion to myofibroblasts, which enhance metastasis[207]. Similarly, loss of PRDX1 converts mammary fibroblasts into a more CAF-like phenotype through the activation of JUN N-terminal kinase (JNK)[208]. Increased ROS and conversion of fibroblasts to myofibroblasts can also be induced by adjacent tumour cells, a response that in turn increases the invasiveness of the cancer cells[209,210]. ROS also mediate the ability of CAFs to promote EMT and stemness in adjacent prostate cancer cells[211].

The ability of CAFs to manipulate ROS levels also influences the immunosuppressive environment of tumours. For example, CAFs can increase oxidative stress in surrounding monocytes, promoting their conversion to myeloid-derived suppressor cells (MDSCs), which inhibit CD8⁺ T cell proliferation. The addition of the antioxidant NAC to a co-culture system can relieve the suppression of T cell proliferation by CAF-induced MDSCs[212]. Consistent with this observation, inhibition of the ROS-producing enzyme NOX4 in CAFs promotes infiltration of CD8⁺ T cells into CAF-rich tumours and decreases tumour growth[213]. CAFs can also induce the formation of neutrophil extracellular traps (NETs), through the induction of ROS in the neutrophils. Although NETs normally function to protect against infection[214,215], tumour-associated neutrophils and NETs have been shown to promote tumour progression and metastasis in various types of cancer[216–218]. Taken together, ROS appear to promote tumour supportive activities of CAFs.

ROS and immune cells

The immune response plays a critical role in determining the course of cancer development and progression, and the effect of ROS on immune cell populations, as well as their ability to produce ROS, can both promote and limit tumorigenesis.

Various components of the innate immune system play key roles in modulating cancer development. Whereas ROS derived from neutrophils can enhance the DNA damage that helps to initiate early tumour development in primary lung cancer[219], the production of ROS by neutrophils that accumulate in the lung has been shown to prevent metastatic breast cancer cell seeding[220], reflecting the different requirement of ROS control at different stages of cancer development. Tumour-associated macrophages (TAMs) adopt an immunosuppressive and angiogenic phenotype that is considered tumour promoting[221,222], a phenotype that is induced in response to ROS[223]. Indeed, the ROS produced by cancer cells themselves can serve to recruit and support TAMs while hindering the infiltration of T cells into the tumour[224–226]. Similarly, ROS produced by neoplastic tissues in a fly model induce changes in the basement membrane that attracts haemocytes (the fly equivalent of macrophages) to support the proliferation and survival of neighbouring cells[227,228]. TAMs also secrete H_2O_2, which lowers the activity of cytotoxic T lymphocytes and natural killer (NK) cells and promotes immunosuppression in patients with cancer[229]. Similarly, ROS contribute to the ability of MDSCs to limit the immune response[230]. Interestingly, MDSCs express high levels of NRF2, allowing them to survive the high ROS environment that they generate to suppress the function of other immune cells[231]. Overall, therefore, ROS are associated with the induction of an immunosuppressive myeloid cell phenotype. However, in a mouse ovarian cancer model, TAMs have been shown to depend on mitophagy as a mechanism to deal with the high ROS environment. In this model, inhibition of mitophagy results in increased ROS and apoptosis in the TAMs, leading to a loss of their ability to inhibit cytotoxic T cell activities — resulting in tumour regression[232].

CD4⁺ T cells and CD8⁺ T cells of the adaptive immune response are key mediators of antitumour immunity and are directly regulated by ROS. ROS are important for T cell function and T cell receptor (TCR) activation induces production of both H_2O_2 and $O_2^{\cdot-}$, which mediate downstream signalling[233–235]. mtROS and mitochondrial respiratory chain activity have also been shown to be important for T cell activation[236,237]. Although these studies and others showed the important role of various kinds of ROS in T cell function[4,238,239], the high levels of ROS encountered by T cells in tumours — resulting from an inadequate nutrient supply, hypoxia, persistent antigen cues, the presence of tumour-associated myeloid cells and the increased ROS production by tumour cells themselves — leads to decreased T cell function[239]. Importantly, the activity of chimeric antigen receptor (CAR) T cells, which are engineered for the treatment of patients with cancer, can be sustained by the expression of catalase to lower ROS[240]. Mitochondrial stress and increased ROS have also been shown to lead to T cell exhaustion[241] that diminishes their ability to control tumour progression. Antioxidants such as NAC decrease T cell exhaustion induced by mtROS[242] and T cell function can be maintained through the overexpression of nuclear factor-κB (NF-κB)-inducing kinase (NIK), which sustains NADPH production through the oxPPP[243]. NIK overexpression therefore enhances the antitumour activity of T cells. Programmed cell death protein 1 (PD1) inhibition, a strategy used to increase T cell activity as a cancer therapy, also decreases ROS levels and maintains T cell survival[244]. Balancing ROS levels and production is therefore vital to maintain T cell survival and function, to allow effective antitumour responses.

The high ROS levels of the TME also impact the activity of regulatory T (T_{reg}) cells, which function to limit CD8⁺ T cell killing. This activity of T_{reg} cells is necessary to prevent autoimmunity and inflammatory disease but can also suppress the antitumour immune response[245,246]. Macrophage-produced ROS are required for the induction of T_{reg} cells[247] and ROS production by NOX2 mediates T_{reg} cell function[248]. Somewhat counterintuitively, a recent study demonstrated that T_{reg} cells show only a weak NRF2 response and so are themselves vulnerable to oxidative stress. In this case, however, the induction of ROS-induced death of T_{reg} cells leads to the release of highly immunosuppressive adenosine that suppresses T cell immunity[249]. Furthermore, as seen repeatedly in other cell types, excessive ROS are detrimental to T_{reg} cell function and may underlie an erosion of T_{reg} cell efficiency during aging that has been associated with decreased activity of GSTP1, a member of the antioxidant GST family[250].

Box 2 | **ROS in the microbiome**

Humans are host to a huge number and diversity of microorganisms that live in or on the body[275]. These microbiota can be found associated with various organs — including the gut, skin, mouth and vagina — and they generally exist in a mutually beneficial relationship with their host[276]. However, there is a growing understanding that the microbiome can influence all stages of cancer development and the response to therapy — in part by affecting the inflammatory and immune systems[277]. Furthermore, the observation that cancers derived from various organs — including the gut, lungs and skin — carry their own microbiota supports the suggestion that these microorganisms can have a direct effect on tumour progression[278,279], although the mechanisms involved remain rather poorly understood[280]. Of interest in the context of this Review is the observation that microbiota produce high levels of reactive oxygen species (ROS). These can directly damage neighbouring cells to promote cancer development[281] and it seems likely that microbiota-derived ROS would induce all of the complex ROS-dependent responses detected in cancer cells. Gut microbiota also strongly influence immune responses and contribute to the ability of tumour-associated myeloid cells to produce ROS and limit tumour growth[282]. These bacteria also produce formylated peptides that have been shown to activate the formyl peptide receptor in stromal (and potentially cancer) cells, leading to the activation of NADPH oxidase (NOX)-dependent ROS production[283,284]. There are clearly many possible ROS-related functions of the microbiota in regulating tumorigenesis that remain to be explored.

Stromal complexity

Taken together, a complex picture emerges in which ROS play an important and varying role in the communication between tumour cells and the various cells of the tumour stroma. This relationship is likely to include other stromal cells such as adipocytes and nerve cells — although this is less well studied — and can even extend beyond the contribution of host cells in the TME, with growing evidence that microorganisms constituting the tumour microbiome also play an important role in ROS generation (BOX 2). ROS are important for the function of most cells in the TME and increased ROS production by different members of this community of cells can promote both pro-tumour and antitumour behaviour. Overall, there is evidence that ROS limitation could slow cancer progression by decreasing the immunosuppressive activity of myeloid cells, while preserving the activity and survival of key mediators of antitumour immunity such as $CD4^+$ and $CD8^+$ T cells. However, as in the cancer cells themselves, the ability to restrain excess ROS generation is important to maintain the survival of the pro-tumour stromal cells, and already high ROS levels seen in the TME may provide a targetable vulnerability.

Determining the ROS response in cancer

The multiple functions of ROS in driving different cell responses are reflected in the widely different reports of how ROS impact cancer development. Despite the complexity, however, it is becoming clear that mechanisms that control the production and response to ROS in both cancer and stromal cells need to be considered to understand the fate of the cancer cells themselves.

It is well established that the type of ROS can be critical to the outcome, with H_2O_2 predominantly leading to protein modification and modulation of signalling events, whereas highly reactive ROS species are more likely to lead to lipid damage and death. However, excessive levels of H_2O_2 will also cause damage and cell death, highlighting a role for ROS levels in determining the outcome[251]. The location and activity of enzymes that produce and regulate different ROS species, and the mechanisms that localize ROS, also have a profound effect on the cell response. An example of the different effects of membrane and mtROS production is seen in a mouse intestinal tumour model, where limiting NOX-driven ROS decreased tumour development while suppressing mtROS promoted tumorigenesis[252]. There are also several examples of systems that selectively impact the mechanisms that mediate cell death in response to ROS, without directly affecting other responses. NADPH can be used to support the activity of ferroptosis suppressor protein 1 (FSP1; also known as AIFM2), an oxidoreductase that suppresses ferroptosis independently of GPX4 by trapping lipid peroxyl radicals, and FSP1 is required to protect from ferroptosis in many cancer cell types[253]. Transient receptor potential cation channel subfamily A member 1 (TRPA1) mediates resistance to cell death following matrix detachment or treatment with ROS-inducing therapies by activating anti-apoptotic pathways[254]. A glutathione-independent role for GCLC in restricting ROS-mediated ferroptosis has also been described recently[255]. Finally, tetrahydrobiopterin (BH4), a cofactor for several metabolic enzymes, can directly protect lipids from oxidation in GPX4-inhibited cancer cells[256]. BH4 is regenerated by dihydrofolate reductase (DHFR), pointing to a mechanism of action for methotrexate, a chemotherapy that inhibits DHFR, in driving the death of susceptible cancer cells[256]. Such a differential ability to protect cells from the toxic effects of damaging ROS without decreasing ROS signalling would be predicted to support tumorigenesis by allowing cancer cells to survive, while maintaining pro-tumorigenic signalling.

The relative robustness of the cancer cell's antioxidant defence will also impact outcome, and this could reflect the organ of origin and the oncogenic alterations carried by the tumour, as well as the cells and environment surrounding the tumour. For example, as discussed above, there is strong evidence to support a metastasis limiting effect of ROS in mouse melanoma and lung cancer models, whereas several mouse pancreatic cancer models indicate a pro-metastatic role for ROS[160,168,177–179,182,187,257]. These observations probably reflect — at least in part — fundamental differences in ROS responses and regulation in the organ from which these tumours originated[258] as well as differences in the support provided by the TME. The ability of CTCs to form clusters containing cancer cells, platelets and various components of the immune system as well as circulating microvesicles[259] are also likely to impact how cancer cells respond to ROS. The route of metastasis through blood or lymph affects the susceptibility of the cancer cells to ROS-induced death[182], and the site of metastasis is also likely to reflect the influence of ROS. For example, in mouse pancreatic cancer models, increased ROS resulting from loss of TIGAR or NRF2 selectively increased lung, but not liver, metastasis[168].

The timing of ROS control is also emerging as an important factor in cancer progression, and different stages of tumorigenesis can be either enhanced or restrained by increased ROS. Several models have shown that adaptation to increased ROS over time (for example,

in response to loss of one arm of the antioxidant defence system) can lead to a change in responses that alter the trajectory of tumour progression. Furthermore, different requirements for ROS regulation at various steps in cancer development result in the selection for ROS modulating adaptations that are dynamic and shift over time. For example, HBB expression, which can limit ROS, is low in primary breast tumour cells, induced in CTCs and then reduced again in metastases[181]. Whereas ROS can promote pre-cancerous acinar to ductal metaplasia lesions in the mouse pancreas[260], increased ROS in response to loss of the antioxidant genes TIGAR or NRF2 retarded early stages of pancreatic cancer development. However, loss of TIGAR in these tumours promoted metastasis but limited proliferation of the metastatic lesion[168]. Reflecting these stage-dependent activities of TIGAR, mouse and human pancreatic tumours show high TIGAR expression in early-stage tumours (limiting ROS), much lower TIGAR levels in invasive tumours at the primary site (increasing ROS) and a recovery of TIGAR expression in secondary lesions growing in the lung[168]. In a mouse breast cancer model[189], inhibition of mitophagy following loss of BNIP3 increased ROS and promoted cancer progression. However, the induction of BNIP3L (also known as NIX) and suppression of mtROS production by oncogenic KRAS was required to support the early stages of pancreatic cancer development[261], consistent with a requirement for ROS limitation during the initial phases of malignant conversion in the pancreas. Although likely to depend on tumour type, the concept that responses to ROS change as tumours progress suggests a requirement for strategies to manipulate ROS differently according to the particular stage of cancer development.

Finally, it has to be acknowledged that much of our information about the role of ROS in cancer development is derived from experimental models, which are often difficult to compare with each other. Some of the apparent contradictory results are likely to be a reflection of differences in the tumour type studied, as well as the experimental system (for example, the use of immunocompetent or immunodeficient mice, subcutaneous or autochthonous tumours, or spontaneous versus experimental metastasis models). Consistency in the models used, along with improved methodologies for detecting specific ROS and targets for ROS modification, will allow for a more accurate analysis of the role of ROS in cancer.

Harnessing ROS regulation for therapy

The nature of the intricacies of ROS responses in cancer argues against the simplistic approach of devising generalized therapies based on ROS regulation. Although ROS can contribute to the promotion of cancer, early expectations that antioxidant treatment would limit tumour development were not borne out — even suggesting that ROS limitation may promote cancer development[262,263]. Our understanding that cancer cells tend to carry high levels of ROS suggests that they may be more likely than normal tissue to undergo cell death in response to further oxidative stress, and indeed many current chemotherapeutic agents can function by increasing the ROS level[63,264] — although these treatments may also increase the cancer risk.

It is also important to consider that systemic cancer therapies designed to manipulate ROS will not only affect the cancer cells but may also regulate the activities of the stromal cells and how they impact tumour survival and metastatic progression. The complex relationship between the type and location of ROS, the effect of ROS on the multiple cell types within the tumour mass and the impact of ROS on tumour dissemination mean that a reductionist approach focusing on one physiological facet in one environment, although very useful in dissecting mechanisms and functions, will not be enough to predict responses in a real-life cancer setting. For example, treatment with long-term fractionated radiation increases the presence of myofibroblasts and CAFs in the tumour environment by increasing mtROS[265], and ROS induced by chemotherapy or antioxidant depletion can also promote the immunosuppressive activity of macrophages[266]. These studies highlight the importance of considering how the therapeutic potential of ROS in the tumour cells may be counterbalanced by undesired collateral responses to ROS in stromal cells.

Studies such as those showing that ROS limit metastasis in a model of lung cancer but promotes metastasis in a pancreatic cancer model induced by the same genetic drivers demonstrate how much remains to be understood[160,168,178,179]. Are these differences due to qualitative differences in the ROS themselves, such as which species are generated or their subcellular localization? Or do lung and pancreatic cells respond to ROS in different ways, allowing pancreatic cells to selectively mitigate cell death responses while retaining signalling capacity? The composition of the lipid membrane, which is one of the targets of ROS-induced cell death, could have a clear impact on how cells respond. Alternatively, different responses could be influenced by the availablilty of ferroptosis-modulating components in the environments of the pancreas and lung — such as seen in lymph and blood[182] — rather than a facet of the tumour cells themselves. Or reflect tumour cell interactions with stromal cells in the TME, or even in circulation during metastasis. Although many questions remain unanswered, it seems clear that the usual blanket approach based on ROS being 'good' or 'bad' for cancer therapy will not bear fruit. Furthermore, an incomplete understanding of the mechanism of function of the purported ROS-limiting therapy can also lead to confusion. For example, whereas treatment with high-dose vitamin C can limit cancer progression, this is not due to the anticipated antioxidant effect of vitamin C but, rather, to the increased ROS resulting from a requirement of cells to reduce large amounts of dehydroascorbate (DHA), an oxidized form of vitamin C that is taken up by cancer cells[267]. Another consideration is that NAC, an antioxidant that is used in many studies of ROS, may have more nuanced activities involving mitochondrial sulfane sulfur production, in addition to being a scavenger through its thiol group or supplying cysteine to generate glutathione[268].

We now know that the effect of ROS — and, by extension, the effect of ROS modulating therapies — will vary considerably depending on the tumour type, location

and stage of cancer development. A therapy that is effective in one tumour type may not work in another, or even at a different time or stage of the same tumour. A considerable concern is that inappropriate application of ROS modulating therapies will not simply be ineffective but may even promote malignant progression. Nevertheless, there are clear differences between cancer and normal cells in their exposure and response to ROS. Continued efforts to understand this complexity will ultimately capitalize on the vulnerabilities of cancer cells to more tailored or refined therapies that target ROS or ROS controlling pathways.

Conclusions

Clearly, ROS are important in the control of cancer development and progression, and ROS production and regulation in cancer and stromal cells play an important role in determining the course of the disease. We have highlighted many of the possible responses to ROS and how these could impact tumorigenesis. The challenge now is to understand how these multiple responses in different cell types are modulated, and how they interact to determine the ultimate outcome.

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Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
K.H.V. is on the Board of Directors and a shareholder of Bristol Myers Squibb, a shareholder of Illumina, Inc., on the Science Advisory Board (with stock or stock options) of PMV Pharma, RAZE Therapeutics, Kovina Therapeutics and Volastra Therapeutics, and a co-founder and consultant of Faeth Therapeutics. She has been in receipt of research funding from Astex Pharmaceuticals and AstraZeneca, and contributed to CRUK Cancer Research Technology filing of patent application WO/2017/144877. E.C.C. declares no competing interests.

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