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Review

p53 and autophagy in cancer: Guardian of the genome meets guardian of the proteome

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ABSTRACT

This review provides a summary of the European Association for Cancer Research 'Cancer Researcher Award' lecture which was presented at the EACR21 meeting in Oslo, Norway, in July 2010. The review focuses on the importance of programmed cell death regulation in tumour development and cancer therapy. Eradication of damaged cells is a principal mechanism of protection against cancer and involves key tumour suppressor proteins such as p53. Cell death-associated tumour suppressors, including p53, are often inactivated during the genesis of cancer and this poses problems for many forms of therapy which require these death proteins for a therapeutic response. The identification therefore of other factors and pathways that regulate cell viability is of prime importance for the development of rationalised new strategies to invoke tumour cell death. Historically, studies of programmed cell death in cancer have focused on the evolutionarily conserved process of apoptosis. More recently, however, attention has also turned to another process termed 'autophagy' which has profound effects on cell viability. Principally, autophagy serves to traffic damaged proteins and organelles to the lysosome for degradation. It functions therefore as a homeostatic mechanism that impinges on both protein and genome integrity. Summarized here are our findings linking p53 to autophagy and how this led to the identification of the human Damage-Regulated Autophagy Modulator (DRAM) family. Further discussion relates to our subsequent studies, together with those of others, that have yielded insights into the selective targeting of autophagy for the treatment of malignant disease.

1. p53 and programmed cell death

Our bodies encounter mutational events on a daily basis which, if left unchecked, may lead to the development of cancer. Cellular mechanisms are therefore in place to counter this possibility. In response to various forms of stress, which may result in damage to DNA and/or proteins, the primary cellular response may be to try to repair the damage. If the damage is too severe however, then the cell may invoke mechanisms to eradicate the damaged cell via pathways of programmed cell death.[1] A central factor controlling these processes is the tumour suppressor, p53. In normal healthy cells p53, which primarily functions as a transcription factor, is kept at low levels through the action of its own target gene Hdm2 (Mdm2 in mice) which directs p53 for ubiquitin-mediated proteasomal degradation.[2,3] In response to various cues including DNA damage, ribosomal stress, hypoxia or oncogene activation, the effects of Hdm2's action are alleviated

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and the levels of p53 accumulate.[1] One of the primary p53 target genes induced following p53 activation is the cyclin-dependent kinase inhibitor, p21 (also known as Waf1, Cip1, Sdi1).[4–6] When p21 levels increase, this causes inhibition of the cyclin-dependent kinases which phosphorylate and inhibit the retinoblastoma protein, pRB. As a result, the cells undergo a reversible cell cycle arrest. During this arrest, repair mechanisms are initiated – partly again through the transcriptional activation of genes by p53.[1] During this period if the damage is successfully repaired, the block to cell cycle progression is alleviated and the cell can undergo replication upon mitogenic stimulation – leading in part to the axiom that p53 is 'Guardian of the genome'.[7]

In some instances, depending on the context or the extent of damage for example, cell cycle arrest and repair is not possible. In these situations signals pervade in the cell which result in programmed cell death. Clearly this is a last resort for the individual cell, but is highly beneficial for the organism as an errant cell which might otherwise go on to form a tumour has been eradicated. Within the cell, two primary cell death pathways exist. The 'extrinsic' pathway which involves members of the TNF receptor superfamily is in essence a cell death priming mechanism (Fig. 1).[1] Only when a receptor on the cell surface engages with a ligand does cell death ensue. This pathway therefore acts to sense adverse conditions, particularly inflammatory states, and is fundamentally dependent on extracellular cues. In contrast, the 'intrinsic' pathway is not dependent on external signals for cell death initiation (Fig. 1).[1] This pathway is the primary pathway that responds to genotoxic stress and is therefore considered to be the principal pathway that responds to chemotherapeutic drugs and irradiation. Ultimately, both the extrinsic and intrinsic pathways converge on caspases (cysteine-aspartic proteases) that digest a variety of substrates to induce the demise of the cell. The cell death invoked in this manner is termed apoptosis and is characterised by chromatin condensation and fragmentation, and membrane blebbing. The dying cell or cell corpse is finally recognised and engulfed by a phagocytic cell resulting in a very 'clean' cell death that does not instigate an inflammatory response.[8]

p53 is engaged in activating both the extrinsic and intrinsic cell death pathways.[1] Reports have shown that p53 can transactivate the death receptors Fas/Apo-1 (the receptor for Fas ligand) and KILLER/DR5 (a receptor for TRAIL (TNF-related apoptosis-induced ligand)) (Fig. 1).[9,10] p53 also activates pro-apoptotic members of the Bcl2 family – Bax, Noxa and Puma – which are involved in permeabilisation of the outer mitochondrial membrane (Fig. 1).[11–14] This is a key event in the intrinsic cell death pathway and in certain extrinsic pathways. p53 also causes cellular perturbations such as endoplasmic reticulum stress via the p53 target gene, Scotin, which ultimately leads to an intrinsic cell death response.[15] In addition to these transcriptional effects, p53 has also been reported to have a more direct role in cell death initiation by

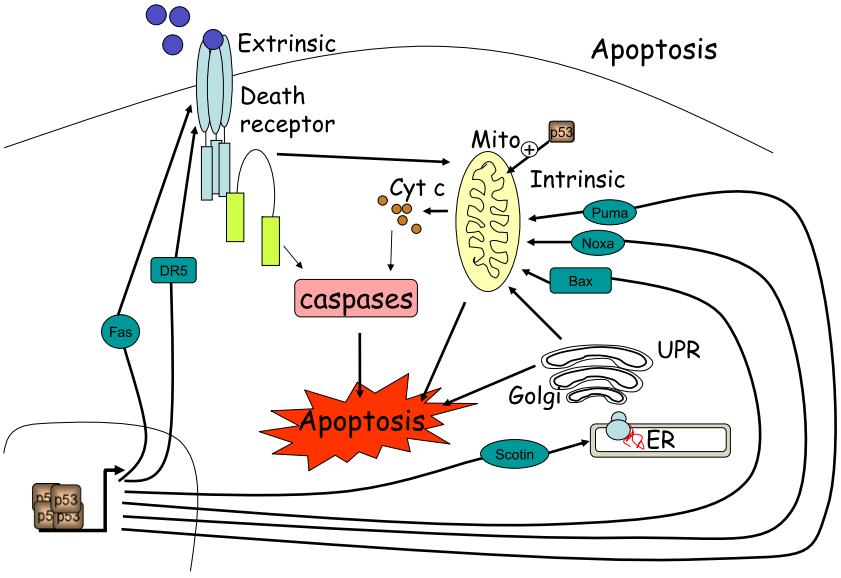

Fig. 1 – p53 activates both the intrinsic and extrinsic apoptotic pathways. Apoptotic cell death can be induced by either the extrinsic or intrinsic pathway. The extrinsic pathway involves receptors of the TNF receptor superfamily at the cell surface. In the presence of their extracellular ligands, these receptors send signals either directly, or indirectly via mitochondria, to cause caspase activation for induction of apoptosis. In contrast, the intrinsic pathway does not depend on extracellular cues and can originate from signals within the cell, e.g. DNA damage. In this pathway, members of the Bcl2 family regulate the permeability of the outer mitochondrial membrane. Upon permeabilisation, apoptogenic factors such as cytochrome c are released resulting in caspase activation. This pathway can also be initiated as a result of stress in the endoplasmic reticulum and the unfolded protein response (UPR). Following its accumulation in the nucleus, p53 can transactivate genes that engage both the extrinsic and intrinsic cell death pathways. p53 can activate, for example, the death receptors, Fas/Apo-1 and KILLER/DR5 and can engage the intrinsic pathway by the activation of factors such as PUMA, NOXA and BAX. p53 can also stimulate activation of the intrinsic pathway via activation of factors such as Scotin. In the absence of transcription of p53 target genes, p53 can also stimulate cell death directly via interaction with Bcl2 family members at mitochondria.

localising to mitochondria and regulating mitochondrial outer membrane permeabilisation directly (Fig. 1).[16–19]

Due to the central role of programmed cell death in tumour suppression, successful tumours must find ways around these safeguard mechanisms and the simplest way is for the tumour cells to mutate or inactivate direct components of a cell death pathway. Many standard forms of chemotherapy, however, require these cell death pathways for their effective action and the lack of a critical cell death regulator can cause *de novo* drug resistance. It has been the aim of our laboratory therefore to identify and understand cell death regulators, with the view that this will lead to better diagnostic tools, better stratification markers and hopefully also novel targets for therapeutic intervention.

2. Autophagy and programmed cell death

In our search for novel cell death regulators, we considered that simply looking for components and regulators of classical apoptotic pathways would limit our chances of finding cell death regulators with clinical potential. We decided therefore to consider other pathways that regulate cell viability. Aside from apoptosis, the other major mechanism of cell death is necrosis.[20] Different to apoptosis, necrosis results in a 'dirtier' form of cell death involving membrane rupture, spillage of cellular constituents into the extracellular space and a potent inflammatory response.[20] In the absence of a 'cleaner' apoptotic death, necrosis is probably the primary form of cell death that occurs following treatment with certain chemotherapeutic drugs.[21] Although clear components regulating necrosis have been defined, the process is often considered a default cell fate and compared with apoptosis has limited points of control.[8,20] As a result, we considered that the ability to identify regulators of necrosis may prove to be difficult.

The process of autophagy has recently risen to prominence as a process which regulates cell viability.[22] Autophagy (which literally means 'Self-eating') is similar to apoptosis in that it is evolutionarily conserved and genetically defined. Macroautophagy is the most extensively studied form of autophagy in mammalian cells and is referred to here simply as 'autophagy'. Ostensively, autophagy is a membrane trafficking process that delivers cytoplasmic constituents to the lysosome for degradation.[23] It is the principal mechanism by which long-lived proteins are degraded and is the only mechanism by which cells can degrade organelles. The process begins with the formation of a membranous structure called a phagophore or isolation membrane. This structure gradually grows to form a double-membraned enclosed vesicle called an autophagosome (Fig. 2).[23] As the structure forms, a cytoplasmic protein, LC3-B, is cleaved and lipidated and becomes an integral component of the autophagosome membrane.[24] Recruitment of this protein to the autophagosome membrane is therefore a marker for the formation or accumulation of these vesicles. Cargoes for degradation can be encapsulated at the time of autophagosome formation or can be delivered to the autophagosomes post formation via adaptor proteins

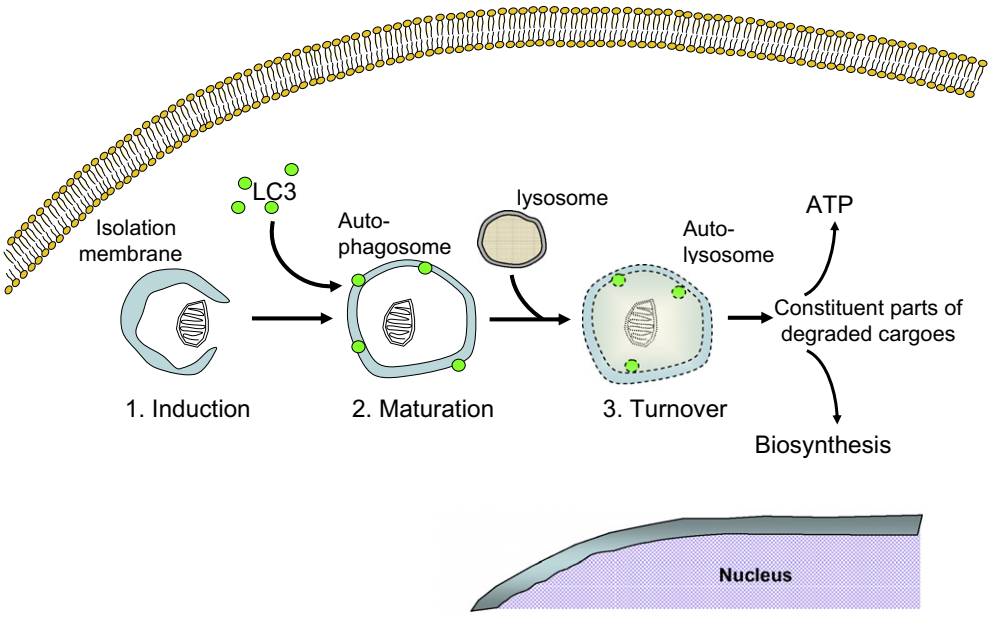

Fig. 2 – The pathways of (Macro)autophagy in mammalian cells. The process of autophagy is considered to be active in all cells. The process initiates with the formation of a membranous structure called a phagophore (or isolation membrane). This structure grows to form a spherical vesicle called an autophagosome which encapsulates bulk cytoplasm and/or organelles. During this process a cytoplasmic protein (LC3-B) becomes cleaved and lipidated and integrates into the autophagosome membrane. Integration of LC3 can be visualised as puncta by either expression of an ectopically tagged form or antibody staining for the endogenous protein and this can then be used as a marker for the formation of autophagosomes. Ultimately, autophagosomes fuse with lysosomes to form autolysosomes in which the contents of the autophagosome are degraded by acidic hydrolases provided by the lysosome. Constituent parts of degraded cargoes can then either be further metabolised to make ATP or utilised for biosynthetic pathways.

that contain motifs that interact with LC3 on the membrane of autophagosomes.[25] Following formation, an autophagosome can undergo fusion with other cellular components including multi-vesicular bodies and endosomes, but ultimately fusion occurs with a lysosome to form an autolysosome (Fig. 2). The constituents of the autophagosome are then broken down by acidic hydrolases provided by the lysosome and are then either recycled into biosynthetic pathways or in some situations further catabolised to produce ATP (Fig. 2).[26]

Autophagy functions at basal levels in virtually all, if not all, cells. The rate and cargoes of autophagy can, however, be changed depending on the situation to respond to the demands of the cell. For example, where autophagy was first genetically defined in yeast, it is clear that in nutrient deprived conditions, autophagy can be activated to produce ATP from internal sources to prevent necrosis for a limited period until nutrient replete conditions are regained.[26] This cell survival mechanism is also critically important for human existence since, if we extrapolate studies of mice deficient in an essential autophagy gene, autophagy can serve to bridge the feeding hiatus that occurs when we switch from umbilical feeding to suckling following birth.[27] In the presence of sufficient external nutrients, it is considered that autophagy in most situations is a homeostatic mechanism that serves, together with the proteasome, to maintain the integrity and fidelity of the cell proteome and organelles. It must be remembered that if damaged proteins and organelles are not removed, then this creates a situation that is in many ways akin to a mutation. Admittedly, the change is not heritable like a genomic alteration, but since cancer is considered a clonal disease arising initially from one rogue cell, then damaged proteins and organelles may well cause predisposition to DNA damage and malignant disease.[28,29] The role of autophagy in guarding the proteome is therefore critical in protecting us against the development of cancer and other forms of human disease.

Autophagy is also a highly adaptable process and the choice of cargo destined for degradation is considered to be paramount for the eventual outcome of the autophagic process. Polarity in the roles of autophagy is perhaps no better exemplified than in its role in regulating cell viability. Although as outlined above, autophagy has clear roles in promoting cell survival in energy deprived states, there is considerable evidence indicating that autophagy in some contexts may also promote cell death. The weight of the current opinion is no longer consistent with the view that autophagy can actually constitute a discrete acute form of death termed 'Autophagic Cell Death'.[30] It is now more so considered that autophagy can be a contributing factor to cell death in certain scenarios in combination with other signals. This may constitute, for example, forms of cell death that require energy where autophagy is the only immediate source. Alternatively, it may be that autophagy acts to facilitate cell death not necessarily in a cell intrinsic manner, but in trans. Studies from developmental systems have indicated that an autophagy competent cell may send permissive signals for death, indicating perhaps that following death, engulfment and digestion of the dead cell can be undertaken.[31] This would be highly understandable in tissue remodelling or cell competition situations during development, but could also be conceivable in mammalian tissue homeostasis and during tumour development. The view of autophagy in cell death has therefore been revised from 'Autophagic Cell Death' to 'Cell Death with Autophagy' and further studies are clearly required to elucidate the role played by autophagy in promotion or facilitation of programmed cell death.[30]

3. p53 and autophagy

Our studies on autophagy were initiated by a screen in our laboratory for factors that are transcriptionally activated by p53 and which may contribute to the ability of p53 to induce programmed cell death. One gene we identified encoded for the previously uncharacterised protein, FLJ11259. This gene was transcriptionally activated by p53 and was also activated by DNA-damaging agents in a p53-dependent manner.[32,33] Our analysis of ectopic FLJ11259 expression revealed that it was not sufficient to induce cell death even though RNAi-mediated knockdown of endogenous FLJ11259 greatly impeded cell death induced by p53. We were intrigued therefore as to the role of FLJ11259 in cell death and since bioinformatics had indicated that FLJ11259 may have a signal peptide for the endoplasmic reticulum we considered that the protein may be localised in the cell within a compartment of the secretory pathway. Localisation studies revealed that FLJ11259 was primarily localised in lysosomes and this naturally caused us to consider whether the protein had an impact on autophagy. Studies using a green fluorescent protein-tagged form of the autophagosome membrane protein LC3 indeed revealed that expression of FLJ11259 results in a massive accumulation of autophagosomes (Fig. 3). Since FLJ11259 is a direct target of p53, we subsequently showed that p53 is a positive regulator of autophagy and that this effect is dependent on FLJ11259. As a result, FLJ11259 was renamed DRAM for Damage-Regulated Autophagy Modulator.[32,33]

Further analysis of DRAM revealed that it was activated not only by p53 but also by the p53 related protein, p73.[34] Different to the situation with p53, however, although p73 was also able to induce autophagy, this effect was independent of DRAM.[34] This indicated that there are additional factors downstream of p73, and potentially also downstream of p53, that mediate the autophagic effects initiated by these factors. In this regard, subsequent work from ourselves and others have identified additional transcriptional targets of p53 that can modulate autophagy.[35–41] Although some of these factors are known to engage pathways impinging on the mTOR kinase, which is a well characterised modulator of autophagy, others, like DRAM, have an as yet undefined action in the control of autophagy (Fig. 3). Future studies on these factors will therefore undoubtedly be insightful as to the way these disparate target genes integrate to regulate autophagy downstream of p53.

Analysis of the human protein database revealed that DRAM actually belongs to a previously undefined protein family.[42,43] There are five obvious DRAM family members in humans with DRAM5 having two distinct splice variants, which we have termed DRAM5a and DRAM5b (Fig. 4).[42] Our analysis of the most closely related human protein to DRAM

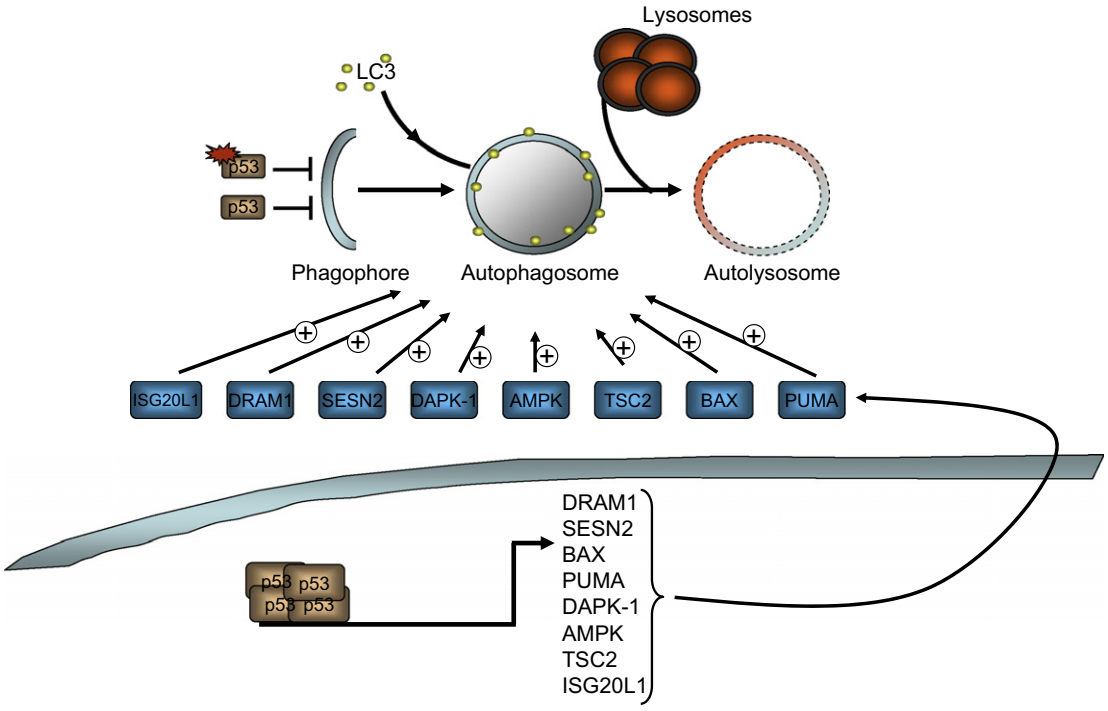

Fig. 3 – p53 engages cellular autophagy in various ways. p53 activates a spectrum of target genes that positively regulate autophagy. These include proteins of known function in nutrient sensing pathways leading to modulation of mTOR (TSC2, AMPK and SESN2). Other p53 target genes which have been reported to be involved in autophagy regulation have also been shown to have roles in cell death (DRAM1, ISG20L1, DAPK-1, BAX and PUMA). Basal levels of p53 are also reported to negatively regulate autophagy in the cytoplasm in the absence of p53 target gene activation. This function has also been reported for tumour-derived mutants of p53.

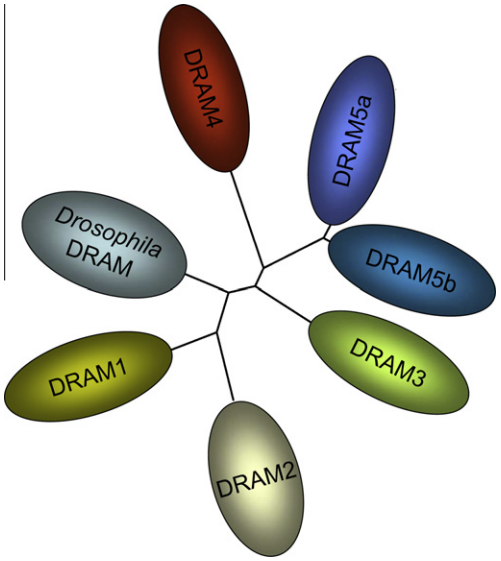

Fig. 4 – The human DRAM family. There are five DRAM-related proteins in human cells. DRAM5 is found as two splice variants in human mRNA, DRAM5a and DRAM5b. Simpler organisms such as *Drosophila* only appear to have one obvious DRAM protein.

(now subsequently called DRAM1), DRAM2, revealed that it is similar to DRAM1 in that it is predominantly localised to lysosomes, but it is different to DRAM1 in that it is not induced by either p53 or p73.[42] Strikingly, we also found that the ectopic expression of DRAM2 does not affect autophagosome accumulation. What then is the role of DRAM2? Does it have a completely different function or is it just unable to modulate autophagy when expressed alone? These findings also raise the question of whether the ability of DRAM proteins to regulate autophagy is an evolutionarily conserved effect or something that has only arisen in higher eukaryotes. Simpler organisms like *Drosophila* only appear to have one DRAM protein and so this enabled us to consider if the ability of DRAM was phylogenetically conserved. In other words, was *Drosophila* DRAM (*Dm*DRAM) like human DRAM1 or DRAM2? Expression of *Dm*DRAM in cells caused a marked accumulation of autophagosomes indicating that the modulation of autophagy by DRAM proteins is likely to be evolutionarily ancient.[42] Additional members of the DRAM family have therefore likely evolved to undertake similar mechanistic roles to DRAM1, but without a clear involvement in the control of autophagy.

4. Targeting autophagy for cancer therapy

The role of autophagy in tumour development is complex.[44,45] Many tumours contain poorly vascularised regions that lack both nutrients and oxygen and it is known that these are associated with high levels autophagosomes.[46] In these settings, it is considered that autophagy is a cytoprotective mechanism for the tumour cells and can therefore be considered oncogenic. There is accumulating evidence, however,

that key autophagy genes are inactivated in major sporadic forms of human cancer and mouse models deficient in autophagy genes have been reported to be tumour prone.[47–50] This points therefore to the fact that autophagy – at some point during tumour development at least – contributes to tumour suppression. The question for autophagy and cancer therapy therefore is: should we treat and if so, when and how? Currently, there is no simple answer to this question and the issue is even more complex when we consider the fact that autophagy protects us against many diseases in addition to cancer. For example, autophagy is critically important for the removal of aggregate-prone proteins that lead to forms of neurodegenerative disease, and mutations in a central autophagy gene are known to be causally linked to the formation of Crohn's disease.[51–55] Autophagy is also known to be critically involved in our immune response to both viral and bacterial infections.[56] So, the issue for cancer therapy is not simply whether we should inhibit or promote autophagy, but can we also do this in a tumour specific manner so not to affect these beneficial forms of autophagy in normal tissues?

In an attempt to address whether selective autophagy targeting was a feasible possibility, we embarked on a screen to identify the signalling pathways that may regulate autophagy in response to specific stimuli. The stimulus we decided to pursue was hypoxia since, although involved in other diseased states such as cardiovascular disease, for the most part hypoxia is a tumour-associated state. Our screen revealed that members of the platelet-derived growth factor receptor family, which are known to be activated in tumours, are involved in an autocrine loop which is required for hypoxia-induced autophagy while being seemingly dispensable for autophagy induced by other stimuli.[57,58] These studies therefore provide a proof-of-principle paradigm that disease-associated forms of autophagy could theoretically be specifically targeted to treat human disease. Ultimately, however, further *in vivo* experiments are required to elucidate not only in which tumour types targeting autophagy would be a viable option but also at what stage or stages of tumour development in any given tissue type is autophagy oncogenic or tumour suppressive. It is without question, however, that autophagy is critical for the survival of at least a sub-set of tumour cells and so it is an issue that is undoubtedly worthy of further pursuit.

Conflict of interest statement

None declared.

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REFERENCES

1. Crighton D, Ryan KM. Splicing DNA-damage responses to tumour cell death. *Biochim Biophys Acta* 2004;**1705**(1):3–15.
2. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature* 1997;**387**(6630):299–303.
3. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997;**387**(6630):296–9.
4. el-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. *Cell* 1993;**75**(4):817–25.
5. Noda A, Ning Y, Venable SF, Pereira-Smith OM, Smith JR. Cloning of senescent cell-derived inhibitors of DNA synthesis using an expression screen. *Exp Cell Res* 1994;**211**(1):90–8.
6. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 1993;**75**(4):805–16.
7. Lane DP. Cancer: p53, guardian of the genome. *Nature* 1992;**358**(6381):15–6.
8. Edinger AL, Thompson CB. Death by design: apoptosis, necrosis and autophagy. *Curr Opin Cell Biol* 2004;**16**(6):663–9.
9. Owen-Schaub LB, Zhang W, Cusack JC, et al. Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. *Mol Cell Biol* 1995;**15**(6):3032–40.
10. Wu GS, Burns TF, McDonald 3rd ER, et al. KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. *Nat Genet* 1997;**17**(2):141–3.
11. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 1995;**80**(2):293–9.
12. Oda E, Ohki R, Murasawa H, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 2000;**288**(5468):1053–8.
13. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell* 2001;**7**(3):683–94.
14. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. *Mol Cell* 2001;**7**(3):673–82.
15. Bourdon JC, Renzing J, Robertson PL, Fernandes KN, Lane DP. Scotin, a novel p53-inducible proapoptotic protein located in the ER and the nuclear membrane. *J Cell Biol* 2002;**158**(2):235–46.
16. Mihara M, Erster S, Zaika A, et al. P53 has a direct apoptogenic role at the mitochondria. *Mol Cell* 2003;**11**(3):577–90.
17. Moll UM, Wolff S, Speidel D, Deppert W. Transcription-independent pro-apoptotic functions of p53. *Curr Opin Cell Biol* 2005;**17**(6):631–6.
18. Chipuk JE, Kuwana T, Bouchier-Hayes L, et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* 2004;**303**(5660):1010–4.
19. Leu JI, Dumont P, Hafey M, Murphy ME, George DL. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol* 2004;**6**(5):443–50.
20. Zong WX, Thompson CB. Necrotic death as a cell fate. *Genes Dev* 2006;**20**(1):1–15.
21. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. *Genes Dev* 2004;**18**(11):1272–82.

22. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008;**451**(7182):1069–75.
23. Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol* 2010;**22**(2):124–31.
24. Kabeya Y, Mizushima N, Ueno T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *Embo J* 2000;**19**(21):5720–8.
25. Kirkin V, McEwan DG, Novak I, Dikic I. A role for ubiquitin in selective autophagy. *Mol Cell* 2009;**34**(3):259–69.
26. Lum JJ, DeBerardinis RJ, Thompson CB. Autophagy in metazoans: cell survival in the land of plenty. *Nat Rev Mol Cell Biol* 2005;**6**(6):439–48.
27. Kuma A, Hatano M, Matsui M, et al. The role of autophagy during the early neonatal starvation period. *Nature* 2004;**432**(7020):1032–6.
28. Mathew R, Kongara S, Beaudoin B, et al. Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes Dev* 2007;**21**(11):1367–81.
29. Karantza-Wadsworth V, Patel S, Kravchuk O, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Dev* 2007;**21**(13):1621–35.
30. Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. *Nat Rev Mol Cell Biol* 2008;**9**(12):1004–10.
31. McPhee CK, Logan MA, Freeman MR, Baehrecke EH. Activation of autophagy during cell death requires the engulfment receptor Draper. *Nature* 2010;**465**(7301):1093–6.
32. Crighton D, Wilkinson S, O'Prey J, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* 2006;**126**(1):121–34.
33. Crighton D, Wilkinson S, Ryan KM. DRAM links autophagy to p53 and programmed cell death. *Autophagy* 2007;**3**(1):72–4.
34. Crighton D, O'Prey J, Bell HS, Ryan KM. P73 regulates DRAM-independent autophagy that does not contribute to programmed cell death. *Cell Death Differ* 2007;**14**(6):1071–9.
35. Yee KS, Wilkinson S, James J, Ryan KM, Vousden KH. PUMA- and Bax-induced autophagy contributes to apoptosis. *Cell Death Differ* 2009;**16**(8):1135–45.
36. Feng Z, Hu W, de Stanchina E, et al. The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. *Cancer Res* 2007;**67**(7):3043–53.
37. Harrison B, Kraus M, Burch L, et al. DAPK-1 binding to a linear peptide motif in MAP1B stimulates autophagy and membrane blebbing. *J Biol Chem* 2008;**283**(15):9999–10014.
38. Martoriati A, Doumont G, Alcalay M, et al. Dapk1, encoding an activator of a p19ARF-p53-mediated apoptotic checkpoint, is a transcription target of p53. *Oncogene* 2005;**24**(8):1461–6.
39. Budanov AV, Karin M. P53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* 2008;**134**(3):451–60.
40. Maiuri MC, Malik SA, Morselli E, et al. Stimulation of autophagy by the p53 target gene sestrin2. *Cell Cycle* 2009;**8**(10):1571–6.
41. Eby KG, Rosenbluth JM, Mays DJ, et al. ISG20L1 is a p53 family target gene that modulates genotoxic stress-induced autophagy. *Mol Cancer* 2010;**9**:95.
42. O'Prey J, Skommer J, Wilkinson S, Ryan KM. Analysis of DRAM-related proteins reveals evolutionarily conserved and divergent roles in the control of autophagy. *Cell Cycle* 2009;**8**(14):2260–5.
43. Kerley-Hamilton JS, Pike AM, Hutchinson JA, Freemantle SJ, Spinella MJ. The direct p53 target gene, FLJ11259/DRAM, is a member of a novel family of transmembrane proteins. *Biochim Biophys Acta* 2007;**1769**(4):209–19.
44. Rosenfeldt MT, Ryan KM. The role of autophagy in tumour development and cancer therapy. *Expert Rev Mol Med* 2009;**11**:e36.
45. Wilkinson S, Ryan KM. Autophagy: an adaptable modifier of tumourigenesis. *Curr Opin Genet Dev* 2010;**20**(1):57–64.
46. Degenhardt K, Mathew R, Beaudoin B, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* 2006;**10**(1):51–64.
47. Qu X, Yu J, Bhagat G, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest* 2003;**112**(12):1809–20.
48. Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci USA* 2003;**100**(25):15077–82.
49. Takahashi Y, Coppola D, Matsushita N, et al. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol* 2007;**9**(10):1142–51.
50. Marino G, Salvador-Montoliu N, Fueyo A, et al. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. *J Biol Chem* 2007;**282**(25):18573–83.
51. Cadwell K, Patel KK, Komatsu M, Virgin HWT, Stappenbeck TS. A common role for Atg16L1, Atg5 and Atg7 in small intestinal Paneth cells and Crohn disease. *Autophagy* 2009;**5**(2):250–2.
52. Cadwell K, Liu JY, Brown SL, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008;**456**(7219):259–63.
53. Saitoh T, Fujita N, Jang MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 2008;**456**(7219):264–8.
54. Hampe J, Franke A, Rosenstiel P, et al. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007;**39**(2):207–11.
55. Sarkar S, Ravikumar B, Rubinsztein DC. Autophagic clearance of aggregate-prone proteins associated with neurodegeneration. *Methods Enzymol* 2009;**453**:83–110.
56. Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. *Nat Rev Immunol* 2007;**7**(10):767–77.
57. Wilkinson S, O'Prey J, Fricker M, Ryan KM. Hypoxia-selective macroautophagy and cell survival signaled by autocrine PDGFR activity. *Genes Dev* 2009;**23**(11):1283–8.
58. Wilkinson S, Ryan KM. Growth factor signaling permits hypoxia-induced autophagy by a HIF1alpha-dependent, BNIP3/3L-independent transcriptional program in human cancer cells. *Autophagy* 2009;**5**(7):1068–9.