Oncogene (2017) **36**, 1315–1327
© 2017 Macmillan Publishers Limited, part of Springer Nature. All rights reserved 0950-9232/17
www.nature.com/onc

REVIEW

Parkin and mitophagy in cancer

JP Bernardini[1,2], M Lazarou[3] and G Dewson[1,2]

Mitophagy, the selective engulfment and clearance of mitochondria, is essential for the homeostasis of a healthy network of functioning mitochondria and prevents excessive production of cytotoxic reactive oxygen species from damaged mitochondria. The mitochondrially targeted PTEN-induced kinase-1 (PINK1) and the E3 ubiquitin ligase Parkin are well-established synergistic mediators of the mitophagy of dysfunctional mitochondria. This pathway relies on the ubiquitination of a number of mitochondrial outer membrane substrates and subsequent docking of autophagy receptor proteins to selectively clear mitochondria. There are also alternate Parkin-independent mitophagy pathways mediated by BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 and Nip-3 like protein X as well as other effectors. There is increasing evidence that ablation of mitophagy accelerates a number of pathologies. Familial Parkinsonism is associated with loss-of-function mutations in PINK1 and Parkin. A growing number of studies have observed a correlation between impaired Parkin activity and enhanced cancer development, leading to the emerging concept that Parkin activity, or mitophagy in general, is a tumour suppression mechanism. This review examines the molecular mechanisms of mitophagy and highlights the potential links between Parkin and the hallmarks of cancer that may influence tumour development and progression.

Oncogene (2017) **36,** 1315–1327; doi:10.1038/onc.2016.302; published online 5 September 2016

MITOPHAGY: MASTER REGULATOR OF MITOCHONDRIAL QUALITY

Mitophagy (mitochondria-specific autophagy) is the fundamental process of clearing damaged or excessive mitochondria via degradation in autophagolysosomes. Degraded mitochondrial components such as protein and lipids can then be recycled. This mitochondrial quality control process is triggered by mitochondrial damage or as a reaction to cellular stress, and is required for the maintenance of a functioning mitochondrial network, cellular metabolism and to prevent the accumulation of damaging mitochondrial DNA (mtDNA) mutations and reactive oxygen species (ROS) (Figure 1). Given its pivotal role in cellular homeostasis, defective mitophagy has been proposed to contribute to a variety of diseases.

Mitochondrial damage has long been associated with Parkinson's disease. The first evidence of this was the observation that MPP+, a metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, induced Parkinson's disease by inhibition of mitochondrial respiratory function.[1,2] Early studies linked the inhibition of mitochondrial respiratory complexes in Parkinson's disease to the generation of cytotoxic ROS[3] and demonstrated that this could be ameliorated by treatment with antioxidants.[4,5] Genetic studies have strengthened the link between Parkinsonism and mitochondrial dysfunction, with causative mutations in genes subsequently found to mediate mitophagy observed in autosomal recessive juvenile Parkinson's disease. These genes include *PARK2*/Parkin,[6] *PARK6*/PINK1[7] and the protein deglycase DJ-1 encoded by the gene *PARK7*,[8] a proposed interacting partner of Parkin.[9,10]

Neurotoxicity in autosomal recessive juvenile Parkinson's disease is associated with homozygous loss of function mutations in the *PARK2*[11,12] or *PARK6*[7,13–15] genes. There are some contrasting reports, however, on whether heterozygous loss of Parkin[16–18] or PINK1[19] also contributes to Parkinson's disease. *Park2*$^{-/-}$ mice do not develop severe neurodegeneration[20] but when crossed with mice with accelerated rates of mtDNA damage, the dopaminergic neurotoxicity observed in Parkinson's disease emerges.[21] Interestingly, however, *Park2*$^{-/-}$ mice do develop hepatocellular carcinoma[22] and are predisposed to a number of tumours upon sublethal irradiation.[23] Together with the frequent hemizygous loss of, or mutations in, *PARK2* or *PARK6* in tumours led to the proposal that PINK1 or Parkin, and mitophagy in general have tumour suppressor function.[24–31] However, with the notable exceptions of melanoma and breast cancer, Parkinson's disease patients are actually significantly less likely to be diagnosed with a number of cancers.[24,32] In this review we offer some ideas as to how these observations might be reconciled.

Parkin-mediated mitophagy also has a proposed role in the innate immune response to intracellular pathogens including *Mycobacterium tuberculosis. M. tuberculosis* is tagged by K63-linked polyubiquitin chains and digested by macro-autophagy.[33,34] *Park2*$^{-/-}$ mice had increased parasite burden and were sensitised to *M. tuberculosis* infection.[35] Given the endosymbiotic origin of mitochondria, the evolution of a conserved mechanism to clear both damaged mitochondria and intracellular pathogens is perhaps not surprising and indeed the machineries regulating mitophagy and xenophagy (autophagy of pathogens) are shared.[33,36–38]

Defects in Parkin-mediated mitophagy have also been linked to cardiomyopathies due to a critical role in cardiovascular development.[39,40] When *Park2* is deleted specifically in

[1]Cell Signaling and Cell Death Division, Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; [2]Department of Medical Biology, University of Melbourne, Parkville, Melbourne, Victoria, Australia and [3]Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Melbourne, Victoria, Australia. Correspondence: Dr G Dewson, Cell Signalling and Cell Death Division, The Walter and Eliza Hall Institute, 1G Royal Parade, Melbourne 3052, Victoria, Australia. E-mail: dewson@wehi.edu.au
Received 16 May 2016; revised 4 July 2016; accepted 4 July 2016; published online 5 September 2016

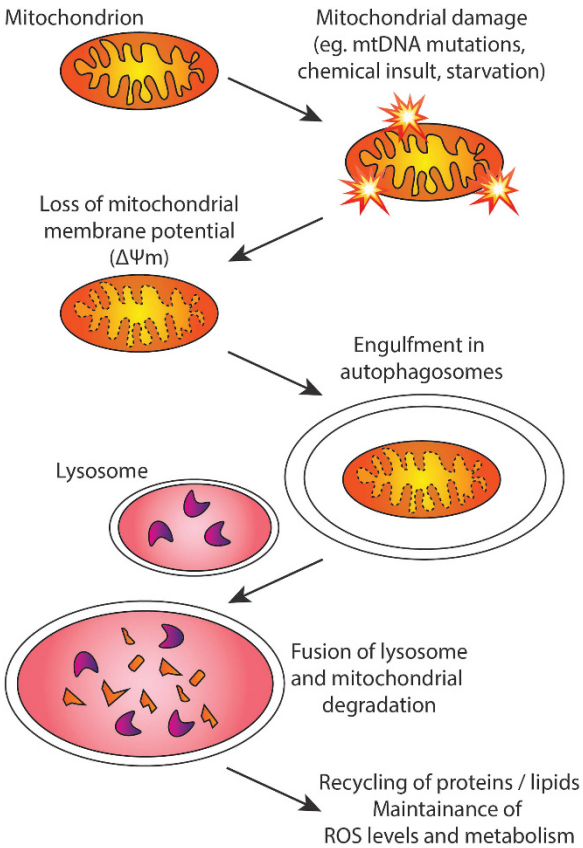

Figure 1. Mitophagic degradation of errant mitochondria. Upon mitochondrial damage induced by mtDNA mutations, starvation or mitochondrial depolarising agents, the loss of mitochondrial membrane potential triggers mitochondrial engulfment in double-membraned autophagosomes. Upon fusion with lysosomes, mitochondria are degraded, their components are recycled and metabolic homeostasis is maintained.

cardiomyocytes (but interestingly not when deleted systemically), the mice show rapid perinatal lethality due to defective cardiomyocyte reprogramming.[40]

These observations highlight the emerging and diverse roles that mitophagy and its mediators have in cellular pathology. In this review we will focus on the current understanding of the role of Parkin and mitophagy in the development, progression and treatment of cancer.

Parkin and PINK1: the critical mediators of mitophagy

The key initiator of mitophagy is the serine/threonine kinase PINK1.[41–44] Under conditions where mitochondria are undamaged, PINK1 is constitutively imported to the mitochondrial inner membrane via the Translocase of the Outer Membrane (TOM) and Translocase of the Inner Membrane (TIM) complexes. Once at the mitochondrial inner membrane, PINK1 is cleaved by the presenilin-associated rhomboid-like protease before degradation by the N-end rule pathway.[45–49] Aberrations in mitochondrial membrane potential (ΔΨm) or the accumulation of unfolded mitochondrial proteins as indicators of mitochondrial damage lead to the stabilisation of PINK1 on the MOM by impairing import to the mitochondrial inner membrane (Figure 2).[49–55] Uncoupling of mitochondrial membrane potential can be pharmacologically mimicked by treatment with the proton ionophore, carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) or respiratory complex inhibitors such as oligomycin and antimycin A.

Stabilised PINK1 at the MOM, with its kinase domain exposed to the cytosol, can then perform its crucial kinase function. Initially, PINK1 was shown to phosphorylate the E3 ubiquitin ligase Parkin.[56,57] Parkin contains an auto-inhibitory ubiquitin-like domain, three Really Interesting New Gene (RING) domains and an in-between RING domain.[58,59] Numerous PINK1-mediated phosphorylation sites on Parkin have been identified, however, phosphorylation of S65 in the Parkin ubiquitin-like domain appears critical as it de-represses its auto-inhibitory activity leading to its activation and further recruitment to the MOM.[57,60] Parkin then ubiquitinates a number of mitochondrial substrates to commit mitochondria to autophagic turnover.[61,62] However, while genetic models confirm that Parkin is downstream of PINK1,[63,64] direct phosphorylation is not sufficient to recruit Parkin to mitochondrial membranes.[65] Instead, a number of groups reported that PINK1 phosphorylates ubiquitin directly, which in turn facilitates Parkin recruitment to mitochondria,[65–68] where its E3 ubiquitin ligase activity is activated by PINK1-mediated phosphorylation. Recent reports suggest that the essential role for PINK1 in Parkin-mediated mitophagy, at least in cardiomyocytes, is due to phosphorylation of mitofusin 2 (Mfn2), a mediator of mitochondrial fusion.[39,40] Phospho-mimetic mutations in Mfn2 allowed spontaneous recruitment of Parkin to mitochondria and when these phosphorylation sites are mutated, mice exhibit perinatal cardiomyopathy due to inefficient mitophagy.[40] Whether this requisite role for phosphorylated Mfn2 as a receptor for Parkin recruitment to damaged mitochondria is ubiquitous or specific to cardiomyocytes is unclear.

Parkin substrates: specific or global ubiquitination?

Parkin seems to be highly promiscuous both in terms of its substrate specificity and the type of ubiquitin modification it confers. Parkin ubiquitinates diverse substrates involved in mitochondrial dynamics, apoptosis and metabolism, highlighting the crosstalk between the mitophagy machinery and a number of other crucial signalling pathways.[61,62] Comprehensive proteomic analysis indicated that Parkin is capable of generating multiple types of polyubiquitin chains on substrates including K6, K11, K48 and K63 linkages.[68,69] Although K48-linked polyubiquitination is a canonical sequence for 26S proteasomal degradation, the other linkages indicate that Parkin may also influence signalling, protein–protein interactions and protein conformation change.[70]

The ubiquitination and degradation of the mitochondrial fusion machinery including the mitofusins is an early event following Parkin recruitment.[61,62] This has been argued to quarantine damaged mitochondria from the remaining mitochondrial network to facilitate specific engulfment of affected mitochondria by the autophagosome.[62] The rapid turnover of certain proteins such as the mitofusins or voltage-dependent anion channel (VDAC) 1 compared with other Parkin substrates suggests the potential for specificity towards particular substrates. However, to date, a single Parkin substrate that is required for mitochondrial clearance has not been identified, leading to the hypothesis that it is the accumulation of ubiquitinated targets that is the defining factor.

Parkin is antagonised by a number of de-ubiquitinating enzymes including ubiquitin-specific proteases (USPs) including USP15[71] and USP30.[72] USP30 removes poly-ubiquitin chains and has been postulated to slow ubiquitin chain growth on mitochondrial substrates of Parkin.[72–74] USP15 has also been argued to antagonise mitophagy by disassembling poly-ubiquitin chains on the surface of mitochondria.[71] By contrast, USP8 has been argued to enhance mitophagy by removing K6-linked ubiquitin chains from Parkin itself.[75]

Downstream consequences: picking up the pieces

A suite of proteins is necessary to facilitate the engulfment and degradation of ubiquitin-decorated mitochondria. A number of

© 2017 Macmillan Publishers Limited, part of Springer Nature.

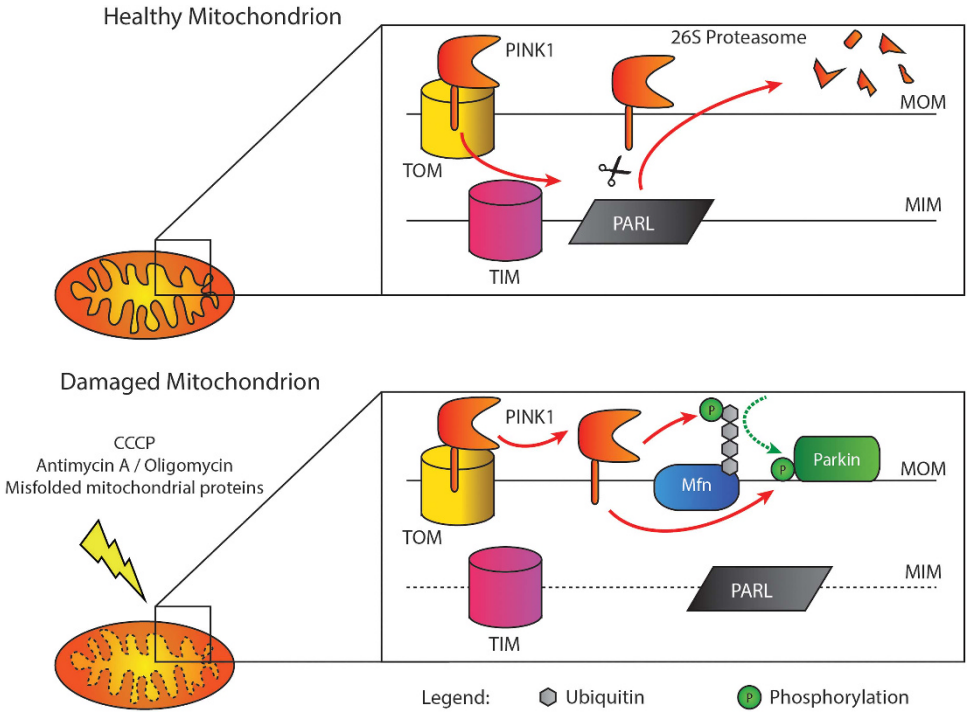

Figure 2. Mitochondrial damage increases PINK1 stability and recruits Parkin. Under homeostatic conditions PINK1 is cleaved by the presenilin-associated rhomboid-like protease located at the mitochondrial inner membrane (MIM) followed by turnover by the 26S proteasome. When mitochondria are damaged (which can be induced experimentally by membrane depolarising agents such CCCP or combined treatment with antimycin A and oligomycin) or accumulate unfolded proteins, PINK1 is stabilised on the mitochondrial outer membrane (MOM) and phosphorylates ubiquitin on S65, allowing Parkin recruitment to phosphorylated ubiquitin. PINK1 then phosphorylates Parkin on S65 in its ubiquitin-like domain and together with Ubiquitin-S65 binding, fully activates Parkin's E3 ubiquitin ligase activity. TIM, translocase of the inner membrane; TOM, translocase of the outer membrane.

receptors have been proposed including the VDAC1/p62[76] axis and other receptors associated with xenophagy.[37] However, further studies have refined the essential receptors for mitophagy to Nuclear Dot Protein 52 (NDP52) and Optineurin and Tax1 Binding Protein 1.[37,77–79] These receptors have a common Microtubule-associated proteins 1A/1B Light Chain 3 (LC3) interacting region (LIR) that links their ability to bind ubiquitinated substrates and LC3, a crucial lipidated inducer of autophagic membrane induction. GABA(A) Receptor-Associated Protein (GABARAP) can also be recruited in much the same way as LC3 to facilitate extension of the autophagosomal membrane during autophagy.[80] Recent work has also shown that TANK-binding Kinase 1 (TBK1) accelerates autophagosome induction by phosphorylating Optineurin, leading to enhanced binding to its ubiquitinated substrates.[38,79] One recently identified autophagy receptor is BCL2L13/Bcl-Rambo. Although first identified as a pro-apoptotic B-Cell Lymphoma 2 (BCL2) family member,[81] BCL2L13 has recently been reclassified as a mitophagy receptor,[82] but unlike NIX and BNIP3, the precise mechanism of its regulation is as yet unknown. It is interesting to consider that three Bcl-2 family orthologues, BCL2L13, NIX and BNIP3 all diverged into mediators of mitophagy.

Following the accumulation of activated mitophagy receptors, LC3 and GABARAP family members bind and promote autophagosome formation in concert with a number of other autophagy effectors including the autophagy initiating Unc-51-like Kinase 1 (ULK1) complex and the ATG12–ATG5–ATG16L complex (reviewed in Lamb *et al.*[83]). Following engulfment of the mitochondrion, the autophagosome fuses with a lysosome allowing low pH-dependent proteolytic clearance of damaged mitochondria (Figure 1). Notably, this lysosomal degradation can be inhibited pharmacologically by the addition of the lysosomal proton pump inhibitor, bafilomycin.[84]

Alternate pathways of mitophagy

Alternative pathways of mitophagy have been well characterised in maturing reticulocytes, skeletal muscle and liver tissue. In these systems Parkin-independent mitophagy occurs via the upregulation of BNIP3 and NIX (Figure 3). These MOM proteins exist as homodimers and in addition have N-terminal LIR that facilitates the recruitment of LC3/GABARAP.[85–88] Both NIX and BNIP3 contain Bcl-2 homology 3 (BH3) domains that are proposed to interact with Bcl-2 and B-Cell Lymphoma Extra Large (Bcl-x_L).[89] Much like Optineurin, phosphorylation of BNIP3 increases its affinity for LC3.[90] Interestingly a number of tissues, including skeletal muscle and reticulocytes, express high levels of BNIP3 and NIX, and the regulation of mitophagy by these receptors seemingly relies on both phosphorylation and direct inhibition by other proteins.[85] FUN14 domain-containing protein 1 (FUNDC1) has been reported to be a mitophagy receptor[91] that is controlled by phosphorylation by ULK1 on S17.[92] There are also other enzymes responsible for negative regulation of FUNDC1 by phosphorylation including Src (on Y18) and Casein Kinase 2 (CK2) (on S13) (Figure 3).[93,94] FUNDC1-mediated mitophagy is initiated by dephosphorylation on S13 by the protein phosphatase Phosphoglycerate Mutase Family Member 5 (PGAM5).[95] The importance of PGAM5 is even more apparent *in vivo*, where its depletion also inhibits CCCP-mediated accumulation of PINK1 at the MOM.[96] *PGAM5* null mice also develop a Parkinson-like phenotype and dopaminergic neurodegeneration.[96] Like BNIP3 and NIX, PGAM5 has been reported to interact with the machineries controlling apoptosis (via Bcl-x_L[97]) and necroptosis.[98] Although the relevance of these interactions remains unclear (PGAM5 is dispensable for necroptosis[99]), interaction with the cell death machinery highlights the potential for coordinated control of mitochondrial homeostasis and cell survival. The ubiquitin ligases Mul1,[100]

© 2017 Macmillan Publishers Limited, part of Springer Nature.

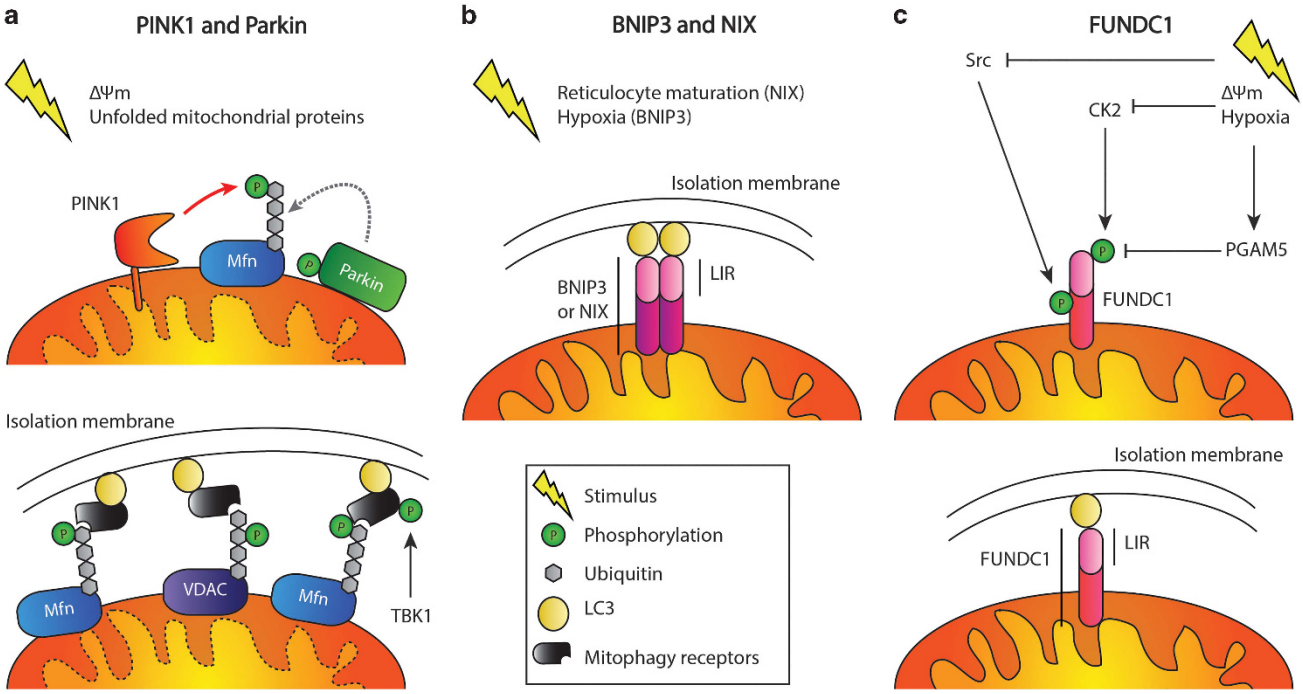

Figure 3. Convergent pathways of mitophagy. (**a**) PINK1 and Parkin: following PINK1-mediated recruitment and activation, Parkin ubiquitinates a number of mitochondrial proteins including the mitofusins (Mfn) and VDAC family members. PINK1 phosphorylates the ubiquitin attached to mitochondrial proteins to recruit mitophagy receptor proteins (including Optineurin and NDP52). This drives ULK1 and LC3 recruitment to extend the isolation membrane and promote autophagosome formation around the damaged mitochondrion. Receptor binding of ubiquitin attached to substrates is enhanced by phosphorylation of the receptors such as Optineurin by TBK1. (**b**) BNIP3 and NIX: BNIP3 and NIX are upregulated in response to hypoxia and reticulocyte maturation respectively. These mitophagy receptors directly bind LC3 via LIR domains and induce isolation membrane recruitment and formation for mitophagy. (**c**) FUNDC1: FUNDC1 is inactivated by phosphorylation by Src and CK2. When dephosphorylated by PGAM5, FUNDC1 can bind LC3 to induce mitochondrial engulfment.

Gp78[101] and Smurf1[102] have also been implicated as effectors of mitophagy.

MITOPHAGY: A CO-ORDINATOR OF MITOCHONDRIAL HOMEOSTASIS

An emerging concept is the complex interplay of mitochondrial signalling pathways. This crosstalk is evident from the apparent 'moonlighting' functions of many effectors of mitochondrial dynamics, apoptosis, metabolism and now mitophagy (Figure 4).[103,104] It is perhaps overly simplistic to view mitophagy as a process in isolation, particularly given recent studies that point to a coordinated response following mitochondrial damage.[44] Here we discuss how the mitophagy machinery is integrally linked with other cellular processes to co-ordinate the survival of the cell following mitochondrial damage.

Intrinsic apoptosis

Apoptosis is critical in development, immune homeostasis and limiting cancer development and has been extensively reviewed elsewhere.[105–107] The role of the Bcl-2 family in mediating the intrinsic pathway of apoptosis is well-understood, but in addition, the pro-survival Bcl-2 proteins have been proposed to regulate autophagy.[108,109] Whether this influence on autophagy is direct or indirect has been questioned,[110] however, emerging evidence also implicates an important role for the Bcl-2 family in regulating mitophagy. As well as the aforementioned ability of Bcl-x_L to indirectly inhibit mitophagy by binding PGAM5, a positive regulator of the mitophagy receptor FUNDC1,[95] Parkin-mediated mitophagy is also antagonised by the overexpression of pro-survival Bcl-2 family members upstream of ubiquitin decoration and mitophagy receptor recruitment.[111] Knockdown of pro-survival proteins or overexpression of pro-apoptotic BH3-only members accelerated Parkin recruitment, and immunoprecipitation and immunofluorescence experiments confirmed that pro-survival proteins bound Parkin and inhibited its recruitment to mitochondria.[111] It remains unclear how Bcl-2 family members inhibit Parkin translocation given that the Bcl-2 family are predominantly associated with the MOM, whereas Parkin is recruited actively from the cytosol during mitophagy. Conversely, Parkin reciprocally modulates apoptotic cell death in response to mitochondrial stressors, as PINK1-induced recruitment of Parkin to mitochondrial membranes was sufficient to sensitise cells to apoptosis specifically induced by CCCP through the degradation of Mcl-1.[112] Other reports proffer a contrasting pro-survival role of Parkin that is perhaps more consistent with the hypothesis that Parkin loss accelerates dopaminergic neuron loss in Parkinson's disease. The pro-apoptotic effector protein Bax is ubiquitinated by Parkin, thus protecting these cells from apoptosis by diminishing functional Bax at mitochondria,[113,114] Parkin has also been argued to mono-ubiquitinate and stabilise Bcl-2.[115] Whether Parkin has a pro-survival or pro-apoptotic role is still debated and indeed whether there is a gene dosage or cell-type specific effect that governs Parkin's influence on cell survival is unclear. However, recent work suggests that Parkin acts as a rheostat to determine cell fate in response to differing degrees of insult and that much of this response is tempered by PINK1 activity.[116] The reclassification of BCL2L13 from a putative BH3-only protein[81] to a mitophagy receptor[82] is a salient example of how overexpression studies should be interpreted cautiously, particularly as evidence is mounting that apoptosis and mitophagy are interconnected and potentially exist in a delicate equilibrium.

© 2017 Macmillan Publishers Limited, part of Springer Nature.

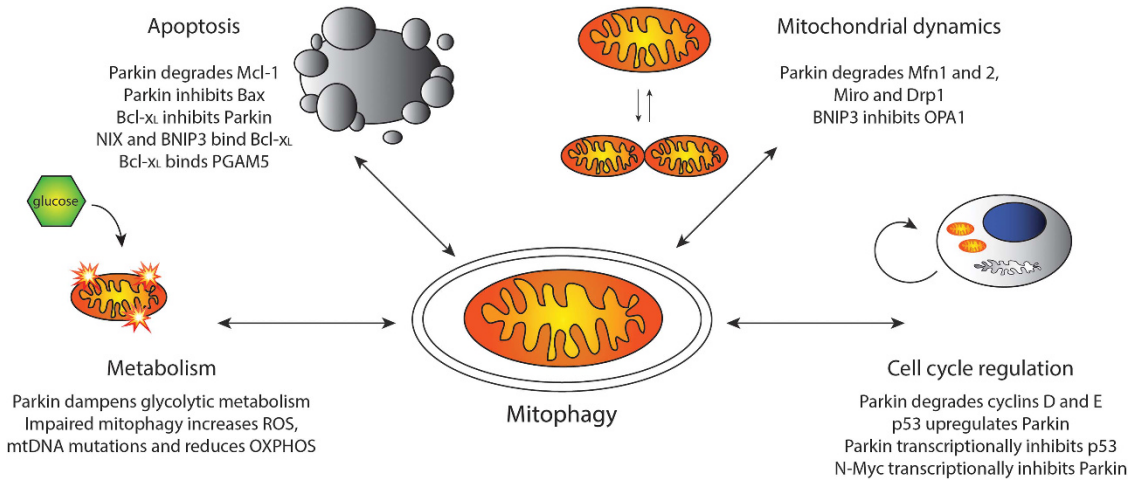

Figure 4. Crosstalk between mitophagy machinery and critical cellular processes. The machinery controlling mitophagy is implicated to influence several fundamental pathways that govern cell fate including apoptosis, mitochondrial fission/fusion and trafficking, metabolism and cell cycle control. The indirect or direct influence on these pathways may contribute to the pathogenesis associated with disabling mitophagy.

Necroptosis

As well as apoptosis, mitophagy has been linked to necroptosis, a programmed necrotic type of cell death that is caspase independent and mediated by Receptor Interacting Protein Kinase (RIPK)3.[117,118] Mitophagy has been implicated to determine whether neuroblastoma cell lines switch from an apoptotic fate to a necroptotic one in response to oxidative stress.[119] In addition, impaired mitophagy and the exhaustion of cellular ATP due to viral infection was shown to promote necrotic cell death,[120] although whether death in this scenario was necroptosis or necrosis was not defined.

Initially, necroptosis was linked to mitochondria by PGAM5-mediated recruitment of an 'attack complex' complex comprising Mixed lineage kinase domain-like (MLKL), RIP1 and RIP3 to mitochondria.[98] This hypothesis was later refined as it was found that necroptosis could proceed in cells that were depleted of their mitochondria.[121] In addition, loss of PINK1 has no effect on tumour necrosis factor-induced necroptosis,[122] suggesting that ROS generation may accompany necroptosis, but does not drive it.[121] It is possible that PGAM5 enhances the clearance of damaged mitochondria, and that its loss indirectly enhances necroptosis due to cytotoxic ROS production.[123] So, although no definitive role for mitophagy in influencing necroptotic cell death has been established, a compromised mitochondrial network may indirectly modulate the extent of necroptosis.

Mitochondrial fission, fusion and transport

Mitochondria are not static organelles, but rather form a dynamic network that constantly undergoes fission and fusion that is critical for efficient energy production and for segregation of mitochondria during cell division. Mitophagy has long been associated with alterations in the mitochondrial fission/fusion machinery. The first evidence came from microscopy studies that showed fragmentation and perinuclear clustering of mitochondria in response to mitochondrial stress,[76,77,124] phenomena that are also apparent during apoptosis. The mitochondrial fusion proteins Mfn1 and Mfn2 are responsible for MOM fusion and cooperate with Optic Atrophy Protein 1 (Opa1) to facilitate the fusion of mitochondria.[125,126] Following a mitochondrial insult, however, Mfn1 and Mfn2 undergo rapid Parkin-dependent degradation.[63,127–129] It is reasonable to assume that the immediate disruption of the fusion machinery is a cytoprotective mechanism to partition damaged mitochondria for autophagic turnover. Indeed ablation of mitochondrial fission prevents effective mitophagic clearance.[130] Dynamin-Related Protein 1 (Drp1), a regulator of fission, is also a proposed substrate of Parkin.[131] This is at odds with the mitochondrial fragmentation observed before mitophagic engulfment, but may indicate a role for Parkin in the homeostatic control of Drp1.[131] Mitochondrial motility is also perturbed by Parkin-mediated mitophagy by the turnover of the Mitochondrial Rho-GTPase (Miro).[132,133] Miro functions as an receptor that bridges kinesins and mitochondria.[134] The PINK1-mediated phosphorylation and subsequent ubiquitination by Parkin tags Miro for degradation and severs the connection between mitochondria and the cytoskeleton, essentially halting mitochondrial motility.[132,133] This presumably is another mechanism by which Parkin paralyses faulty mitochondria for subsequent autophagic clearance. Interestingly, BNIP3 also reportedly inhibits Opa1 and is thus thought to promote mitochondrial fission.[135,136]

Cell cycle and proliferation

Cyclin E, a regulator of Cyclin Dependent Kinase 2 (CDK2), has been identified as a substrate of Parkin, and its turnover was implicated as the major factor responsible for Parkin's putative tumour suppressor function.[137] This phenomenon of Parkin-dependent degradation of cyclin E was found to be compromised by expression of Parkin point mutants associated with glioblastoma, lung and colon cancers.[138] In addition, Parkin is implicated as a master cell cycle regulator that also downregulates cyclin D.[139] This potential impact on cell cycle control may in part explain the apparent paradox that deleted or mutated Parkin provides a selective advantage to tumours while promoting neuronal cell death. Proliferating tumour cells with predominantly hemizygous loss of Parkin may have impaired turnover of cell cycle regulators leading to enhanced proliferation. In contrast neuronal cells are post-mitotic and so cannot dilute the effect of accumulating damaged mitochondria due to the homozygous loss of *PARK2* and Parkin-mediated mitophagy. Curiously *Park2*$^{-/-}$ mice do not show any accumulation of cyclin E in post-mitotic neurons.[140]

There is also a reported reciprocal regulation between p53 and Parkin. *Park2* is a p53 target gene,[23] but Parkin has also been reported to downregulate p53 transcriptionally via its RING1 domain, independent of its E3 ligase activity.[141] Consequently, p53 was elevated in the brains of autosomal recessive juvenile Parkinson's disease patients harbouring mutations in Parkin. The physiological relevance of this transcriptional repression of p53 by

© 2017 Macmillan Publishers Limited, part of Springer Nature.

Parkin is currently unclear, but appears to be cell-type dependent,[23] and is potentially at odds with the proposed and accepted tumour suppressor functions of Parkin and p53 respectively.

Metabolism, bioenergetics and ROS generation

Parkin has a central role in balancing cellular metabolism. Its loss results in a switch to aerobic glycolysis known as the Warburg effect and is characteristic of many solid tumours,[23] and excessive production of ROS from damaged mitochondria. Parkin contributes to metabolic control by p53 by effecting the proteasomal degradation of metabolic enzymes responsible for glycolysis.[23] Indeed a number of these enzymes have been identified as substrates of Parkin, but it is not necessarily clear how they contribute to Parkin-dependent regulation of metabolism. For example, the enzyme Pyruvate Dehydrogenase Alpha 1 (PDHA1) is a suppressor of oxidative phosphorylation that is positively regulated by Parkin, somewhat discordant with the premise that Parkin restricts glycolysis.[23,25,142] Another interesting observation is that Parkin contributes to lipid metabolism by stabilising the lipid transporter, CD36.[143] Parkin has also been shown to target and downregulate Pyruvate Kinase M2 (PKM2),[144] an important enzyme that facilitates glycolysis in tumour cells.

Parkin's apparent tumour suppressor role in dampening glycolytic metabolism is complemented by its ability to dampen ROS production at mitochondria. The generation of ROS and subsequent oxidative damage to mitochondria and cells remains a hallmark of tumour progression. Mitochondria generate a small amount of ROS in a healthy setting due to inefficiencies in the electron transport chain during oxidative phosphorylation. Residual pools of reduced glutathione act as a buffer to neutralise any ROS. However, in scenarios when the electron transport chain is blocked, ROS overwhelm cytoprotective antioxidant mechanisms leading to damage of both mitochondrial and genomic DNA (reviewed in Sabharwal and Schumacker[145]). In the absence of the mitophagy machinery, damaged mitochondria produce cytotoxic ROS. It is interesting to note that ROS at lower levels can also act as potent mitogens and thus trigger cell growth,[146] perhaps linking elevated ROS in mitophagy-deficient cells with enhanced tumour development. Parkin loss is responsible for the accumulation of ROS in the brains of patients with both diagnosed and asymptomatic Parkinson's disease as well as in *Park2*$^{-/-}$ mice.[2,142,147] In the brains of *Park2*$^{-/-}$ mice, there is a depletion of the cytoprotective antioxidant glutathione by the accumulation of ROS generated by uncoupled mitochondria.[142,148] This *Park2*$^{-/-}$ phenotype recapitulates that observed in juvenile Parkinsonism caused by the loss of E3 ligase activity in Parkin point mutants.[149] It is likely that the switching of cells to a glycolytic metabolism is a necessary effect of Parkin's inability to clear damaged mitochondria that are unable to undergo effective oxidative phosphorylation. In the context of tumour progression, mtDNA damage can deplete the levels of mitochondrially-encoded metabolic proteins including the mitochondrial respiratory complex members and as such, acts as a positive feedback loop to generate more damaging ROS through electron transport chain uncoupling. In much the same way, a high concentration of cellular ROS can damage genomic DNA, potentially promoting oncogenic mutations, although this mutation rate is highest at the mitochondria.[150] Such genomic instability has been identified as a hallmark of cancer and as such, the appropriate clearance of mitochondria may be critical to prevent the transformation of cells.

Mutations in respiratory complex II components not only inhibit the electron transport chain, they also stabilise Hypoxia Inducible Factor-α (HIF-α),[151] causing a transcriptional shift from oxidative phoposphorylation to glycolytic metabolism.[152] The PI3K pathway, a mitogenic pathway commonly upregulated in tumours, is also more active in glycolytic cells.[153] The switch to aerobic glycolysis is accompanied by an array of complex bioenergetic changes (reviewed in Wallace[146]). Maintaining a functional mitochondrial network and removing errant mitochondria by mitophagy may be crucial to prevent this metabolic switch that drives tumour growth.

Parkin also has a role in promoting mitochondrial biogenesis by binding Mitochondrial Transcription Factor A (TFAM) and inducing the expression of mitochondrial genes including essential respiratory complexes in proliferating cells.[154] Although Parkin has been shown to interact with genomic DNA,[141] it is yet to be elucidated how Parkin is able to interact with mtDNA in the mitochondrial matrix when Parkin has been well described to accumulate at the mitochondrial outer membrane following mitochondrial damage. It may well be that the role of Parkin differs substantially between replicating and post-mitotic cells the result of its inhibition or mutation is profoundly context dependent. The contribution of mitophagy to the many signalling pathways discussed here highlights the central role that mitochondrial quality control has in the appropriate control of cell death, metabolism and mitochondrial dynamics, which cause or contribute to a range of pathologies when abrogated.

MITOPHAGY: ROLE IN CANCER

Although aberrant mitophagy is causally linked to the development of autosomal recessive juvenile Parkinsonism through homozygous loss of *PARK6*/PINK1 or *PARK2*/Parkin, increasing evidence indicates that defects in the mitophagy machinery may also have a role in the progression of cancer (Table 1). Copy number variations in *PARK2* are common in a range of tumours. Although certain cancers, such as sarcomas and uterine cancer have amplifications in *PARK2*, the majority of tumours with lesions in *PARK2,* including ovarian,[26–29] breast[27] and lung cancers,[30,31] harbour deletions or loss of function mutations. However, as the *PARK2* gene is located on a common fragile site on chromosome 6

Table 1. Alterations to *PARK2* (Parkin) and *PARK6* (PINK1) in different tumours

Cancer	*Gene*	*Mutation*		*Ref.*
Ovarian	*PARK2*	Copy number loss	Loss of *PARK2* locus in patient samples due to 6q25-27 deletion	27,29
			Targeted exonic deletion in *PARK2* in patient samples	28
Lung	*PARK2*	Non-synonymous mutation	Parkin mutations in patient samples (A46T, D243N, N254S, H279P)	136
Glioblastoma/glioma	*PARK2*	Non-synonymous mutation	Parkin mutations in patient samples (I2V, R42C, T173A, R275Q, E344G, V380L, D394N)	138,161
	PARK2	Copy number loss	Homozygous and heterozygous loss in patient samples	138
	PARK2	Dysregulated expression	Reduced Parkin expression correlates with glioma grade	162
Colorectal	*PARK2*	Copy number loss	Homozygous and heterozygous loss in patient samples	138,159
			Heterozygous Parkin null mice accelerate intestinal adenoma when crossed to APC^{min} mice	159
Breast	*PARK2*	Copy number loss	Loss of *PARK2* locus in patient samples due to 6q25-27 deletion	27,172–176
Neuroblastoma	*PARK6/PINK1*	Non-synonymous mutation	PINK1 mutations in patient samples (R279H, L437P)	164

© 2017 Macmillan Publishers Limited, part of Springer Nature.

(FRA6E, 6q26), its deletion does not necessarily imply a causal role in these tumours. Indeed the loss of *PARK2* at this fragile site may be a consequence of replication stress in certain tumours.[155,156] However, that specific point mutations or focal deletions in *PARK2* are detected in a variety of tumours, support Parkin as a potential driver mutation within this fragile site.[138] It is also puzzling that although homozygous deletion or loss of function mutations are deleterious, at least in differentiated neurons, hemizygous deletion or mutation seemingly provides a tumour cell with a growth advantage. This discordance between similar genetic lesions promoting diametrically opposed cell fates has been hypothesised to be a cell type-specific or a gene dosage effect.[157,158] It may also be the case that Parkin functions differently in post-mitotic cells and replicating cells. This may explain the observation that Parkin in post-mitotic neurons has an apparent pro-survival role whereas it limits growth in rapidly dividing cancers. Further studies need to be conducted to determine whether this dual role for Parkin is purely a result of defective mitophagy or whether it implicates a mitophagy-independent role for Parkin. Here we review the emerging evidence that mitophagy defects promote the development of specific tumours.

Colorectal cancer

Evidence suggests that as many as 33% of colorectal carcinomas exhibit heterozygous loss of *PARK2*.[159] Another study further refined this assertion, showing that 25% of colorectal carcinoma tumours had focal intragenic deletion of *PARK2*, thus identifying this gene as a strong candidate for the progression of colorectal cancer.[138] As colorectal carcinoma is characterised by genomic instability the loss of *PARK2* in these tumours may be incidental. However, loss of a single allele of *Park2* was sufficient to accelerate the progression of spontaneous colorectal tumours in the Apc^{min} mouse model,[159] implicating *PARK2* mutations as drivers of tumour progression and not merely a consequence of genomic instability, suggesting that Parkin's E3 ligase activity is a *bona fide* tumour suppressive mechanism. Another study found that a patient diagnosed with colorectal cancer had a focal deletion in *PARK2* in their liver metastatic tumour, whereas the progenitor primary tumour did not.[160] This anecdotally raises the possibility that *PARK2* deletion or loss of function mutation provides a selective advantage for some metastases.

Glioblastoma and neuroblastoma

Another cancer where aberrant Parkin is frequently observed is glioblastoma multiforme. Using data from the Cancer Genome Atlas, it was discovered that approximately a quarter of glioblastoma multiforme samples had either heterozygous or homozygous loss of *PARK2*,[138] with discrete regions of chromosome 6 selectively deleted.[161] The range of alterations included intragenic deletions in the *PARK2* gene and *PARK2* point mutations.[138] Interestingly, these mutations correlated with the known disease-causing mutations in autosomal recessive Parkinson's disease, clustering in the RING and ubiquitin-like domains of Parkin that are required for substrate binding, ubiquitination machinery recruitment and ubiquitin transfer.[138] In addition, in the absence of somatic mutations in *PARK2*, it is the loss of p53-mediated transcription of Parkin that is proposed to enhance glioma development.[162] The tumourigenesis of glioblastoma may be particularly sensitive to Parkin loss given the protein's high expression level in the brain. PINK1 is also implicated in glioblastoma multiforme, with genetic silencing of PINK1 shown to arrest the growth of glioblastoma cells, potentially via the Notch signalling pathway.[163] This study is seemingly at odds with the observation that Parkin is a tumour suppressor in glioblastoma, and may highlight a role for PINK1 in the Notch signalling pathway that is independent of its role in Parkin-mediated mitophagy.

Neuroblastoma is a solid extracranial tumour arising from the sympathetic nervous system. Recently, mutations in the mitophagy machinery have been identified in neuroblastoma patients including in *PARK6* (encoding PINK1).[164] Two mutations that are commonly observed in autosomal recessive Parkinson's disease were also identified in the neuroblastoma samples tested.[164] One subtype of poor prognosis neuroblastoma is characterised by amplification of the oncogene *MYCN*. The levels of N-Myc and Parkin protein are inversely related in neuroblastoma cell lines,[165] and chromatin immunoprecipitation experiments indicated that N-Myc directly represses Parkin by binding to the promoter region of the *PARK2* gene.[165] N-Myc is highly expressed in proliferating cells and notably downregulated in post-mitotic cells.[166] Conversely, Parkin is prominently expressed in post-mitotic neurons and accumulates in certain subsets of neurons as they mature and differentiate,[167,168] all of which indirectly suggest a potential inverse relationship between Parkin and N-Myc. Whether N-Myc-mediated regulation of Parkin and mitophagy is important in the development and progression of high-risk neuroblastoma remains to be established.

Lung cancer

As with glioblastoma, the loss of Parkin is a common mutation in lung cancers.[138] Both the upregulation of cyclin E[138] and ROS accumulation due to autophagy inhibition[169] have been purported drivers of tumour progression in lung cancer. *Atg5*-deficient, KRAS-driven lung cancers show a reduced clearance of damaged mitochondria and a concomitant impairment of mitochondrial oxidative phosphorylation.[170] Although this study ablated *Atg5*, a downstream effector of autophagosome membrane formation and extension in autophagy, the resulting phenotype of accumulation of ROS from damaged mitochondria phenocopies *Park2* null cells. Interestingly, however, these *Atg5* null tumours were less aggressive in mice, despite an accelerated onset of the disease.[170] The lung cancer basal epithelial cell line, A549 also has impaired mitophagic clearance, perhaps as a result of the downregulation of the fission protein Drp1.[171] As mentioned previously, appropriate fission and quarantine of damaged organelles from the mitochondrial network is required for mitophagy.[130] Induction of autophagy in the same cell line with a chemical inducer also arrested tumour proliferation and decreased survival,[172] but whether this is a mitophagy-specific response is still unclear. Another study found that mtDNA mutations in complex-I occurred in patients with lung cancer with no history of smoking. However, for patients with KRAS mutations, the correlation with increased mtDNA mutations was not significant,[173] highlighting the potential for defects in mitochondrial metabolism in promoting lung cancer.

Breast cancer

The ablation of mitophagy also affects the progression of breast cancer. The *PARK2*-containing FRA6E fragile region is commonly lost in certain breast cancers.[174,175] Supporting the role of Parkin as a tumour suppressor, copy number alterations in *PARK2* are seen at a relatively high frequency in aggressive triple-negative tumours.[176] Indeed, cyclins D and E, two reported substrates of Parkin, are upregulated in breast cancers.[177–180] Re-expression of Parkin in a number of breast cancer cell lines, including MCF7 cells, reduced the ability of these cells to form colonies in a clonogenic assay. This indicates that deletion of *PARK2* specifically caused the growth advantage, at least *in vitro*, and argues that the loss of the functional *PARK2* gene is not merely a passenger mutation, but an important driver of breast cancer.[138] Interestingly, mitochondrial metabolism is also a significant driver in breast cancer, with the growth of aggressive triple negative breast cancer lines arrested

© 2017 Macmillan Publishers Limited, part of Springer Nature.

by restoration of complex-I activity in an autophagy dependent-manner.[181]

Mitophagy also has a role in breast cancer by influencing adjacent stromal cells that alter the tumour microenvironment. The upregulation of BNIP3 and NIX in stromal fibroblasts accompanies a switch in cellular metabolism to produce more energy rich metabolites, including L-lactate, to promote tumour growth.[182] This was attributed to a mitophagic decrease in mitochondrial load and concomitant switching to a glycolytic metabolism.[182] Furthermore, loss of the TFAM in breast cancer-associated fibroblasts causes mitochondrial dysfunction.[183] This in turn generates hydrogen peroxide from damaged mitochondria and a switch to aerobic glycolysis and concomitant production of L-lactate, both of which promote adjacent tumour growth.[183] BNIP3 was also shown to suppress tumour progression in an aggressive mouse model of breast cancer.[184] As with Parkin loss in certain contexts, BNIP3 loss promoted tumour growth and metastasis by switching cells towards a tumourigenic glycolytic metabolism and increasing ROS production.[184] Thus, the ablation of both PINK1/Parkin-mediated mitophagy and alternate forms of mitophagy can contribute to the progression of breast cancers.

Ovarian cancer

Ovarian cancer is associated with downregulation of the general autophagy machinery, particularly Beclin1 and LC3.[185] And as noted in other tumours, reduced autophagic flux correlates with tumour progression in ovarian cancers.[185] Interestingly, the targeted inhibition of oxidative phosphorylation in ovarian cancer stem cells increased mitochondrial ROS generation, triggering cell death via inhibition of the mammalian target of rapamycin.[186] Chemical induction of autophagy halts ovarian tumour proliferation in a number of cell lines, but the macro-autophagic mechanism proposed is via endoplasmic reticulum stress, not mitochondrial stress.[187] This highlights, as others have observed, the difficulty in pinpointing the tumour suppressive mechanism of autophagy compared with mitophagy.[188] Although the loss of heterozygosity of the mitophagy receptor NIX was associated with late stage ovarian tumours, no conclusive link between ovarian cancer and loss of NIX has been reported.[189] Similar to breast cancer, the release of hydrogen peroxide from ovarian cancer cells promoted mitophagy in adjacent stromal cells, likely by the upregulation of NIX.[190] Ovarian cancer shares some key traits with both neuroblastoma and breast cancer in that N-Myc[191] and cyclin E[177] are both upregulated in a subset of these tumours. N-Myc has been shown to be a transcriptional repressor of Parkin[165] while cyclin E accumulates when functional Parkin is lost.[138] It is intriguing to speculate that Parkin may indirectly act as a potential cell cycle regulator in ovarian cancer.

Melanoma

Although patients with Parkinson's disease have a lower overall incidence of many cancers than the broader population the incidence of melanoma is higher.[24,192] Ultraviolet stress in melanocytes upregulates melanin, which is a known ROS scavenger and addition of melanin to melanoma cells decreases the net rate of both mitochondrial fission and mitophagy.[193] Superoxide damage is a major stressor in Ultraviolet-exposed melanocytes. The tumour suppressor p14^{ARF} has been shown to translocate to dysfunctional mitochondria in melanocytes to reduce superoxide levels and guard against metabolic reprogramming.[194] Together with Parkin-mediated mitochondrial clearance, this provides a rationale for why mitochondrial quality control may be an important tumour suppressor mechanism in melanoma.

Interestingly melanomas have been recently described as 'addicted' to autophagy, with disruption to the process by genetic or pharmacological inhibition contributing to tumour arrest.[195–199] This is phenocopied by BNIP3 depletion,[200] suggesting that mitophagy specifically contributes to melanoma progression, although alternative roles of BNIP3 are also implicated. How this is reconciled with the observation that Parkinson's patients, with impaired mitochondrial quality control, are sensitised to melanoma, remains unclear, but has been recently linked to the inactivating mutations in *PARK2*.[201] Notably there is a flux between autophagic dependence in developing melanoma, with early transformation associated with downregulated autophagic machinery and late stage metastatic disease showing a reliance on it.[195] Clearly, much is still to be resolved regarding the 'addiction' of melanoma to autophagy, and any specific role for mitophagy is currently unclear.

KRAS-driven tumours

KRAS-driven lung cancers, like melanomas, are an interesting exception to the hypothesis that mitophagy is able to suppress tumour progression. When mitophagic clearance is inhibited by conditional depletion of the essential autophagy effector, Atg5, the progression of lung tumours driven by mutated *KRAS* was impaired.[170] Despite this, the initial stages of the disease upon Cre-mediated deletion of *Atg5* are more severe, with mice having a higher tumour burden compared with controls.[170] Knock-out of *Atg7*, which functions with Atg5 during LC3/GABARAP lipidation, also impaired tumour development in the same model of lung cancer.[202] Similarly, *KRAS*-driven pancreatic cancers with ablated autophagy do not progress to high-grade neoplasias.[203] When p53 is abrogated in these cells, however, the disease progresses much more rapidly when autophagy is inhibited.[203] Recently, *KRAS*-mutant tumours were found to be highly and selectively sensitive to vitamin C-induced oxidative stress due to the rapid depletion of glutathione.[204] This suggests that *KRAS*-driven tumours might be sensitive to Parkin loss as they are highly glycolytic and overtly sensitive to oxidative stress.

OUTSTANDING QUESTIONS

The molecular mechanism of PINK1/Parkin-mediated mitophagy is becoming ever clearer, however many questions still remain including how the process contributes to disease, particularly tumour progression. It is unclear how Parkin tags defective mitochondria for clearance. Why are certain substrates such as Mfn1 and Mfn2 targeted and degraded so rapidly by Parkin compared with other substrates? Is this an epiphenomenon or an important determinant of mitochondrial turnover? How is Parkin activated to ubiquitinate non-mitochondrial targets including cyclins and do other kinases activate Parkin other than PINK1? The complex nature of the crosstalk between mitophagy and other cellular pathways still has to be interrogated. The degree to which mitophagy contributes to the control of mitochondrial dynamics, apoptosis, metabolism and cell growth warrants further investigation in different cellular contexts to determine their co-ordinated control. This insight may reveal new avenues to target diseases that are characterised by defective mitochondrial clearance, but may also reveal unappreciated consequences of directly targeting such processes, for example the apoptotic machinery, in the clinic.

A major paradox is the role of Parkin in promoting cell survival in neurons while fulfilling a tumour-suppressive role in numerous cancers. Similar anomalies need to be resolved between different cancer types, as loss of the mitophagy machinery can accelerate the progression of certain cancers while others appear 'addicted' to mitophagy. Evidence from melanomagenesis further highlights that autophagy itself is in flux in cancers, with its up- and downregulation at different stages of disease.

Although many studies have examined the role autophagy plays in cancer progression, few delineate whether this autophagic turnover is mitophagy or otherwise. Is the predominating

© 2017 Macmillan Publishers Limited, part of Springer Nature.

influence of defective mitophagy enhanced tumour progression due to a metabolic switch to glycolysis or an increased mutation load due to increased ROS generation? Alternatively, is tumour progression driven by the loss of Parkin's influence on cellular pathways independent of mitochondrial clearance, for example the appropriate turnover of cell cycle regulators? Given that defective mitophagy can influence diverse cellular pathways that are hallmarks or enabling characteristics of tumours[205] it is possible that appropriate mitochondrial clearance contributes to the suppression of tumours on numerous levels that are likely very much context dependent.

Targeting the mitophagy machinery therapeutically is a promising strategy for the treatment of a host of pathologies including Parkinson's disease, tuberculosis and cancer. Negative regulators of mitophagy, such as USP30, represent targets whose inhibition can enhance mitophagy and so potentially suppress the progression of Parkinson's disease or suppress tumour growth. By the same token, the inhibition of mitophagy in *KRAS*-driven cancers and melanoma may be a viable strategy to sensitise these tumours to therapeutics. The challenge in stimulating mitophagy in tumour cells is that many have a reduced pool of functional mitophagy effectors, such as Parkin, due to loss of heterozygosity or loss-of-function mutations. This challenge might be overcome in a number of ways. As mentioned, the inhibition of negative regulators is a promising avenue to enhance the mitophagic response. The mitophagy effectors can also be enhanced by stabilisation of PINK1 at mitochondrial membranes, perhaps by inhibiting PINK1 mitochondrial import and proteolysis, thus allowing accumulation of Parkin at the MOM for the induction of mitophagy. Perhaps even the stimulation of alternate mitophagy pathways via BNIP3 and NIX in tumour cells can complement the loss of PINK1/Parkin mediated mitophagy in both cancer as well as Parkinsonism. The influence of mitophagy on the progression of a variety of cancers makes the development of mitophagy enhancers a promising avenue to explore in the treatment of cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors acknowledge Paul Ekert and Jamie Fletcher for advice and discussions in the preparation of the manuscript. GD is supported by an Australian Research Council Future Fellowship (#FT100100791). Supported through operational infrastructure grants through the Victorian State Government Operational Infrastructure Support and the Australian Government NHMRC IRIISS.

REFERENCES

1 Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 1983; **219**: 979–980.
2 Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD. Mitochondrial complex I deficiency in Parkinson's disease. *Lancet* 1989; **1**: 1269.
3 Cleeter MW, Cooper JM, Schapira AH. Irreversible inhibition of mitochondrial complex I by 1-methyl-4-phenylpyridinium: evidence for free radical involvement. *J Neurochem* 1992; **58**: 786–789.
4 Sershen H, Mason MF, Hashim A, Lajtha A. Effect of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on age-related changes in dopamine turnover and transporter function in the mouse striatum. *Eur J Pharmacol* 1985; **113**: 135–136.
5 Wagner GC, Jarvis MF, Carelli RM. Ascorbic acid reduces the dopamine depletion induced by MPTP. *Neuropharmacology* 1985; **24**: 1261–1262.
6 Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S *et al.* Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 1998; **392**: 605–608.
7 Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S *et al.* Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 2004; **304**: 1158–1160.
8 Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E *et al.* Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. *Science* 2003; **299**: 256–259.
9 Xiong H, Wang D, Chen L, Choo YS, Ma H, Tang C *et al.* Parkin, PINK1, and DJ-1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. *J Clin Investig* 2009; **119**: 650–660.
10 Moore DJ, Zhang L, Troncoso J, Lee MK, Hattori N, Mizuno Y *et al.* Association of DJ-1 and parkin mediated by pathogenic DJ-1 mutations and oxidative stress. *Hum Mol Genet* 2005; **14**: 71–84.
11 Lucking CB, Abbas N, Durr A, Bonifati V, Bonnet AM, de Broucker T *et al.* Homozygous deletions in parkin gene in European and North African families with autosomal recessive juvenile parkinsonism. The European Consortium on Genetic Susceptibility in Parkinson's Disease and the French Parkinson's Disease Genetics Study Group. *Lancet* 1998; **352**: 1355–1356.
12 Hattori N, Kitada T, Matsumine H, Asakawa S, Yamamura Y, Yoshino H *et al.* Molecular genetic analysis of a novel Parkin gene in Japanese families with autosomal recessive juvenile parkinsonism: evidence for variable homozygous deletions in the Parkin gene in affected individuals. *Ann Neurol* 1998; **44**: 935–941.
13 Valente EM, Bentivoglio AR, Dixon PH, Ferraris A, Ialongo T, Frontali M *et al.* Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35–1p36. *Am J Hum Genet* 2001; **68**: 895–900.
14 Hatano Y, Sato K, Elibol B, Yoshino H, Yamamura Y, Bonifati V *et al.* PARK6-linked autosomal recessive early-onset parkinsonism in Asian populations. *Neurology* 2004; **63**: 1482–1485.
15 Hatano Y, Li Y, Sato K, Asakawa S, Yamamura Y, Tomiyama H *et al.* Novel PINK1 mutations in early-onset parkinsonism. *Ann Neurol* 2004; **56**: 424–427.
16 Kim SY, Seong MW, Jeon BS, Kim SY, Ko HS, Kim JY *et al.* Phase analysis identifies compound heterozygous deletions of the PARK2 gene in patients with early-onset Parkinson disease. *Clin Genet* 2012; **82**: 77–82.
17 Kay DM, Stevens CF, Hamza TH, Montimurro JS, Zabetian CP, Factor SA *et al.* A comprehensive analysis of deletions, multiplications, and copy number variations in PARK2. *Neurology* 2010; **75**: 1189–1194.
18 Foroud T, Uniacke SK, Liu L, Pankratz N, Rudolph A, Halter C *et al.* Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease. *Neurology* 2003; **60**: 796–801.
19 Hedrich K, Hagenah J, Djarmati A, Hiller A, Lohnau T, Lasek K *et al.* Clinical spectrum of homozygous and heterozygous PINK1 mutations in a large German family with Parkinson disease: role of a single hit? *Arch Neurol* 2006; **63**: 833–838.
20 Perez FA, Palmiter RD. Parkin-deficient mice are not a robust model of parkinsonism. *Proc Natl Acad Sci USA* 2005; **102**: 2174–2179.
21 Pickrell AM, Huang CH, Kennedy SR, Ordureau A, Sideris DP, Hoekstra JG *et al.* Endogenous parkin preserves dopaminergic substantia nigral neurons following mitochondrial DNA mutagenic stress. *Neuron* 2015; **87**: 371–381.
22 Fujiwara M, Marusawa H, Wang HQ, Iwai A, Ikeuchi K, Imai Y *et al.* Parkin as a tumor suppressor gene for hepatocellular carcinoma. *Oncogene* 2008; **27**: 6002–6011.
23 Zhang C, Lin M, Wu R, Wang X, Yang B, Levine AJ *et al.* Parkin, a p53 target gene, mediates the role of p53 in glucose metabolism and the Warburg effect. *Proc Natl Acad Sci USA* 2011; **108**: 16259–16264.
24 Inzelberg R, Jankovic J. Are Parkinson disease patients protected from some but not all cancers? *Neurology* 2007; **69**: 1542–1550.
25 Xu L, Lin DC, Yin D, Koeffler HP. An emerging role of PARK2 in cancer. *J Mol Med* 2014; **92**: 31–42.
26 Saito S, Sirahama S, Matsushima M, Suzuki M, Sagae S, Kudo R *et al.* Definition of a commonly deleted region in ovarian cancers to a 300- kb segment of chromosome 6q27. *Cancer Res* 1996; **56**: 5586–5589.
27 Cesari R, Martin ES, Calin GA, Pentimalli F, Bichi R, McAdams H *et al.* Parkin, a gene implicated in autosomal recessive juvenile parkinsonism, is a candidate tumor suppressor gene on chromosome 6q25–6q27. *Proc Natl Acad Sci USA* 2003; **100**: 5956–5961.
28 Denison SR, Wang F, Becker NA, Schule B, Kock N, Phillips LA *et al.* Alterations in the common fragile site gene Parkin in ovarian and other cancers. *Oncogene* 2003; **22**: 8370–8378.
29 Denison SR, Callahan G, Becker NA, Phillips LA, Smith DI. Characterization of FRA6E and its potential role in autosomal recessive juvenile parkinsonism and ovarian cancer. *Genes Chromosom Cancer* 2003; **38**: 40–52.
30 Kong FM, Anscher MS, Washington MK, Killian JK, Jirtle RL. M6P/IGF2R is mutated in squamous cell carcinoma of the lung. *Oncogene* 2000; **19**: 1572–1578.
31 Picchio MC, Martin ES, Cesari R, Calin GA, Yendamuri S, Kuroki T *et al.* Alterations of the tumor suppressor gene Parkin in non-small cell lung cancer. *Clin Cancer Res* 2004; **10**: 2720–2724.
32 Olsen JH, Friis S, Frederiksen K, McLaughlin JK, Mellemkjaer L, Moller H. Atypical cancer pattern in patients with Parkinson's disease. *Br J Cancer* 2005; **92**: 201–205.

© 2017 Macmillan Publishers Limited, part of Springer Nature.

33 Watson RO, Manzanillo PS, Cox JS. Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell* 2012; **150**: 803–815.
34 Bento CF, Empadinhas N, Mendes V. Autophagy in the fight against tuberculosis. *DNA Cell Biol* 2015; **34**: 228–242.
35 Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, Rae CS *et al.* The ubiquitin ligase parkin mediates resistance to intracellular pathogens. *Nature* 2013; **501**: 512–516.
36 Lazarou M. Keeping the immune system in check: a role for mitophagy. *Immunol Cell Biol* 2015; **93**: 3–10.
37 Lazarou M, Sliter DA, Kane LA, Sarraf SA, Wang C, Burman JL *et al.* The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. *Nature* 2015; **524**: 309–314.
38 Richter B, Sliter DA, Herhaus L, Stolz A, Wang C, Beli P *et al.* Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. *Proc Natl Acad Sci USA* 2016; **113**: 4039–4044.
39 Chen Y, Dorn GW 2nd. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. *Science* 2013; **340**: 471–475.
40 Gong G, Song M, Csordas G, Kelly DP, Matkovich SJ, Dorn GW 2nd. Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice. *Science* 2015; **350**: aad2459.
41 Durcan TM, Fon EA. The three 'P's of mitophagy: PARKIN, PINK1, and post-translational modifications. *Genes Dev* 2015; **29**: 989–999.
42 Pickrell AM, Youle RJ. The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. *Neuron* 2015; **85**: 257–273.
43 Trempe JF, Fon EA. Structure and function of Parkin, PINK1, and DJ-1, the three musketeers of neuroprotection. *Front Neurol* 2013; **4**: 38.
44 Scarffe LA, Stevens DA, Dawson VL, Dawson TM. Parkin and PINK1: much more than mitophagy. *Trends Neurosci* 2014; **37**: 315–324.
45 Meissner C, Lorenz H, Weihofen A, Selkoe DJ, Lemberg MK. The mitochondrial intramembrane protease PARL cleaves human Pink1 to regulate Pink1 trafficking. *J Neurochem* 2011; **117**: 856–867.
46 Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, Loh SH *et al.* PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet* 2011; **20**: 867–879.
47 Whitworth AJ, Lee JR, Ho VM, Flick R, Chowdhury R, McQuibban GA. Rhomboid-7 and HtrA2/Omi act in a common pathway with the Parkinson's disease factors Pink1 and Parkin. *Dis Models Mech* 2008; **1**: 168–174 discussion 173.
48 Yamano K, Youle RJ. PINK1 is degraded through the N-end rule pathway. *Autophagy* 2013; **9**: 1758–1769.
49 Jin SM, Lazarou M, Wang C, Kane LA, Narendra DP, Youle RJ. Mitochondrial membrane potential regulates PINK1 import and proteolytic destabilization by PARL. *J Cell Biol* 2010; **191**: 933–942.
50 Vives-Bauza C, Zhou C, Huang Y, Cui M, de Vries RL, Kim J *et al.* PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc Natl Acad Sci USA* 2010; **107**: 378–383.
51 Matsuda N, Sato S, Shiba K, Okatsu K, Saisho K, Gautier CA *et al.* PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J Cell Biol* 2010; **189**: 211–221.
52 Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J *et al.* PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol* 2010; **8**: e1000298.
53 Jin SM, Youle RJ. The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria. *Autphagy* 2013; **9**: 1750–1757.
54 Lazarou M, Jin SM, Kane LA, Youle RJ. Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. *Dev Cell* 2012; **22**: 320–333.
55 Okatsu K, Uno M, Koyano F, Go E, Kimura M, Oka T *et al.* A dimeric PINK1-containing complex on depolarized mitochondria stimulates Parkin recruitment. *J Biol Chem* 2013; **288**: 36372–36384.
56 Riley BE, Lougheed JC, Callaway K, Velasquez M, Brecht E, Nguyen L *et al.* Structure and function of Parkin E3 ubiquitin ligase reveals aspects of RING and HECT ligases. *Nat Commun* 2013; **4**: 1982.
57 Kondapalli C, Kazlauskaite A, Zhang N, Woodroof HI, Campbell DG, Gourlay R *et al.* PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open Biol* 2012; **2**: 120080.
58 Trempe JF, Sauve V, Grenier K, Seirafi M, Tang MY, Menade M *et al.* Structure of parkin reveals mechanisms for ubiquitin ligase activation. *Science* 2013; **340**: 1451–1455.
59 Hristova VA, Beasley SA, Rylett RJ, Shaw GS. Identification of a novel Zn^{2+}-binding domain in the autosomal recessive juvenile Parkinson-related E3 ligase parkin. *J Biol Chem* 2009; **284**: 14978–14986.
60 Kazlauskaite A, Kondapalli C, Gourlay R, Campbell DG, Ritorto MS, Hofmann K *et al.* Parkin is activated by PINK1-dependent phosphorylation of ubiquitin at Ser65. *Biochem J* 2014; **460**: 127–139.
61 Sarraf SA, Raman M, Guarani-Pereira V, Sowa ME, Huttlin EL, Gygi SP *et al.* Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. *Nature* 2013; **496**: 372–376.
62 Chan NC, Salazar AM, Pham AH, Sweredoski MJ, Kolawa NJ, Graham RL *et al.* Broad activation of the ubiquitin-proteasome system by Parkin is critical for mitophagy. *Hum Mol Genet* 2011; **20**: 1726–1737.
63 Ziviani E, Tao RN, Whitworth AJ. Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. *Proc Natl Acad Sci USA* 2010; **107**: 5018–5023.
64 Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH *et al.* Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature* 2006; **441**: 1162–1166.
65 Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA *et al.* PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol* 2014; **205**: 143–153.
66 Kazlauskaite A, Martinez-Torres RJ, Wilkie S, Kumar A, Peltier J, Gonzalez A *et al.* Binding to serine 65-phosphorylated ubiquitin primes Parkin for optimal PINK1-dependent phosphorylation and activation. *EMBO Rep* 2015; **16**: 939–954.
67 Koyano F, Okatsu K, Kosako H, Tamura Y, Go E, Kimura M *et al.* Ubiquitin is phosphorylated by PINK1 to activate parkin. *Nature* 2014; **510**: 162–166.
68 Ordureau A, Sarraf SA, Duda DM, Heo JM, Jedrychowski MP, Sviderskiy VO *et al.* Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis. *Mol Cell* 2014; **56**: 360–375.
69 Ordureau A, Heo JM, Duda DM, Paulo JA, Olszewski JL, Yanishevski D *et al.* Defining roles of PARKIN and ubiquitin phosphorylation by PINK1 in mitochondrial quality control using a ubiquitin replacement strategy. *Proc Natl Acad Sci USA* 2015; **112**: 6637–6642.
70 Komander D, Rape M. The ubiquitin code. *Annu Rev Biochem* 2012; **81**: 203–229.
71 Cornelissen T, Haddad D, Wauters F, Van Humbeeck C, Mandemakers W, Koentjoro B *et al.* The deubiquitinase USP15 antagonizes Parkin-mediated mitochondrial ubiquitination and mitophagy. *Hum Mol Genet* 2014; **23**: 5227–5242.
72 Bingol B, Tea JS, Phu L, Reichelt M, Bakalarski CE, Song Q *et al.* The mitochondrial deubiquitinase USP30 opposes parkin-mediated mitophagy. *Nature* 2014; **510**: 370–375.
73 Cunningham CN, Baughman JM, Phu L, Tea JS, Yu C, Coons M *et al.* USP30 and parkin homeostatically regulate atypical ubiquitin chains on mitochondria. *Nat Cell Biol* 2015; **17**: 160–169.
74 Liang JR, Martinez A, Lane JD, Mayor U, Clague MJ, Urbe S. USP30 deubiquitylates mitochondrial Parkin substrates and restricts apoptotic cell death. *EMBO Rep* 2015; **16**: 618–627.
75 Durcan TM, Tang MY, Perusse JR, Dashti EA, Aguileta MA, McLelland GL *et al.* USP8 regulates mitophagy by removing K6-linked ubiquitin conjugates from parkin. *EMBO J* 2014; **33**: 2473–2491.
76 Geisler S, Holmstrom KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ *et al.* PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol* 2010; **12**: 119–131.
77 Narendra D, Kane LA, Hauser DN, Fearnley IM, Youle RJ. p62/SQSTM1 is required for Parkin-induced mitochondrial clustering but not mitophagy; VDAC1 is dispensable for both. *Autophagy* 2010; **6**: 1090–1106.
78 Wong YC, Holzbaur EL. Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *Proc Natl Acad Sci USA* 2014; **111**: E4439–E4448.
79 Heo JM, Ordureau A, Paulo JA, Rinehart J, Harper JW. The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. *Mol Cell* 2015; **60**: 7–20.
80 Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y, Yoshimori T. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J Cell Sci* 2004; **117**: 2805–2812.
81 Kataoka T, Holler N, Micheau O, Martinon F, Tinel A, Hofmann K *et al.* Bcl-rambo, a novel Bcl-2 homologue that induces apoptosis via its unique C-terminal extension. *J Biol Chem* 2001; **276**: 19548–19554.
82 Murakawa T, Yamaguchi O, Hashimoto A, Hikoso S, Takeda T, Oka T *et al.* Bcl-2-like protein 13 is a mammalian Atg32 homologue that mediates mitophagy and mitochondrial fragmentation. *Nat Commun* 2015; **6**: 7527.
83 Lamb CA, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol* 2013; **14**: 759–774.
84 Yoshimori T, Yamamoto A, Moriyama Y, Futai M, Tashiro Y. Bafilomycin A1, a specific inhibitor of vacuolar-type H(+)-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. *J Biol Chem* 1991; **266**: 17707–17712.
85 Boland ML, Chourasia AH, Macleod KF. Mitochondrial dysfunction in cancer. *Front Oncol* 2013; **3**: 292.

© 2017 Macmillan Publishers Limited, part of Springer Nature.

86 Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A *et al.* Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep* 2010; **11**: 45–51.
87 Schwarten M, Mohrluder J, Ma P, Stoldt M, Thielmann Y, Stangler T *et al.* Nix directly binds to GABARAP: a possible crosstalk between apoptosis and autophagy. *Autophagy* 2009; **5**: 690–698.
88 Hanna RA, Quinsay MN, Orogo AM, Giang K, Rikka S, Gustafsson AB. Microtubule-associated protein 1 light chain 3 (LC3) interacts with Bnip3 protein to selectively remove endoplasmic reticulum and mitochondria via autophagy. *J Biol Chem* 2012; **287**: 19094–19104.
89 Ray R, Chen G, Vande Velde C, Cizeau J, Park JH, Reed JC *et al.* BNIP3 heterodimerizes with Bcl-2/Bcl-X(L) and induces cell death independent of a Bcl-2 homology 3 (BH3) domain at both mitochondrial and nonmitochondrial sites. *J Biol Chem* 2000; **275**: 1439–1448.
90 Zhu Y, Massen S, Terenzio M, Lang V, Chen-Lindner S, Eils R *et al.* Modulation of serines 17 and 24 in the LC3-interacting region of Bnip3 determines pro-survival mitophagy versus apoptosis. *J Biol Chem* 2013; **288**: 1099–1113.
91 Liu L, Feng D, Chen G, Chen M, Zheng Q, Song P *et al.* Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. *Nat Cell Biol* 2012; **14**: 177–185.
92 Wu W, Tian W, Hu Z, Chen G, Huang L, Li W *et al.* ULK1 translocates to mitochondria and phosphorylates FUNDC1 to regulate mitophagy. *EMBO Rep* 2014; **15**: 566–575.
93 Chen G, Han Z, Feng D, Chen Y, Chen L, Wu H *et al.* A regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. *Mol Cell* 2014; **54**: 362–377.
94 Liu L, Sakakibara K, Chen Q, Okamoto K. Receptor-mediated mitophagy in yeast and mammalian systems. *Cell Res* 2014; **24**: 787–795.
95 Wu H, Xue D, Chen G, Han Z, Huang L, Zhu C *et al.* The BCL2L1 and PGAM5 axis defines hypoxia-induced receptor-mediated mitophagy. *Autophagy* 2014; **10**: 1712–1725.
96 Lu W, Karuppagounder SS, Springer DA, Allen MD, Zheng L, Chao B *et al.* Genetic deficiency of the mitochondrial protein PGAM5 causes a Parkinson's-like movement disorder. *Nat Commun* 2014; **5**: 4930.
97 Lo SC, Hannink M. PGAM5, a Bcl-XL-interacting protein, is a novel substrate for the redox-regulated Keap1-dependent ubiquitin ligase complex. *J Biol Chem* 2006; **281**: 37893–37903.
98 Wang Z, Jiang H, Chen S, Du F, Wang X. The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways. *Cell* 2012; **148**: 228–243.
99 Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S *et al.* The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity* 2013; **39**: 443–453.
100 Yun J, Puri R, Yang H, Lizzio MA, Wu C, Sheng ZH *et al.* MUL1 acts in parallel to the PINK1/parkin pathway in regulating mitofusin and compensates for loss of PINK1/parkin. *eLife* 2014; **3**: e01958.
101 Fu M, St-Pierre P, Shankar J, Wang PT, Joshi B, Nabi IR. Regulation of mitophagy by the Gp78 E3 ubiquitin ligase. *Mol Biol Cell* 2013; **24**: 1153–1162.
102 Orvedahl A, Sumpter Jr R, Xiao G, Ng A, Zou Z, Tang Y *et al.* Image-based genome-wide siRNA screen identifies selective autophagy factors. *Nature* 2011; **480**: 113–117.
103 Green DR, Galluzzi L, Kroemer G. Cell biology. Metabolic control of cell death. *Science* 2014; **345**: 1250256.
104 Li MX, Dewson G. Mitochondria and apoptosis: emerging concepts. *F1000Prime Rep* 2015; **7**: 42.
105 Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat Rev Mol Cell Biol* 2014; **15**: 49–63.
106 Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 2008; **9**: 47–59.
107 Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008; **9**: 231–241.
108 Maiuri MC, Le Toumelin G, Criollo A, Rain JC, Gautier F, Juin P *et al.* Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. *Embo J* 2007; **26**: 2527–2539.
109 Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N *et al.* Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 2005; **122**: 927–939.
110 Lindqvist LM, Heinlein M, Huang DC, Vaux DL. Prosurvival Bcl-2 family members affect autophagy only indirectly, by inhibiting Bax and Bak. *Proc Natl Acad Sci USA* 2014; **111**: 8512–8517.
111 Hollville E, Carroll RG, Cullen SP, Martin SJ. Bcl-2 family proteins participate in mitochondrial quality control by regulating Parkin/PINK1-dependent mitophagy. *Mol Cell* 2014; **55**: 451–466.
112 Carroll RG, Hollville E, Martin SJ. Parkin sensitizes toward apoptosis induced by mitochondrial depolarization through promoting degradation of Mcl-1. *Cell Rep* 2014; **9**: 1538–1553.
113 Johnson BN, Berger AK, Cortese GP, Lavoie MJ. The ubiquitin E3 ligase parkin regulates the proapoptotic function of Bax. *Proc Natl Acad Sci USA* 2012; **109**: 6283–6288.
114 Charan RA, Johnson BN, Zaganelli S, Nardozzi JD, LaVoie MJ. Inhibition of apoptotic Bax translocation to the mitochondria is a central function of parkin. *Cell Death Dis* 2014; **5**: e1313.
115 Chen D, Gao F, Li B, Wang H, Xu Y, Zhu C *et al.* Parkin mono-ubiquitinates Bcl-2 and regulates autophagy. *J Biol Chem* 2010; **285**: 38214–38223.
116 Zhang C, Lee S, Peng Y, Bunker E, Giaime E, Shen J *et al.* PINK1 triggers autocatalytic activation of Parkin to specify cell fate decisions. *Curr Biol* 2014; **24**: 1854–1865.
117 Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W *et al.* Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. *J Exp Med* 1998; **187**: 1477–1485.
118 Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S *et al.* Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nat Immunol* 2000; **1**: 489–495.
119 Radogna F, Cerella C, Gaigneaux A, Christov C, Dicato M, Diederich M. Cell type-dependent ROS and mitophagy response leads to apoptosis or necroptosis in neuroblastoma. *Oncogene* 2015; **35**: 2839–2853.
120 Xia M, Meng G, Jiang A, Chen A, Dahlhaus M, Gonzalez P *et al.* Mitophagy switches cell death from apoptosis to necrosis in NSCLC cells treated with oncolytic measles virus. *Oncotarget* 2014; **5**: 3907–3918.
121 Tait SW, Oberst A, Quarato G, Milasta S, Haller M, Wang R *et al.* Widespread mitochondrial depletion via mitophagy does not compromise necroptosis. *Cell Rep* 2013; **5**: 878–885.
122 Remijsen Q, Goossens V, Grootjans S, Van den Haute C, Vanlangenakker N, Dondelinger Y *et al.* Depletion of RIPK3 or MLKL blocks TNF-driven necroptosis and switches towards a delayed RIPK1 kinase-dependent apoptosis. *Cell Death Dis* 2014; **5**: e1004.
123 Lu W, Sun J, Yoon JS, Zhang Y, Zheng L, Murphy E *et al.* Mitochondrial protein PGAM5 regulates mitophagic protection against cell necroptosis. *PLoS One* 2016; **11**: e0147792.
124 Okatsu K, Saisho K, Shimanuki M, Nakada K, Shitara H, Sou YS *et al.* p62/SQSTM1 cooperates with Parkin for perinuclear clustering of depolarized mitochondria. *Genes Cells* 2010; **15**: 887–900.
125 Detmer SA, Chan DC. Functions and dysfunctions of mitochondrial dynamics. *Nat Rev Mol Cell Biol* 2007; **8**: 870–879.
126 Otera H, Mihara K. Molecular mechanisms and physiologic functions of mitochondrial dynamics. *J Biochem* 2011; **149**: 241–251.
127 Gegg ME, Cooper JM, Chau KY, Rojo M, Schapira AH, Taanman JW. Mitofusin 1 and mitofusin 2 are ubiquitinated in a PINK1/parkin-dependent manner upon induction of mitophagy. *Hum Mol Genet* 2010; **19**: 4861–4870.
128 Poole AC, Thomas RE, Yu S, Vincow ES, Pallanck L. The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/parkin pathway. *PLoS One* 2010; **5**: e10054.
129 Tanaka A, Cleland MM, Xu S, Narendra DP, Suen DF, Karbowski M *et al.* Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J Cell Biol* 2010; **191**: 1367–1380.
130 Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G *et al.* Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 2008; **27**: 433–446.
131 Wang H, Song P, Du L, Tian W, Yue W, Liu M *et al.* Parkin ubiquitinates Drp1 for proteasome-dependent degradation: implication of dysregulated mitochondrial dynamics in Parkinson disease. *J Biol Chem* 2011; **286**: 11649–11658.
132 Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D *et al.* PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell* 2011; **147**: 893–906.
133 Birsa N, Norkett R, Wauer T, Mevissen TE, Wu HC, Foltynie T *et al.* Lysine 27 ubiquitination of the mitochondrial transport protein Miro is dependent on serine 65 of the Parkin ubiquitin ligase. *J Biol Chem* 2014; **289**: 14569–14582.
134 Fransson S, Ruusala A, Aspenstrom P. The atypical Rho GTPases Miro-1 and Miro-2 have essential roles in mitochondrial trafficking. *Biochem Biophys Res Commun* 2006; **344**: 500–510.
135 Landes T, Emorine LJ, Courilleau D, Rojo M, Belenguer P, Arnaune-Pelloquin L. The BH3-only Bnip3 binds to the dynamin Opa1 to promote mitochondrial fragmentation and apoptosis by distinct mechanisms. *EMBO Rep* 2010; **11**: 459–465.
136 Quinsay MN, Lee Y, Rikka S, Sayen MR, Molkentin JD, Gottlieb RA *et al.* Bnip3 mediates permeabilization of mitochondria and release of cytochrome c via a novel mechanism. *J Mol Cell Cardiol* 2010; **48**: 1146–1156.

© 2017 Macmillan Publishers Limited, part of Springer Nature.

137 Staropoli JF, McDermott C, Martinat C, Schulman B, Demireva E, Abeliovich A. Parkin is a component of an SCF-like ubiquitin ligase complex and protects postmitotic neurons from kainate excitotoxicity. *Neuron* 2003; **37**: 735–749.
138 Veeriah S, Taylor BS, Meng S, Fang F, Yilmaz E, Vivanco I *et al.* Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. *Nat Genet* 2010; **42**: 77–82.
139 Gong Y, Zack TI, Morris LG, Lin K, Hukkelhoven E, Raheja R *et al.* Pan-cancer genetic analysis identifies PARK2 as a master regulator of G1/S cyclins. *Nat Genet* 2014; **46**: 588–594.
140 Ko HS, von Coelln R, Sriram SR, Kim SW, Chung KK, Pletnikova O *et al.* Accumulation of the authentic parkin substrate aminoacyl-tRNA synthetase cofactor, p38/JTV-1, leads to catecholaminergic cell death. *J Neurosci: Off J Soc Neurosci* 2005; **25**: 7968–7978.
141 da Costa CA, Sunyach C, Giaime E, West A, Corti O, Brice A *et al.* Transcriptional repression of p53 by parkin and impairment by mutations associated with autosomal recessive juvenile Parkinson's disease. *Nat Cell Biol* 2009; **11**: 1370–1375.
142 Palacino JJ, Sagi D, Goldberg MS, Krauss S, Motz C, Wacker M *et al.* Mitochondrial dysfunction and oxidative damage in parkin-deficient mice. *J Biol Chem* 2004; **279**: 18614–18622.
143 Kim KY, Stevens MV, Akter MH, Rusk SE, Huang RJ, Cohen A *et al.* Parkin is a lipid-responsive regulator of fat uptake in mice and mutant human cells. *J Clin Investig* 2011; **121**: 3701–3712.
144 Liu K, Li F, Han H, Chen Y, Mao Z, Luo J *et al.* Parkin regulates the activity of pyruvate kinase M2. *J Biol Chem* 2016; **291**: 10307–10317.
145 Sabharwal SS, Schumacker PT. Mitochondrial ROS in cancer: initiators, amplifiers or an Achilles' heel?. *Nat Rev Cancer* 2014; **14**: 709–721.
146 Wallace DC. Mitochondria and cancer. *Nat Rev Cancer* 2012; **12**: 685–698.
147 Dexter DT, Sian J, Rose S, Hindmarsh JG, Mann VM, Cooper JM *et al.* Indices of oxidative stress and mitochondrial function in individuals with incidental Lewy body disease. *Ann Neurol* 1994; **35**: 38–44.
148 Itier JM, Ibanez P, Mena MA, Abbas N, Cohen-Salmon C, Bohme GA *et al.* Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. *Hum Mol Genet* 2003; **12**: 2277–2291.
149 Hyun DH, Lee M, Hattori N, Kubo S, Mizuno Y, Halliwell B *et al.* Effect of wild-type or mutant Parkin on oxidative damage, nitric oxide, antioxidant defenses, and the proteasome. *J Biol Chem* 2002; **277**: 28572–28577.
150 Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci USA* 1997; **94**: 514–519.
151 Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT. Loss of the SdhB, but Not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol Cell Biol* 2008; **28**: 718–731.
152 Semenza GL. Regulation of cancer cell metabolism by hypoxia-inducible factor 1. *Semin Cancer Biol* 2009; **19**: 12–16.
153 Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. *Genes Dev* 2009; **23**: 537–548.
154 Kuroda Y, Mitsui T, Kunishige M, Shono M, Akaike M, Azuma H *et al.* Parkin enhances mitochondrial biogenesis in proliferating cells. *Hum Mol Genet* 2006; **15**: 883–895.
155 Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. *Science* 2008; **319**: 1352–1355.
156 Burrell RA, McClelland SE, Endesfelder D, Groth P, Weller MC, Shaikh N *et al.* Replication stress links structural and numerical cancer chromosomal instability. *Nature* 2013; **494**: 492–496.
157 Plun-Favreau H, Lewis PA, Hardy J, Martins LM, Wood NW. Cancer and neurodegeneration: between the devil and the deep blue sea. *PLoS Genet* 2010; **6**: e1001257.
158 Morris LG, Veeriah S, Chan TA. Genetic determinants at the interface of cancer and neurodegenerative disease. *Oncogene* 2010; **29**: 3453–3464.
159 Poulogiannis G, McIntyre RE, Dimitriadi M, Apps JR, Wilson CH, Ichimura K *et al.* PARK2 deletions occur frequently in sporadic colorectal cancer and accelerate adenoma development in Apc mutant mice. *Proc Natl Acad Sci USA* 2010; **107**: 15145–15150.
160 Vakiani E, Janakiraman M, Shen R, Sinha R, Zeng Z, Shia J *et al.* Comparative genomic analysis of primary versus metastatic colorectal carcinomas. *J Clin Oncol* 2012; **30**: 2956–2962.
161 Mulholland PJ, Fiegler H, Mazzanti C, Gorman P, Sasieni P, Adams J *et al.* Genomic profiling identifies discrete deletions associated with translocations in glioblastoma multiforme. *Cell Cycle* 2006; **5**: 783–791.
162 Viotti J, Duplan E, Caillava C, Condat J, Goiran T, Giordano C *et al.* Glioma tumor grade correlates with parkin depletion in mutant p53-linked tumors and results from loss of function of p53 transcriptional activity. *Oncogene* 2014; **33**: 1764–1775.
163 Lee KS, Wu Z, Song Y, Mitra SS, Feroze AH, Cheshier SH *et al.* Roles of PINK1, mTORC2, and mitochondria in preserving brain tumor-forming stem cells in a noncanonical Notch signaling pathway. *Genes Dev* 2013; **27**: 2642–2647.
164 Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D *et al.* The genetic landscape of high-risk neuroblastoma. *Nat Genet* 2013; **45**: 279–284.
165 West AB, Kapatos G, O'Farrell C, Gonzalez-de-Chavez F, Chiu K, Farrer MJ *et al.* N-myc regulates parkin expression. *J Biol Chem* 2004; **279**: 28896–28902.
166 Strieder V, Lutz W. Regulation of N-myc expression in development and disease. *Cancer Lett* 2002; **180**: 107–119.
167 Wang M, Suzuki T, Kitada T, Asakawa S, Minoshima S, Shimizu N *et al.* Developmental changes in the expression of parkin and UbcR7, a parkin-interacting and ubiquitin-conjugating enzyme, in rat brain. *J Neurochem* 2001; **77**: 1561–1568.
168 Kitada T, Asakawa S, Minoshima S, Mizuno Y, Shimizu N. Molecular cloning, gene expression, and identification of a splicing variant of the mouse parkin gene. *Mamm Genome: Off J Int Mamm Genome Soc* 2000; **11**: 417–421.
169 Kaminskyy VO, Piskunova T, Zborovskaya IB, Tchevkina EM, Zhivotovsky B. Suppression of basal autophagy reduces lung cancer cell proliferation and enhances caspase-dependent and -independent apoptosis by stimulating ROS formation. *Autophagy* 2012; **8**: 1032–1044.
170 Rao S, Tortola L, Perlot T, Wirnsberger G, Novatchkova M, Nitsch R *et al.* A dual role for autophagy in a murine model of lung cancer. *Nat Commun* 2014; **5**: 3056.
171 Thomas KJ, Jacobson MR. Defects in mitochondrial fission protein dynamin-related protein 1 are linked to apoptotic resistance and autophagy in a lung cancer model. *PLoS One* 2012; **7**: e45319.
172 Hung JY, Hsu YL, Li CT, Ko YC, Ni WC, Huang MS *et al.* 6-Shogaol, an active constituent of dietary ginger, induces autophagy by inhibiting the AKT/mTOR pathway in human non-small cell lung cancer A549 cells. *J Agric Food Chem* 2009; **57**: 9809–9816.
173 Dasgupta S, Soudry E, Mukhopadhyay N, Shao C, Yee J, Lam S *et al.* Mitochondrial DNA mutations in respiratory complex-I in never-smoker lung cancer patients contribute to lung cancer progression and associated with EGFR gene mutation. *J Cell Physiol* 2012; **227**: 2451–2460.
174 Noviello C, Courjal F, Theillet C. Loss of heterozygosity on the long arm of chromosome 6 in breast cancer: possibly four regions of deletion. *Clin Cancer Res* 1996; **2**: 1601–1606.
175 Orphanos V, McGown G, Hey Y, Boyle JM, Santibanez-Koref M. Proximal 6q, a region showing allele loss in primary breast cancer. *Br J Cancer* 1995; **71**: 290–293.
176 Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y *et al.* The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature* 2012; **486**: 395–399.
177 Courjal F, Louason G, Speiser P, Katsaros D, Zeillinger R, Theillet C. Cyclin gene amplification and overexpression in breast and ovarian cancers: evidence for the selection of cyclin D1 in breast and cyclin E in ovarian tumors. *Int J Cancer* 1996; **69**: 247–253.
178 Bartkova J, Lukas J, Muller H, Lutzhoft D, Strauss M, Bartek J. Cyclin D1 protein expression and function in human breast cancer. *Int J Cancer* 1994; **57**: 353–361.
179 Buckley MF, Sweeney KJ, Hamilton JA, Sini RL, Manning DL, Nicholson RI *et al.* Expression and amplification of cyclin genes in human breast cancer. *Oncogene* 1993; **8**: 2127–2133.
180 Gillett C, Fantl V, Smith R, Fisher C, Bartek J, Dickson C *et al.* Amplification and overexpression of cyclin D1 in breast cancer detected by immunohistochemical staining. *Cancer Res* 1994; **54**: 1812–1817.
181 Santidrian AF, Matsuno-Yagi A, Ritland M, Seo BB, LeBoeuf SE, Gay LJ *et al.* Mitochondrial complex I activity and NAD+/NADH balance regulate breast cancer progression. *J Clin Investig* 2013; **123**: 1068–1081.
182 Chiavarina B, Whitaker-Menezes D, Migneco G, Martinez-Outschoorn UE, Pavlides S, Howell A *et al.* HIF1-alpha functions as a tumor promoter in cancer associated fibroblasts, and as a tumor suppressor in breast cancer cells: autophagy drives compartment-specific oncogenesis. *Cell Cycle* 2010; **9**: 3534–3551.
183 Balliet RM, Capparelli C, Guido C, Pestell TG, Martinez-Outschoorn UE, Lin Z *et al.* Mitochondrial oxidative stress in cancer-associated fibroblasts drives lactate production, promoting breast cancer tumor growth: understanding the aging and cancer connection. *Cell Cycle* 2011; **10**: 4065–4073.
184 Chourasia AH, Tracy K, Frankenberger C, Boland ML, Sharifi MN, Drake LE *et al.* Mitophagy defects arising from BNip3 loss promote mammary tumor progression to metastasis. *EMBO Rep* 2015; **16**: 1145–1163.
185 Shen Y, Li DD, Wang LL, Deng R, Zhu XF. Decreased expression of autophagy-related proteins in malignant epithelial ovarian cancer. *Autophagy* 2008; **4**: 1067–1068.

© 2017 Macmillan Publishers Limited, part of Springer Nature.

186 Alvero AB, Montagna MK, Holmberg JC, Craveiro V, Brown D, Mor G. Targeting the mitochondria activates two independent cell death pathways in ovarian cancer stem cells. *Mol Cancer Ther* 2011; **10**: 1385–1393.
187 Kandala PK, Srivastava SK. Regulation of macroautophagy in ovarian cancer cells in vitro and in vivo by controlling glucose regulatory protein 78 and AMPK. *Oncotarget* 2012; **3**: 435–449.
188 Chourasia AH, Boland ML, Macleod KF. Mitophagy and cancer. *Cancer Metab* 2015; **3**: 4.
189 Lai J, Flanagan J, Phillips WA, Chenevix-Trench G, Arnold J. Analysis of the candidate 8p21 tumour suppressor, BNIP3L, in breast and ovarian cancer. *Br J Cancer* 2003; **88**: 270–276.
190 Martinez-Outschoorn UE, Balliet RM, Lin Z, Whitaker-Menezes D, Howell A, Sotgia F *et al.* Hereditary ovarian cancer and two-compartment tumor metabolism: epithelial loss of BRCA1 induces hydrogen peroxide production, driving oxidative stress and NFkappaB activation in the tumor stroma. *Cell Cycle* 2012; **11**: 4152–4166.
191 Helland A, Anglesio MS, George J, Cowin PA, Johnstone CN, House CM *et al.* Deregulation of MYCN, LIN28B and LET7 in a molecular subtype of aggressive high-grade serous ovarian cancers. *PLoS One* 2011; **6**: e18064.
192 Moller H, Mellemkjaer L, McLaughlin JK, Olsen JH. Occurrence of different cancers in patients with Parkinson's disease. *BMJ* 1995; **310**: 1500–1501.
193 Zanchetta LM, Garcia A, Lyng F, Walsh J, Murphy JE. Mitophagy and mitochondrial morphology in human melanoma-derived cells post exposure to simulated sunlight. *Int J Radiat Biol* 2011; **87**: 506–517.
194 Christensen C, Bartkova J, Mistrik M, Hall A, Lange MK, Ralfkiaer U *et al.* A short acidic motif in ARF guards against mitochondrial dysfunction and melanoma susceptibility. *Nat Commun* 2014; **5**: 5348.
195 Maes H, Agostinis P. Autophagy and mitophagy interplay in melanoma progression. *Mitochondrion* 2014; **19 Pt A**: 58–68.
196 Marino ML, Pellegrini P, Di Lernia G, Djavaheri-Mergny M, Brnjic S, Zhang X *et al.* Autophagy is a protective mechanism for human melanoma cells under acidic stress. *J Biol Chem* 2012; **287**: 30664–30676.
197 Sheen JH, Zoncu R, Kim D, Sabatini DM. Defective regulation of autophagy upon leucine deprivation reveals a targetable liability of human melanoma cells in vitro and in vivo. *Cancer Cell* 2011; **19**: 613–628.
198 Tormo D, Checinska A, Alonso-Curbelo D, Perez-Guijarro E, Canon E, Riveiro-Falkenbach E *et al.* Targeted activation of innate immunity for therapeutic induction of autophagy and apoptosis in melanoma cells. *Cancer Cell* 2009; **16**: 103–114.
199 Xie X, White EP, Mehnert JM. Coordinate autophagy and mTOR pathway inhibition enhances cell death in melanoma. *PLoS One* 2013; **8**: e55096.
200 Maes H, Van Eygen S, Krysko DV, Vandenabeele P, Nys K, Rillaerts K *et al.* BNIP3 supports melanoma cell migration and vasculogenic mimicry by orchestrating the actin cytoskeleton. *Cell Death Dis* 2014; **5**: e1127.
201 Hu HH, Kannengiesser C, Lesage S, Andre J, Mourah S, Michel L *et al.* PARKIN inactivation links Parkinson's disease to melanoma. *J Natl Cancer Inst* 2016; **108**: djv340.
202 Guo JY, Karsli-Uzunbas G, Mathew R, Aisner SC, Kamphorst JJ, Strohecker AM *et al.* Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev* 2013; **27**: 1447–1461.
203 Rosenfeldt MT, O'Prey J, Morton JP, Nixon C, MacKay G, Mrowinska A *et al.* p53 status determines the role of autophagy in pancreatic tumour development. *Nature* 2013; **504**: 296–300.
204 Yun J, Mullarky E, Lu C, Bosch KN, Kavalier A, Rivera K *et al.* Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* 2015; **350**: 1391–1396.
205 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646–674.

© 2017 Macmillan Publishers Limited, part of Springer Nature.