DNA damage and the balance between survival and death in cancer biology

Wynand P. Roos, Adam D. Thomas and Bernd Kaina

Abstract | DNA is vulnerable to damage resulting from endogenous metabolites, environmental and dietary carcinogens, some anti-inflammatory drugs, and genotoxic cancer therapeutics. Cells respond to DNA damage by activating complex signalling networks that decide cell fate, promoting not only DNA repair and survival but also cell death. The decision between cell survival and death following DNA damage rests on factors that are involved in DNA damage recognition, and DNA repair and damage tolerance, as well as on factors involved in the activation of apoptosis, necrosis, autophagy and senescence. The pathways that dictate cell fate are entwined and have key roles in cancer initiation and progression. Furthermore, they determine the outcome of cancer therapy with genotoxic drugs. Understanding the molecular basis of these pathways is important not only for gaining insight into carcinogenesis, but also in promoting successful cancer therapy. In this Review, we describe key decision-making nodes in the complex interplay between cell survival and death following DNA damage.

Chemical carcinogens in the environment and those produced in the body by cellular and gut microbiota metabolism[1], radicals produced by activated immune cells such as monocytes and macrophages[2], ultraviolet (UV) radiation[3], ionizing radiation[4], and many pharmaceuticals, notably genotoxic anticancer drugs[5], attack DNA and produce a variety of DNA lesions[6] (FIG. 1a). These lesions give rise to gene mutations and chromosomal damage, which are causal events in oncogenic transformation and tumour progression[7]. To limit genomic instability, cells are equipped with DNA damage response (DDR) pathways and DNA repair proteins to remove and tolerate DNA lesions[8]. Unrepaired damage can be toxic, promoting pathways of cell elimination such as apoptotic and necrotic death[9] that are also thought to function as tumour suppressor pathways[10]. Cell death following DNA damage is a regulated process during which 'decisions' are made at the molecular level that dictate cell fate — to survive or to die — with the outcome determined by a threshold of pro-survival factors versus pro-death factors[11]. Understanding how the cell overrides DNA repair with cell death has huge implications for potentiating the tumour cell-killing effect of genotoxic chemotherapy.

Institute of Toxicology, University Medical Center, Obere Zahlbacher Strasse 67, D-55131 Mainz, Germany.
Correspondence to B.K.
kaina@uni-mainz.de

doi:10.1038/nrc.2015.2
Published online 18 Dec 2015

The DDR in survival and death

The toxicity of DNA adducts is typically due to the arrest of polymerases during DNA replication or transcription (FIG. 1b). Some adducts (for example, *N*3-methyladenine) that are induced by simple alkylating agents, UV-radiation-induced photoproducts, intrastrand and interstrand crosslinks produced by some chemical weapons (such as mustard gas) and genotoxic anticancer drugs (for example, cisplatin, cyclophosphamide and melphalan), bulky adducts from polycyclic aromatic hydrocarbons, heterocyclic amines, aflatoxins and vinyl chloride[12,13], and DNA breaks induced by topoisomerase inhibitors are direct steric hindrances affecting the progression of both DNA and RNA polymerases. Smaller adducts such as O^6-alkylguanine and *N*7-alkylguanine can indirectly hinder DNA polymerase progression if their respective repair pathway (mismatch repair (MMR) and base excision repair (BER)) is operational during DNA synthesis[14,15]. Such DNA polymerase-stalling events will initiate DDR signalling to stabilize the stalled replication fork, and will initiate cell cycle arrest, DNA repair and replication restart once the damage has been repaired. If fork restart is unsuccessful, prolonged polymerase stalling could lead to the collapse of the fork and to the formation of a potentially toxic one-ended DNA double-strand break (DSB)[16], that is, a DSB with no other DNA end to which to be ligated. Although DSBs are major toxic events that follow DNA damage, in tumours they are the likely source of genomic changes once pro-death pathways have been deactivated[17]. Following DSB induction, cell fate decisions are made post-DDR activation[18]. A central question is whether the

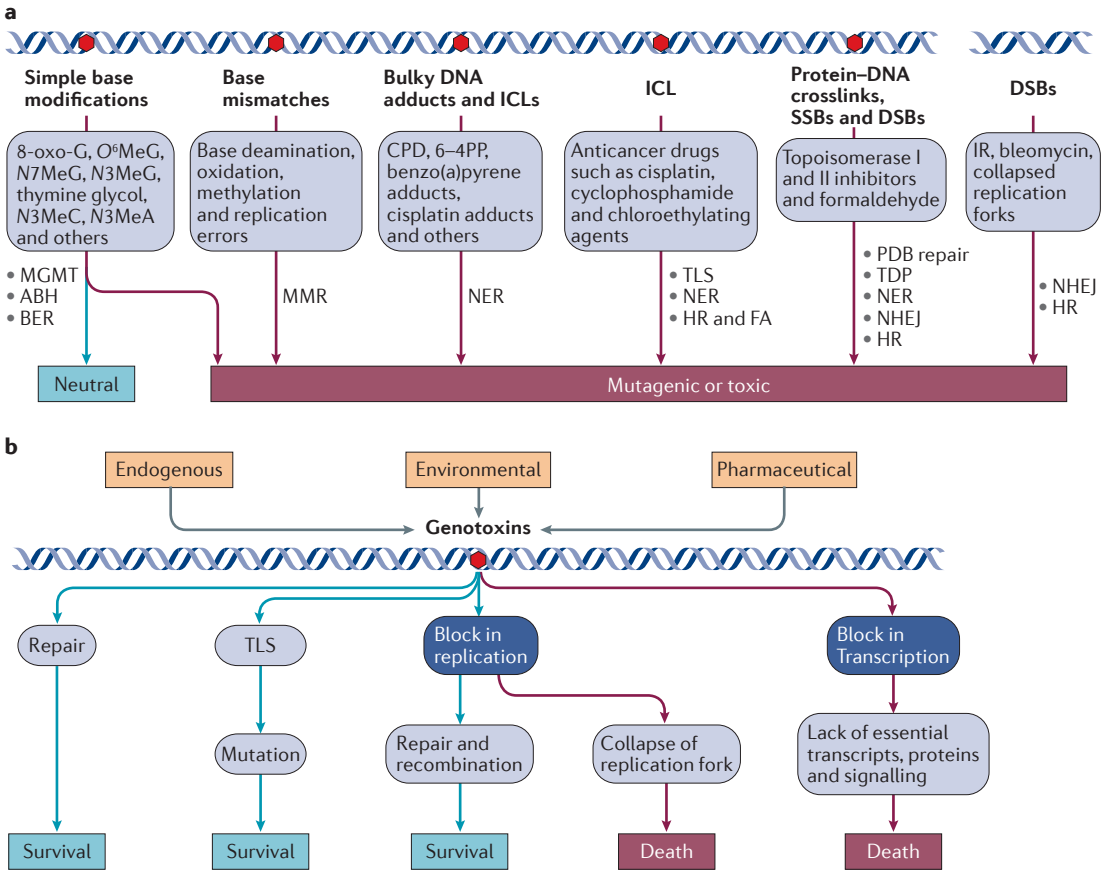


Figure 1 | **Cellular consequences of DNA damage. a** | Simple base modifications are corrected by *O*[6]-methylguanine-DNA methyltransferase (MGMT), alkB homologue (ABH) family members and base excision repair (BER). Base mismatches are repaired by mismatch repair (MMR). Bulky adducts are removed by nucleotide excision repair (NER). Interstrand and intrastrand crosslinks (ICLs) are repaired by the concerted efforts of translesion synthesis (TLS), NER, homologous recombination (HR) and Fanconi anaemia (FA) repair pathways. Protein–DNA crosslinks are repaired by the combined action of protein-linked DNA break (PDB) repair, NER, non-homologous end joining (NHEJ) and HR, and DNA double-strand breaks (DSBs) are repaired by NHEJ or HR. Unrepaired DNA lesions can be neutral, mutagenic or toxic. **b** | Many sources give rise to DNA damage, which can cause mutation and DNA recombination, and can prevent DNA replication or block RNA transcription. Depending on the type of DNA lesion and the cellular background DNA damage is either tolerated or lethal. 6–4PP, 6–4 pyrimidine photoproduct; 8-oxo-G, 8-oxo-guanine; CPD, cyclobutane pyrimidine dimer; IR, ionizing radiation; Me, methyl; SSB, single-strand break; TDP, tyrosyl-DNA phosphodiesterase.

DNA adducts themselves are able to activate cell death pathways or whether DSBs are the principal apoptotic trigger. Evidence suggests that DSBs are strong activators of apoptosis[19], and most DNA base damage (for example, small adducts induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) and simple alkylating agents) induces necrosis[20]. The induction of necrosis is a consequence of poly(ADP-ribose) polymerase 1 (PARP1) activation during the repair of, for example, *N*-methylpurines and 8-oxo-guanine by BER. PARP1 activation depletes the cellular NAD^+ and ATP pools, which triggers necrotic death (also known as parthanatos)[21]. Incidentally, parthanatos, like apoptosis, is thought to be a protective mechanism that counteracts carcinogenesis if the need for the repair of small DNA adducts exceeds the repair capacity of the cell[22]. It should be noted that DNA repair by non-homologous end joining (NHEJ), homologous recombination (HR), MMR, BER, nucleotide excision repair (NER) and protein-linked DNA break (PDB) repair in combination with DDR signalling and damage tolerance mechanisms[23–25] are key cell survival pathways (FIG. 1b). However, it is not solely the lack of repair that initiates death programmes, but is rather a series of molecular interactions intertwined with the DDR that switches off repair and stimulates cell death.

In order for the cell to respond to critical DNA damage, the damage must first be detected, typically once it has manifested as a DSB or a replication fork-stalling event. Three interlinked sensor systems have been described that have the ability to detect a single DSB within minutes of its formation[26]. These immediate-early sensors in the DDR are the PI3K-related kinases (PIKKs): ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK). From these sensors, through a series of kinase reactions the DDR cascade becomes activated, targeting many dual-function proteins that are key nodes in promoting

survival or cell death. These proteins signal downstream checkpoint activation to prevent cell cycle progression and the recruitment of DNA repair proteins to facilitate DSB repair via the stimulation of NHEJ or HR (for example, BRCA1 and Fanconi anaemia complementation group D2 (FANCD2))[12], depending on the cell cycle phase. In this capacity of promoting repair and cell cycle arrest, ATM and ATR are unquestionably pro-survival, as corresponding knockdown cell lines are clearly more sensitive to DSBs following DNA damage[27]. Therefore, one would predict a low penetrance of dominant-negative ATM and ATR mutations in cancer. However, contrary to expectation, ATM is frequently mutated in tumours[28], which is accompanied by a gain of therapeutic resistance[29]. As ATM and ATR promote cell death in instances of high DSB levels[30], either through the activation of the p53-dependent[31] and caspase 2-dependent[32] apoptosis pathways (FIG. 2) or through the activation of E2F1, p73 and CHK1 (reviewed in REF. 33) in a cell type-specific manner[34], loss of ATM will render tumours resistant to these cell death pathways.

Patients with ataxia telangiectasia, which is caused by inactivating mutations in *ATM*, have a particularly increased risk of breast cancer[35] and a moderately increased risk of colon cancer[36], and patients with functionally compromised ATR also show an increased risk of malignancy[37]. Although ATM seems to be more frequently altered than ATR[38] in tumours, it is clear that both ATM and ATR are necessary for maintaining genome stability either by stimulating DNA repair or by signalling death[39] in compromised cells. However, as the tumours in these patients still contain either mutated ATM or mutated ATR, it suggests that the ability to survive extends beyond the capacity of these kinases to recognize and signal the downstream repair of DSBs or the stabilization of stalled replication forks. One explanation is that there are many sub-pathways for circumventing DNA polymerase stalling and DSB repair, so it is unlikely that DSBs will remain unrepaired in ATM- or ATR-mutated cells[40], but they may not be faithfully repaired, which may contribute to genomic instability. One hypothesis states that ATM- and ATR-mediated recombination repair operates at stalled replication forks and may compete via proliferating cell nuclear antigen (PCNA) ubiquitylation with translesion synthesis (TLS; which can be error-prone)[41] to prevent fork collapse, thereby promoting cell survival through lesion bypass. ATM and ATR can switch the balance to cell death by favouring the activation of pro-death functions (discussed further below) such as Ser46 phosphorylation of p53. Therefore, the regulation of survival and death functions is not strictly separable but embedded in complex networks with regulatory feedback and feedforward loops.

Survival and death strategies

Survival strategies include the repair of pre-toxic lesions that rests on the constitutive and induced activities of several repair pathways (FIG. 1a), bypass of pre-toxic lesions by TLS, autophagy, senescence, inhibition of apoptosis by anti-apoptotic factors such as survivin, inhibitors of apoptosis (IAPs) and X-linked inhibitor of apoptosis proteins (XIAPs) (FIG. 2), and activation of transcription factors such as nuclear factor-κB (NF-κB) and AKT (also known as PKB) regulating the expression of pro-survival genes (BOX 1). Cellular death strategies include apoptosis (FIG. 2), regulated necrosis (parthanatos, necroptosis and ferroptosis)[42], checkpoint adaptation following DNA damage[43] and mitotic death[44]. These pro-death and pro-survival pathways, and how they interact, are discussed in more detail below.

p53 in the balance between survival and death following DNA damage. Different levels of damage may trigger different responses; this paradigm states that low levels of DNA damage trigger repair and survival mechanisms and high levels trigger cell death. It is reasonable to hypothesize that p53 is central in this situation given the roles of p53 target genes in survival and death[39] (FIG. 2). How can the cell quantify DNA damage, and how are the decisions made? Theoretical models predict different activation levels of p53 that are dependent on different DNA damage levels, which are controlled by the conflicting actions of positive (ATM/ATR–CHK1/CHK2–p53) and negative (MDM2) feedback loops[45–47]. Simply, low DNA damage levels transiently activate p53 and high DNA damage levels lead to sustained p53 activation. The different stabilization levels of p53 may contribute to the differential expression of pro-survival and pro-apoptosis genes, as the affinity of p53 for its binding sites in promoters is high for genes that are associated with cell cycle arrest (for example, *CDKN1A*, which encodes p21) and is low for genes that are associated with apoptosis (for example, p53-induced gene 3 (PIG3; also known as TP53I3))[48]. Promoter selectivity by p53 is also dependent on RNA and chromatin-interacting partners. Thus, by using an inducible knockout mouse model it was shown that the expression of *Cdkn1a* in normal cells is dependent on the RNA helicase p68 (also known as DDX5)[49], whereas in lung and breast cancer cells the expression of PIG3 and p53 regulated apoptosis inducing protein 1 (p53AIP1) relies on cellular apoptosis susceptibility protein (CAS; also known as EXP2 and CSE1L)[50]. Furthermore, the direct interaction between p53 and apoptosis-stimulating of p53 protein 1 (ASPP1) and ASPP2 (REF. 51) triggers apoptosis, but its interaction with inhibitor of ASPP protein (iASPP)[52] prevents apoptosis. Crosstalk between pro-survival pathways, for example, NF-κB (BOX 1) and p53, also influences the role of p53 as a pro-survival or a pro-death factor. Clearly, the regulation of p53 and the resulting downstream effects are complex and are very likely to be cell-type and cancer-type specific.

In addition to the above-mentioned mechanisms, the change in function of p53 from 'arrestor' and 'repairer' to 'killer' is dependent upon various post-translational amino-terminal phosphorylations and carboxy-terminal acetylations of p53 (REF. 53) (FIG. 3). Phosphorylation at Ser15, Ser37, Thr18 and Ser20 by ATM, ATR, CHK1, CHK2 and DNA-PK, among others, uncouples p53 from its inhibitory MDM2 binding partner and aids in the nuclear sequestration and transcriptional activation of p53 that is necessary for its arrestor functions,

Ataxia telangiectasia
A heritable disorder caused by mutated *ATM*. Patients suffering from this disease are predisposed to cancer and are very sensitive to ionizing radiation.

Necroptosis
A form of regulated necrosis that the cell uses when apoptosis cannot be initiated. During necroptosis the content of the cell is released into the extracellular space and causes inflammation.

Ferroptosis
A regulated, non-apoptotic form of cell death that is dependent on iron.

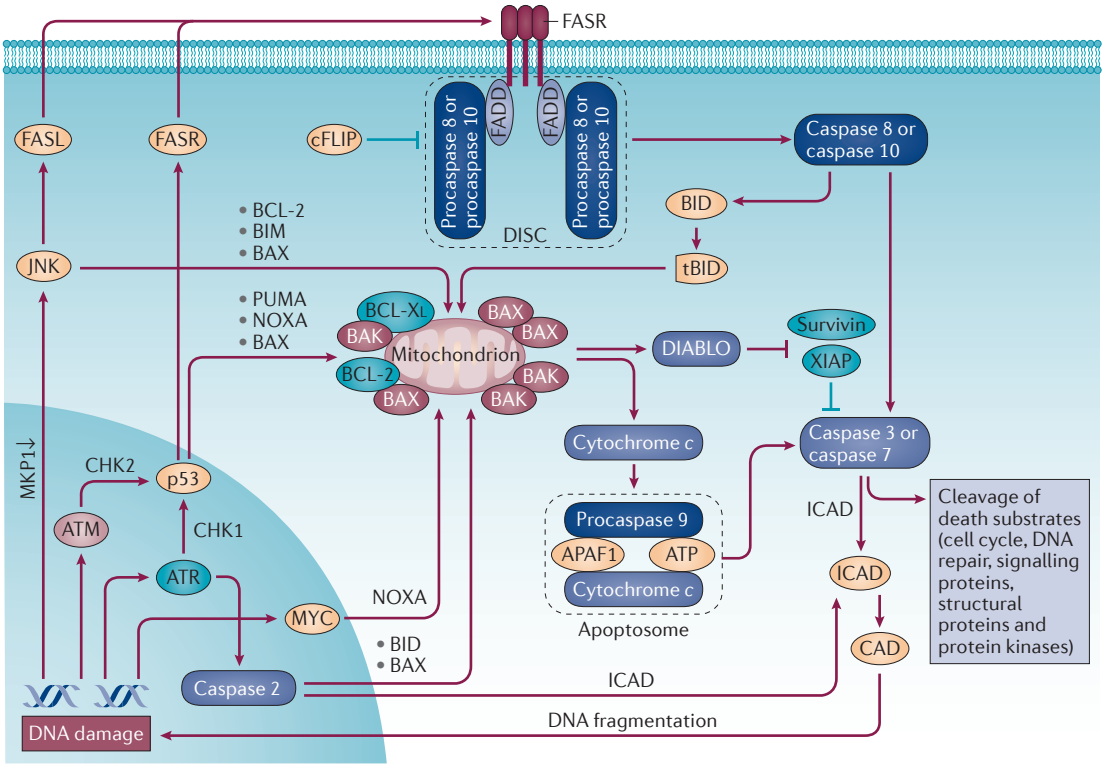


Figure 2 | **DNA damage-dependent apoptosis.** DNA damage is able to activate both the extrinsic (death receptor) and the intrinsic (mitochondria-dependent) apoptosis pathways. Pro-apoptosis components of the DNA damage response are JUN N-terminal kinase (JNK), p53, caspase 2 and possibly MYC. They activate the pro-apoptosis proteins Fas ligand (FASL), Fas receptor (FASR), BCL-2-interacting mediator of cell death (BIM; also known as BCL2L11), BAX, p53 upregulated modulator of apoptosis (PUMA; also known as BBC3), NOXA (also known as PMAIP1) and BH3 death domain interacting protein (BID) and downregulate the anti-apoptosis protein BCL-2. This causes the activation of protease activity of caspases and the DNase activity of caspase-activated DNases (CADs), cleaving the DNA. DNA damage may also inhibit the transcription of MAPK phosphatase 1 (MKP1), which leads to increased JNK phosphorylation and activating protein 1 (AP-1) activity that drives FASL and the extrinsic apoptosis pathway. APAF1, apoptotic peptidase activating factor 1; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; cFLIP, cellular FLICE-like inhibitory protein (also known as CFLAR); DISC, death-inducing signalling complex; FADD, FAS-associated death domain; ICAD, inhibitor of caspase-activated DNase; tBID, truncated BID; XIAP, X-linked inhibitor of apoptosis.

for example, the induction of *CDKN1A*[54]. The phosphorylation of Ser46 is specifically linked to the killer function of p53 (REF. 55), whereby it is postulated to alter promoter selection and favour the induction of apoptotic genes such as *NOXA* (also known as *PMAIP1*)[56], *PTEN*[57] and *TP53AIP1* (REF. 58) causing apoptosis in response to chemical agents and physical stress[56–58]. There are a number of candidate kinases that have been shown to interact with p53 and to phosphorylate Ser46, such as homeodomain-interacting protein kinase 2 (HIPK2)[59], p38 (REF. 60), protein kinase Cδ (PKCδ)[61], p53-dependent damage-inducible nuclear protein 1 (p53DINP1; also known as TP53INP1)[62] and dual specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2)[63]. ATM and ATR are thought to signal upstream of Ser46 phosphorylation (possibly via XIAP-associated factor 1 (XAF1)[64]) where they phosphorylate Ser19 of seven in absentia homologue 1 (SIAH1), an E3 ubiquitin ligase. This modification disrupts the HIPK2–SIAH1 complex to stabilize HIPK2 (REF. 65) and allows its association with promyelocytic leukaemia protein (PML; a potent pro-apoptotic tumour suppressor), p300, CREB-binding protein (CBP; also known as CREBBP) and p53 in nuclear PML bodies, with the net effect being the upregulation of pro-apoptotic p53 targets[66].

Incidentally, the dephosphorylation of p53 (at Ser46 and other sites) is thought to be dependent upon the p53-induced phosphatase WIP1 (also known as PPM1D)[67]. Therefore, opposing feedback loops of p53 (Ser46) phosphorylation and dephosphorylation may exist, and an imbalance favouring HIPK2 stabilization, perhaps initiated by high and/or persistent levels of activated ATM and/or ATR (in situations of high levels of damage) may switch the cell to apoptosis over repair. Nonetheless, it is clear that the regulation of p53 is highly complex and requires further investigation to understand how the decision is made between survival and death following DNA damage.

In cancer biology, the apoptotic switch may be kept inactive owing to the increased SIAH1 E3 ubiquitin ligase activity that forms part of the hypoxic response, which is coordinated by hypoxia-inducible factors (HIFs)[68]. Interestingly, there may be a link between mutation of isocitrate dehydrogenase 1 (IDH1) and increased HIF1

PML bodies
Heterogeneous nuclear structures that range in size from 0.2 to 1 micrometre. They are found in most mammalian cell nuclei and have been ascribed functions in tumour suppression and transcription regulation, among others.

activity in gliomagenesis[69]. It is enticing to postulate the involvement of SIAH1-mediated HIPK2 degradation, which results from increased HIF1 activity, as a resistance mechanism to chemotherapy-induced cell death of solid hypoxic tumours. Perhaps the effectors discussed here are also involved in cancer pathogenesis, as observed for WIP1 overexpression[70]. Of course, the transcription of pro-apoptotic p53 target genes will need to overcome the threshold set by the anti-apoptotic proteins BCL-2 (REF. 71), survivin and XIAP[72] in order to activate the cell death machinery (FIG. 2). Tumour cells with high levels of these anti-apoptosis proteins have a lower or an abrogated apoptotic response following chemotherapy[71,73]. Therefore, adjuvant cancer therapy that targets the SIAH E3 ubiquitin ligases in tumour cells may be a rewarding approach[74].

Transcriptome damage and cell death. It is important to consider the stability, the inducibility and the regulation of the transcriptome in triggering cell death following DNA

Box 1 | **Activation of transcription factors regulating survival functions**

There is a plethora of transcription factors involved in regulating survival. We discuss two of these factors below, nuclear factor-κB (NF-κB) and AKT.

Pro-survival role of NF-κB

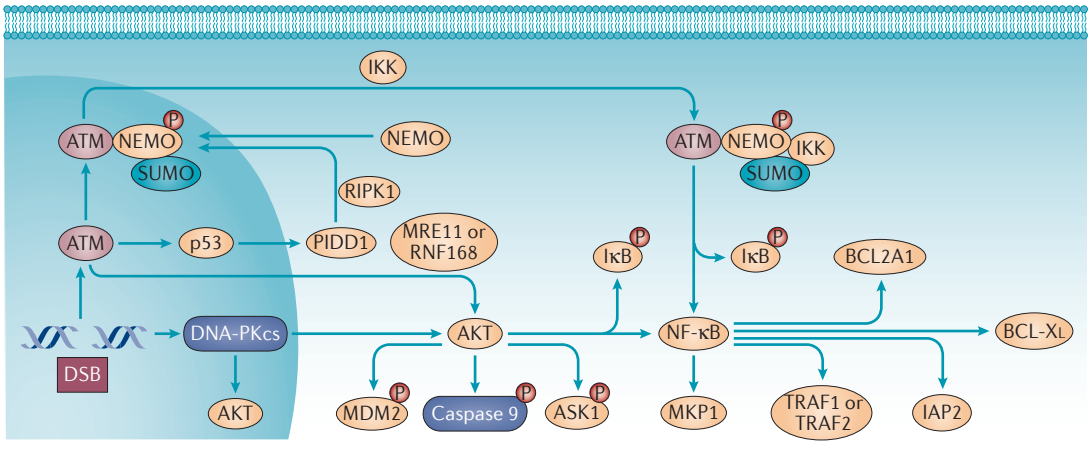
An increased body of evidence supports the model that NF-κB can be activated by DNA double-strand breaks (DSBs)[186]. For the activation of NF-κB via the ataxia telangiectasia mutated (ATM)/ataxia telangiectasia and Rad3-related (ATR)–CHK1/CHK2–p53 axis, it has been proposed that DSBs cause the nuclear localization of NF-κB essential modulator (NEMO)[187] via p53-induced death domain-containing protein 1 (PIDD1) in complex with receptor-interacting serine/threonine-protein kinase 1 (RIPK1; also known as RIP1) that promotes sumoylation of NEMO[188]. Thus, RIPK1-mediated NEMO sumoylation is essential for DNA damage-dependent activation of NF-κB[189]. DSBs also activate ATM, which complexes with and phosphorylates NEMO[187]. The ATM–NEMO complex is shuttled to the cytoplasm[190] where it binds to the inhibitor of NF-κB kinase (IKK) complex, which subsequently phosphorylates IκB, thereby leading to the transcriptional activation of NF-κB pro-survival target genes. Among others, NF-κB transcribes *BCL2L1* (which encodes BCL-XL), *BCL2A1* (also known as *BFL1*)[191], inhibitor of apoptosis protein 2 (*IAP2*; also known as *BIRC2*)[192], TNF receptor-associated factor 1 (*TRAF1*) and *TRAF2* (REF. 193) (see the figure). Transcriptional activation of NF-κB, therefore, suppresses the activation of both mitochondrial- and death receptor (TNFR)-mediated apoptosis. In addition, NF-κB also upregulates MAPK phosphatase 1 (MKP1)[194] to counteract JUN N-terminal kinase (JNK) phosphorylation and JNK-driven apoptosis, observed after sustained DNA damage induction (FIG. 2). Consequently, preventing the activation of NF-κB during treatment with DNA-damaging agents such as topoisomerase I inhibitors results in increased apoptosis and reduced tumour growth *in vivo*[195].

Pro-survival role of AKT

The AKT kinase suppresses apoptosis in a PI3K-dependent manner[196] by phosphorylating, and therefore inhibiting, pro-apoptotic proteins or by activating an anti-apoptosis system. Thus, AKT phosphorylates BCL-2-associated agonist of cell death (BAD)[150], apoptosis signal-regulating kinase 1 (ASK1; also known as MAP3K5)[151], human caspase 9 (REF. 152) and the E3 ubiquitin ligase MDM2 (REF. 153), thereby suppressing apoptosis (see the figure). AKT also suppresses apoptosis by promoting the degradation of IκB, which leads to the activation of NF-κB and to the suppression of apoptosis via the transcription of anti-apoptotic genes (see the figure).

Depending on the DNA-damaging agent, AKT can be activated in a manner dependent on DNA-dependent protein kinase (DNA-PK)[146], ATM[147], ATR[148] and/or MRE11 (REF. 149). Phosphorylated AKT (Ser472) colocalizes with phosphorylated ATM (Ser1981) at DNA damage-induced γH2AX foci[149], and this colocalization is facilitated by the action of the E3 ubiquitin ligase RNF168. AKT also influences the stabilization of p53 following activation of the DNA damage response (DDR) as AKT contributes to p53 degradation, probably through its action with MDM2 (REF. 146). Suppression of apoptosis by AKT leads to increased survival on DNA damage, which has been shown in numerous cancers[197].

In addition to the role of AKT described above, AKT was also shown to directly stimulate DNA repair. Thus, following DNA damage, AKT binds to DNA-PK, phosphorylates it and regulates the accumulation of DNA-PK at damaged sites to improve the efficiency of DSB re-joining by non-homologous end joining (NHEJ)[198]. AKT, therefore, regulates both DSB repair and survival and, conversely, the p53-dependent and p53-independent responses linked to cell death (see the figure).



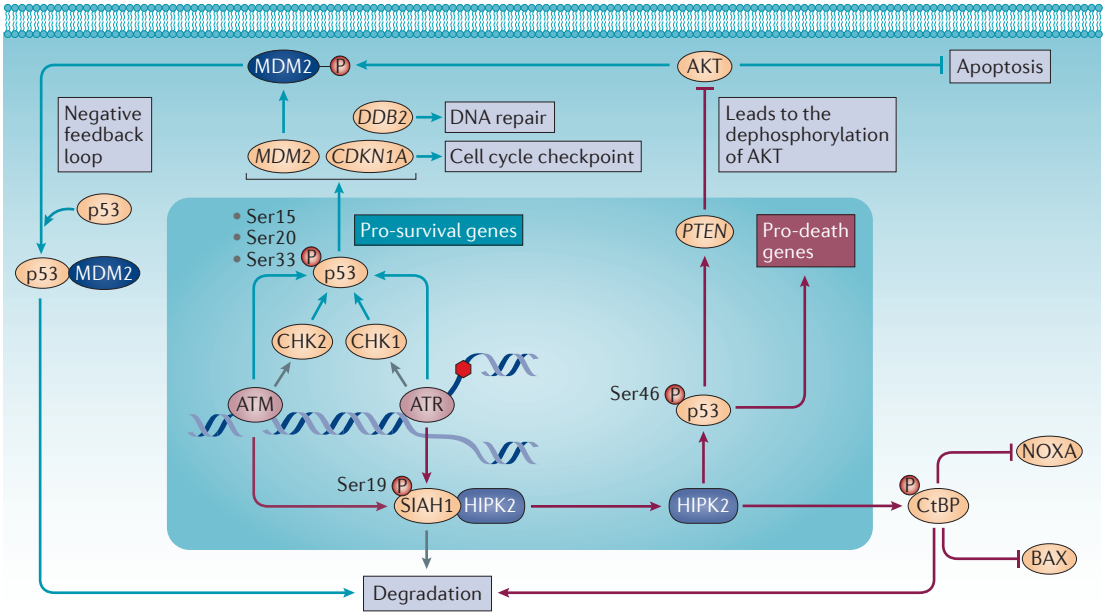


Figure 3 | **The DNA damage response activates both pro-survival and pro-death signalling.** Pro-survival signalling: ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) phosphorylate p53, thereby leading to the expression of pro-survival genes such as cyclin-dependent kinase inhibitor 1A (*CDKN1A*), DNA binding protein 2 (*DDB2*) and the E3 ubiquitin ligase *MDM2*. This is very likely an early response that is required for checkpoint activation and DNA repair. Pro-death signalling: ATM and ATR cause the stabilization of homeodomain-interacting protein kinase 2 (HIPK2) that stimulates apoptosis by phosphorylating Ser46 of p53, which results in its targeting to pro-apoptosis genes. HIPK2 also causes the degradation of carboxy-terminal binding protein (CtBP) that leads to the activation of pro-apoptotic proteins NOXA and BAX. This may be a late response when DNA repair has failed. SIAH1, seven in absentia homologue 1.

damage and not just DNA damage. On the one hand, the inhibition of RNA polymerase II during transcription is a known apoptotic trigger[75], but, on the other hand, epigenetic deregulation during cancer formation of pro-survival and anti-survival factors also affects cell death[76].

First, considering the inhibition of RNA polymerase II, the model states that, in general, pro-survival factors are transcribed from large genes (many kilobases in size), whereas pro-death factors are transcribed from small genes (few kilobases)[77]. Consequently, DNA adducts that inhibit transcription are considered to be more likely to inhibit the transcription of pro-survival genes, as larger genes are greater targets for damage than smaller genes. In this case, unrepaired transcription-blocking adducts prevent the transcription of pro-survival factors, shifting the balance to cell death.

This mechanism can tilt the balance between survival and death, whereby transcriptional inhibition triggers phosphorylation and the accumulation of p53 either through ATM- and/or ATR-directed signalling[78] or indirectly through the inhibition of *MDM2* transcription[79] (FIG. 3). The same transcription-blocking DNA damage could also prevent the transcription of pro-survival genes that are regulated by p53 such as damage-specific DNA binding protein 2 (*DDB2*) and xeroderma pigmentosum complementation group C (*XPC*)[80–82] and thereby affect the survival–death balance.

Bulky DNA adducts are particularly effective transcription inhibitors. Such adducts are repaired by transcription-coupled NER (TC-NER)[83] following RNA polymerase stalling. When TC-NER fails to remove bulky adducts, an accumulation of phospho-p53 (Ser15) is observed[84]. Whether the same is seen for phospho-p53 (Ser46) is unknown. The possibility that DNA damage-dependent transcriptional inhibition activates p53-triggered apoptosis is particularly important in cancer biology, as one would expect higher transcription rates given the increased proliferation (and metabolic) rates of cancer cells.

Another example of the interplay between transcription and DNA damage was provided by studies with cells deficient in the transcription factor FOS. Following UV radiation and treatment with agents that induce bulky DNA lesions, FOS is promptly induced in an immediate-early[85] and late[86] response. A hallmark of FOS-deficient cells is their hypersensitivity to genotoxins that cause the formation of bulky DNA lesions, such as UV radiation and benzo(a)pyrene[87,88], which is related to severe replication and transcription blockade[89]. The repair of DNA adducts is slow in FOS-deficient cells because they fail to express the NER proteins XPF and XPG, which are regulated via FOS-activating protein 1 (AP-1)[89]. The immediate-early induction of FOS causes the stimulation of *XPF* and *XPG* transcription, which is important because it maintains the NER capacity of the cell. This early response is known as maintenance regulation and compensates for a shortage of these unstable NER gene transcripts[90]. XPF and XPG are endonucleases that cleave the DNA 5′ and 3′ from the damage[91], and it is reasonable to suppose that cells express

them at a low basal level in order to avoid undesirable genomic changes. A fine-tuned (homeostatic) upregulation of these repair genes, depending on the genotoxic stress level, is obviously important in order to maintain genomic stability. Notably, priming the system with a low dose of a DNA-damaging agent prevents cell death that is triggered by a subsequent challenge dose[90]. This indicates a survival adaptation resulting from late FOS activation[86]. Therefore, the balance between survival and death might be different following acute and chronic genotoxic exposure.

The induction of FOS following DNA damage is part of a general stress response that involves the activation of the JUN N-terminal kinase (JNK) and p38 kinase, which results in an increase in AP-1 activity. NER-defective mutants display sustained JNK and p38 kinase activation, indicating that DNA damage is responsible for the response[92]. The resulting long-term activation of AP-1 results in an increased expression of Fas ligand (FASL; also known as FASLG)[93] that drives the extrinsic apoptosis pathway (FIG. 2).

Collectively, unrepaired toxic DNA lesions, which are usually substrates of NER, elicit a sustained upregulation of JNK and p38 kinases[92], a pro-death switch, by causing the downregulation of MAPK phosphatase 1 (MKP1; also known as DUSP1) — probably as a result of transcription blockade — which deactivates JNK and p38 (REF. 94) (FIG. 2). This in turn leads to late FASL upregulation that is controlled by JUN–ATF2 (REF. 95). In this case, the balance between survival and death following DNA damage rests on the proper resynthesis of the repair proteins XPF and XPG, the level of MKP1 downregulation, the duration of JNK and p38 kinase activation, the level of FASL induction and the availability of the FAS (also known as CD95 and APO1) death receptor system, which is often silenced in tumours, such as in melanomas[96]. In this context, for DNA damage that induces the upregulation of FOS and XPF–XPG-mediated NER repair, the levels of FOS in cancer cells may have a deciding role. It is conceivable that the manipulation of this system bears a potential for cancer therapy.

Changes in the transcriptome that are caused by epigenetic alterations should also be taken into account when considering the response of cells to DNA-damaging agents as epigenetics has a role in regulating DNA repair and the activation and execution of the death pathways. For example, in tumours, notably gliomas, the DNA repair gene O^6-methylguanine-DNA methyltransferase (*MGMT*) is frequently silenced by the hypermethylation of CpG islands in its promoter region[97]. MGMT removes alkyl adducts from the O^6-position of guanine, thereby preventing the cascade of events leading to DDR activation and cell death. Therefore, gliomas in which *MGMT* is silenced respond to alkylating chemotherapeutics[98]. Silencing of the factors that are required for apoptosis has also been reported in other cancers. As discussed above, the sustained stabilization of p53 caused by unrepaired DNA damage triggers apoptosis. A critical target gene of p53 is hypermethylated in cancer 1 (*HIC1*), which encodes a transcriptional repressor of sirtuin 1 (SIRT1). SIRT1 deacetylates p53, thereby negatively regulating its transcriptional activity[99]. Therefore, the often observed epigenetic silencing of *HIC1* in cancer cells removes the transcriptional suppression of *SIRT1* and leads to premature p53 inactivation, which counteracts p53-triggered apoptosis[100]. In addition, epigenetic silencing of the factors that are directly involved in apoptosis execution is also found in cancers. For example, melanomas contain silenced apoptotic peptidase activating factor 1 (*APAF1*)[101] and caspase 8 promoters[96], rendering them resistant to genotoxic therapies.

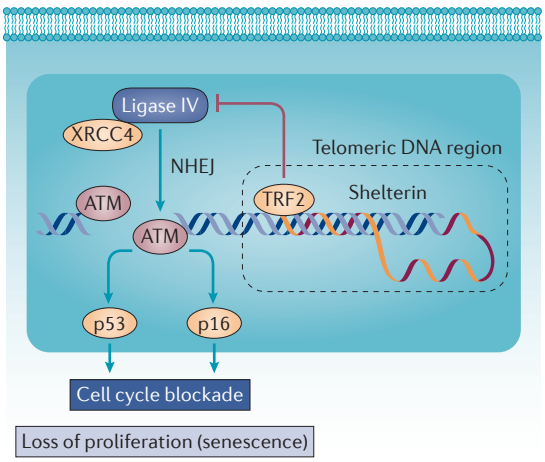


Figure 4 | **DNA damage-dependent senescence.** At telomeres, the shelterin protein telomeric repeat-binding factor 2 (TRF2) inhibits completion of non-homologous end joining (NHEJ) double-strand break repair by inhibiting ligase IV. This leads to continuous ataxia telangiectasia mutated (ATM) signalling to p53 and/or p16 and an irreversible cell cycle block.

DNA damage-triggered senescence. DNA damage is an inducer of cellular senescence[102]. Current knowledge of senescence originates from the initial observation that human diploid fibroblasts (HDFs) can only undergo a limited number of cell divisions (replicative senescence) and that HDFs exposed to high doses of DNA-damaging agents such as ionizing radiation or mitomycin C (MMC) stop proliferating while remaining metabolically active[103]. This phenomenon of DNA damage-induced senescence has been harnessed for generating cell feeder layers[103].

There is evidence that the signal for DNA damage-induced senescence originates from DSBs in telomeres, which activate continuous DDR signalling[102] and thus activate a cell cycle arrest in a p53-dependent[104] or p16 (also known as INK4A)-dependent[105] manner (FIG. 4). A fundamental question is why DSBs in genomic DNA only transiently activate the DDR but DSBs in telomeric DNA activate continuous DDR signalling. The answer lies in the observation that DSBs in or next to telomeres are not repaired. Telomeric repeat-binding factor 2 (TRF2), a component of shelterin that binds to double-stranded TTAGGG repeats in telomeres *in vitro*, localizes to all telomeres *in vivo*[106] and inhibits the

End-to-end fusion
The ends of linear DNA are protected by telomeres. When double-strand breaks (DSBs) occur in telomeres and telomeres are partially lost, DSB repair can fuse two broken telomeric ends. This can be seen in mitosis as two chromosomes connected at their ends.

completion of DSB repair by NHEJ by preventing the recruitment of ligase IV to DSBs that are located in or next to telomeric DNA[107] (FIG. 4). Therefore, owing to persistent DSBs in telomeres, the DDR is continually active, the cell cycle arrest is prolonged and the cell enters a quiescent state, but in genomic DNA DSBs are repaired and DDR activation and cell cycle arrest are transient. Thus, TRF2 protects human telomeres from end-to-end fusion[108], which may result in malignant transformation[109]. This explains why high doses of DNA-damaging agents (for example, >70 Gy of ionizing radiation) are required for generating viable, non-proliferating HDFs, as the probability for DSBs forming in telomeres is a fraction of the probability of their formation in the rest of the genome. It is important to note that p53 is required for activating this pathway, which explains why HDFs do not die following high-dose treatment, whereas human transformed cells that harbour activated RAS or mutated p53 undergo apoptotic death rather than senescence[110].

Notably, already moderately toxic doses of chemical genotoxins are able to trigger senescence. Thus, temozolomide, a methylating agent that is used in glioma therapy, was shown to induce ATM and ATR activation and senescence in a sub-fraction of the cell population already at low clinical concentrations[111]. It is therefore unlikely that unrepaired DSBs in telomeres are the only mechanism by which senescence is induced following the exposure of cells to DNA-damaging agents. Thus, intriguing unanswered questions that still need to be addressed include: how do low-dose genotoxins induce senescence in a telomere-independent manner, and are these silenced cells able to re-enter a proliferative state? If cells re-enter proliferation, the implication would be far-reaching, as tumour cells would escape therapy by entering a non-proliferating state that is reversed after the therapy cycle has been completed.

Autophagy in the balance between survival and death following DNA damage. The regulation of autophagy by DNA damage occurs on three levels. DNA damage prevents the suppression of autophagy by mTOR complex 1 (mTORC1) while activating the autophagy-related protein 1 homologue (ATG1; also known as ULK1) and the Beclin 1 complexes. This is accomplished via several pathways involving PARP1, JNK, ATM and p53 (FIG. 5).

In autophagy, p53 has multiple roles that are mediated through the transcriptional activation of *PTEN*[112] and the lysosomal protein DNA damage-regulated autophagy modulator protein 1 (DRAM1)[113]. The transcriptional activation of *PTEN* by p53 counteracts the suppression of autophagy by preventing the activation of AKT by receptor tyrosine kinases (RTKs). There are, therefore, opposing effects at play between the activation of AKT by growth factors[114] and the DNA damage-dependent inactivation of AKT by PTEN[115]. DRAM1 does not seem to have a role in autophagy initiation. The three splice variants (SV1 (REF. 113), SV4 and SV5 (REF. 116)) of *DRAM1* localize to the lysosomes or endosomes (SV1) or to the peroxisomes and autophagosomes (SV4 and SV5). As none of these organelles has a role in the initiation of autophagy, DRAM1 expression by p53 probably prepares the cells for dealing with the increased load of autophagy that is induced by DNA damage.

PARP1 is a chromatin-associated enzyme that is involved in BER of small adducts such as those induced by alkylating agents and ROS[117]. It modifies several proteins by poly(ADP-ribosyl)ation and in the process consumes large amounts of NAD^+. Treating PARP1 wild-type cells with doxorubicin, a topoisomerase II inhibitor that also induces oxidative stress[118], causes cells to indirectly activate autophagy, by the depletion of ATP and NAD^+. When PARP1 is knocked out, doxorubicin no longer triggers a strong autophagy response[119]. Interestingly, PARP1 also regulates starvation-triggered autophagy. When PARP1 is inhibited or deleted, the onset of starvation-triggered autophagy is delayed[120]. Starvation increases the intracellular ROS levels[121] and the DNA damage caused by ROS leads to the depletion of energy by PARP1 activation. Therefore, the repair of DNA damage during which PARP1 becomes activated is a pre-requisite for the optimal induction of autophagy[120]. All DNA-damaging agents, such as UV-A, that cause the formation of ROS, trigger autophagy[122]. Therefore, it seems that DNA damage and autophagy are tightly connected through PARP1.

DDR signalling can directly activate autophagy. In this context, ATM activates AMPK, which subsequently leads to the activation of autophagy[123]. Autophagy has been observed following the induction of DNA damage that does not require PARP1 for its repair. An example is DSBs that arise from the processing of O^6MeG[124] and that lead to the activation of ATR and ATM[125]. O^6MeG-triggered autophagy is dependent on ATM[111]. The use of ionizing radiation has expanded our understanding of the role of ATM in autophagy. DSBs that are induced by ionizing radiation activate ATM, which in turn activates the tumour suppressor tuberin (TSC2) via the serine/threonine-protein kinase 11 (STK11) and AMPK metabolic pathway to repress the autophagy suppressor mTORC1 (REFS 126,127) and activate the ATG1 complex. Interestingly, chaperone-mediated autophagy has been shown to have a role in the negative feedback regulation of the DDR as it causes the degradation of activated CHK1 (REF. 128).

The Beclin 1 complex can be inhibited by BCL-2 (REF. 129). BCL-2 is a downstream target of phosphorylation by JNK[130], thus BCL-2 is no longer able to inhibit the Beclin 1 complex and pre-autophagosomal membrane structures can form. As discussed above, MKP1 inactivates JNK and, therefore, the downregulation of MKP1 resulting from transcription-blocking DNA lesions will lead to sustained JNK activation. Therefore, it can be hypothesized that all transcription-blocking DNA lesions (above a particular threshold) bear the potential to activate the Beclin 1 complex (FIG. 5).

At low DNA damage levels, autophagy is a survival mechanism. With high damage levels, however, autophagy becomes a process that is out of control, and the balance is shifted to death. In molecular terms,

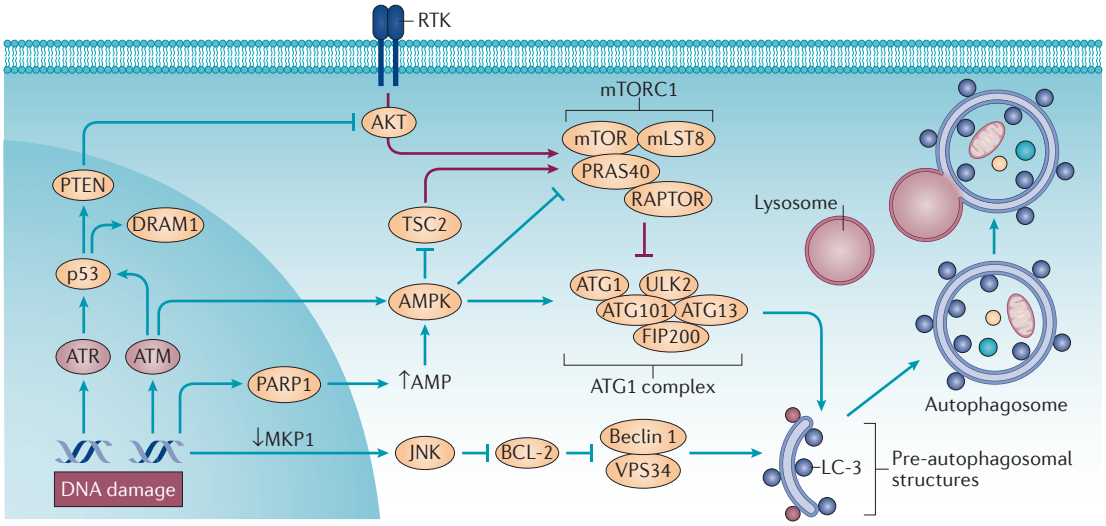


Figure 5 | **DNA damage-dependent autophagy.** Under optimal growth conditions, autophagy is suppressed owing to signalling from receptor tyrosine kinases (RTKs) through the PI3K–AKT pathway to mTOR complex 1 (mTORC1). mTORC1 comprises mTOR, mammalian lethal with SEC13 protein 8 (mLST8), PRAS40 (also known as AKT1S1) and regulatory-associated protein of mTOR (RAPTOR). AKT suppresses autophagy by phosphorylating PRAS40 directly or indirectly through the phosphorylation of tuberin (TSC2) (at Ser939, Ser1086/Ser1088 and Thr1422). The ATG1 complex, comprising autophagy-related protein 1 homologue (ATG1; also known as ULK1) and ULK2, ATG13 and FIP200 (also known as RB1CC1), is a principal initiator of autophagy. mTORC1 inhibits the ATG1 complex by direct interaction of RAPTOR with ATG1–ULK2 and mTOR phosphorylation of ATG13 and ATG1–ULK2. These phosphorylations suppress ATG1–ULK2 kinase activity and, as ATG1–ULK2–AGT13–FIP200 kinase activity is required for autophagy, this prevents autophagy initiation. Contrary to the suppression of autophagy by AKT and mTORC1, autophagy can be activated by the energy-sensitive kinase AMP-dependent protein kinase (AMPK). As shown, AMPK is activated by high cellular levels of AMP and deactivated by high ATP levels. Once activated, AMPK phosphorylates RAPTOR and TSC2 (at Thr1227 and Ser1345), thereby removing the suppression of autophagy by mTORC1. AMPK furthermore phosphorylates ATG1, thereby stimulating the activity of the ATG1 complex and promoting autophagy. Activated ATG1–ULK2 phosphorylates both FIP200 and ATG13, which then lead to the translocation of the entire complex to the pre-autophagosomal membrane and to autophagy induction. The canonical initiation of the pre-autophagosomal membrane structures is mediated by Beclin 1 in complex with VPS34 (also known as PIK3C3), a class III PI3K. DNA damage leads to the activation of autophagy by inactivating mTORC1 and activating the ATG1 and the Beclin 1 complexes. ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related; DRAM, damage-regulated autophagy modulator protein; JNK, JUN N-terminal kinase; MAPK1, MAPK phosphatase 1; PARP1, poly(ADP-ribose) polymerase 1.

persistent DNA damage leads to the long-lasting activation of ATM that causes the repression of mTORC1 and the induction of autophagy[127] that is not switched off[131]. Under these conditions, excessive autophagy will cause the cell to cannibalize itself and die. Within this context, ATM can be activated by forkhead box protein O3 (FOXO3)[132], which is normally bound to DNA. Upon DNA damage, it dissociates from the DNA, interacts with ATM and contributes to the sustained activation of the ATM/ATR–CHK1/CHK2–p53 axis. In addition, FOXO3 also regulates the transcription of *LC3* (also known as *MAP1LC3A*) and BCL-2/adenovirus E1B 19 kDa protein-interacting protein 3 (*BNIP3*), both of which are required for autophagy[132]. As FOXO3 activates both the p53-dependent cell death and autophagy pathway, it may have a role at the interface between survival and death. Studies have shown that death by autophagy in malignant cells following treatment with arsenic trioxide (As_2O_3)[133]. Arsenic trioxide affects the function of PML bodies[59], which are important in the phosphorylation of p53 at Ser46 by HIPK2. Therefore, a link may exist, centring on PML bodies[134], between autophagy cell death and phospho-p53 (Ser46). Although survival through autophagy may be a tumour-promoting mechanism[135] and a target for therapy[136], autophagy-mediated cell death may be an important tumour suppressor[137] that also contributes to the outcome of cancer therapy.

Interplay between survival and death

The crosstalk between the major end points autophagy, necrosis and apoptosis following genotoxic insult is complex and not fully understood. Some aspects have already been discussed above and so we focus here on examples that underline this complexity. On a basic level, DNA damage activates the cell cycle checkpoints, stops cell cycle progression and activates DNA repair via the ATM/ATR–CHK1/CHK2–p53 axis. Upon damage resolution, the ATM/ATR–CHK1/CHK2–p53 cascade is switched off by dephosphorylation[67], and cell death by necrosis or apoptosis does not occur. The complexity arises from the observation that the DDR simultaneously activates pro-death mechanisms such as apoptosis[138] and necroptosis[21] and pro-survival mechanisms including DNA repair[90] and autophagy[111], often via the same signalling proteins and pathways. Within this context, thresholds have an important role.

Thresholds in cell death are established by downstream mechanisms that inhibit pro-death pathways such as apoptosis. Important death modulators are survivin, IAPs and XIAP. Survivin, together with IAP and XIAP, inhibits caspases[72,139], thus preventing cell death by apoptosis. Survivin is highly expressed in replicating tissues during embryonic and fetal development[140] and is also found to be overexpressed in tumours[141]. Knockout of survivin sensitizes cells to DNA damage-triggered apoptosis as the threshold of activation is reduced[142]. During embryonic development, cells in tissues undergo continuous cycles of cell division. Replication stress that is caused by endogenous DNA damage[143] could potentially drive these cells into apoptosis. Survivin prevents this by suppressing the premature activation of apoptosis by inhibiting caspase activation. In tumours, the same mechanism leads to resistance to anticancer drugs[144]. Therefore, in order for apoptosis to occur, sufficient caspases must be activated to overcome the threshold preventing apoptosis that is established by the anti-apoptosis proteins survivin and XIAPs. Interestingly, as no threshold for the protective function of autophagy has been described, it is reasonable to posit that autophagy protects the cell from apoptosis at low DNA damage levels. In addition to overexpressing survivin, cancers have been shown to modulate the threshold for cell death activation by other means. For example, melanomas suppress the expression of the pro-apoptosis proteins APAF1 (REF. 101) and caspase 8 (REF. 96), and lymphomas express high levels of anti-apoptosis proteins such as BCL-2 (REF. 145). The creation of this threshold can be strengthened by active signalling. Thus, depending on the DNA-damaging agent, AKT can be activated in a manner dependent on DNA-PK[146], ATM[147], ATR[148] and/or MRE11 (REF. 149). AKT strengthens the threshold via its activating or inhibitory action on BCL-2-associated agonist of cell death (BAD)[150], apoptosis signal-regulating kinase 1 (ASK1; also known as MAP3K5)[151], caspase 9 (REF. 152) and MDM2 (REF. 153) (BOX 1). Upon persistent DNA damage and sustained DDR and apoptosis signalling, sufficient caspases become activated to overcome the inhibitory threshold, and these caspases cleave Beclin 1 (REF. 154), thus preventing further autophagy initiation and the protection gained from it. Importantly, the cleavage fragment of Beclin 1 interacts with mitochondria and further stimulates apoptosis. The threshold has thus been exceeded and the cell is able to enter the apoptotic pathway.

Upon DNA damage, if the cell is unable to activate the required amount of caspases to switch from autophagy to apoptosis, and the DNA damage is not resolved, the cell can die by regulated necrosis. It is important to note that most studies on regulated necrotic cell death have been carried out in systems in which apoptosis was inhibited, either chemically using small-molecule inhibitors[155] or by knockout[156] or knockdown[157] of death receptors and associated domains. Under these conditions, cells preferentially undergo regulated necrosis following DNA damage. This is in agreement with the biological role of regulated necrosis in nature. Cells infected with viruses that contain proteins that inhibit apoptosis trigger regulated necrosis as an alternative cell death mechanism[158]. In a similar manner, cancer cells that show apoptosis resistance are susceptible to the induction of necrosis[159]. Notably, apoptosis is an energy-dependent process whereas necrosis requires much less energy[160]. Excessive PARP1 activation that is caused by high amounts of base damage or DNA single-strand breaks will therefore switch the cell from apoptosis to regulated necrosis[161]. Consequently, two crucial factors for the initiation of regulated necrosis over apoptosis are, first, the effectiveness of the apoptosis machinery within a cell and, second, the available energy required for apoptosis initiation and completion.

In this scenario, two forms of regulated necrosis, namely, necroptosis and parthanatos, are briefly discussed. Necroptosis depends on the activation of receptor-interacting serine/threonine-protein kinase 3 (RIPK3)[162] by tumour necrosis factor receptor signalling via RIPK1 (REF. 163), cytosolic DNA via DNA-dependent activator of IFN-regulatory factors (DAI; also known as ZBP1 and DLM1)[164] and by lipopolysaccharides or double-stranded RNA via the Toll-like receptors and the action of TIR domain-containing adaptor molecule 1 (TICAM1)[165]. Caspase 8 and cellular FLICE-like inhibitory protein (cFLIP; also known as CFLAR)[166,167] inactivate RIPK3; therefore, inhibitors of caspase 8, such as IAP1 and IAP2 (REF. 168), will stimulate necroptosis while inhibiting apoptosis. RIPK3 in conjunction with RIPK1, DAI or TICAM1 causes the trimerization and plasma membrane localization of mixed lineage kinase domain-like protein (MLKL)[169] and, consequently, the loss of the osmotic homeostasis of the cell.

The term parthanatos was coined to describe the form of necrosis that is triggered owing to the hyperactivation of PARP1 (REF. 170). PARP1 hyperactivation leads to the depletion of cellular NAD^+ (REF. 171) that causes a catastrophic failure in ATP production via oxidative phosphorylation[172] and glycolysis[173]. Parthanatos is the most common form of regulated necrosis that is triggered by DNA damage. Whether necroptosis is triggered by DNA damage is largely unknown. Parthanatos is induced by genotoxins such as ionizing radiation[174], chemical ROS producers, and methylating and ethylating agents[21]. Massive amounts of DNA damage will lead to excessive PARP1 activation and consequently to the loss of cellular NAD^+ and ATP. Given the central role that ATP has in both apoptosis and necrosis, it is reasonable to state that the cellular energy status is an important factor involved in the decision of which pathway will be activated following DNA damage[175]. In support of this, autophagy and the energy sensor AMPK have a central role in preventing DNA damage-triggered parthanatos[176]. Following the induction of DNA damage, multiple energy-dependent processes (for example, BER) become activated that deplete cellular NAD^+ and ATP levels. The increase in AMP will activate AMPK to facilitate the restoration of ATP levels via autophagy and other mechanisms. When DNA damage overwhelms the capability of the cell to repair it, or when the energy-dependent processes that are activated by the DNA damage deplete ATP and NAD^+

Jasplakinolide
A drug originally isolated from a sponge used for the stabilization and polymerization of the actin filament. It triggers apoptosis in a DNA damage-independent manner.

(PARP1-mediated repair) beyond the ability of AMPK to restore sufficient energy to sustain life, the cell will die. In this scenario, the activation of AMPK protects against DNA damage-triggered cell death by activating autophagy or stimulating apoptosis instead of parthanatos by providing sufficient ATP for apoptosis execution.

Shifting the balance from death to survival

Apoptosis is characterized by several features (mitochondrial membrane dysfunction, cell shrinkage, membrane changes and activation of caspases) that are indicative of early and late apoptosis[177]. Can the process that triggers apoptosis be reverted if DNA damage signalling is interrupted? A point of no return was postulated, although this precise point has not been clearly defined[178]. This model of cell death interruption was verified by the demonstration that transient treatment with jasplakinolide induces mitochondrial damage and caspase activation, and that this could be reverted by interrupting the treatment. However, the process of apoptosis was no longer reversible when nuclear fragmentation occurred[179], supporting the idea that there is a point of no return in the pathway shifting cells from survival to death. This point is probably determined by a sufficiently high level of executing caspases and caspase-activated DNases (CADs) needed to cleave the genomic DNA completely. This may be influenced by anti-caspases such as survivin or XIAPs, which are overexpressed in cancers[73]. Rescue from apoptosis (known as anastasis) might be a tumour-relevant process, as it has been shown that incomplete apoptotic DNA cleavage may result in chromosomal aberrations and genomic instability[179]. Whether the process of DNA damage-induced apoptosis can be abrogated by the attenuation of the damage-triggered signalling following damage repair is an intriguing open question.

Conclusions

DNA damage triggers a plethora of cellular responses. Thus, it activates cell cycle checkpoints that provide time for the cell to repair the damage before it interferes with the replication machinery. If repair fails or DNA repair is saturated, the remaining DNA damage impedes replication and transcription, and thereby the activated DDR signals downstream cell death pathways. The competence of a cell to survive DNA damage is therefore proportional to the amount of critical DNA damage, the repair capacity of the cell, the proliferation level, the status of p53 and key DDR proteins including ATM, ATR and DNA-PK, the effectiveness of activating DNA repair genes (which is dependent on epigenetic silencing and cellular transcription factors), and the execution of downstream cell death pathways. The DNA repair capacity differs greatly between cell types; for example, human embryonic stem cells repair most types of DNA lesion more effectively than differentiated cell types[180], whereas monocytes and muscle cells are defective in BER[181,182], and some cancers show upregulation of repair, for example, metastatic melanoma[183], and highly variable MGMT repair activity, such as in gliomas[97,98]. The current paradigm states, in simple terms, that a low level of DNA damage activates DNA repair (hallmarked by the upregulation of repair genes such as *XPF*, *XPG*, *DDB2*, *XPC*, *XRCC1* and others[90]), whereas at high DNA damage levels DNA repair is saturated, and unrepaired DNA damage activates one of the death programmes, including apoptosis, regulated necrosis and extensive self-cannibalism by autophagy. It is not well understood how the cell switches between these pathways, but it seems that the phosphorylation status of p53 and anti-apoptosis thresholds are key nodes in determining whether a cell lives or dies. The functions of ATM and ATR extend into each possible outcome following DNA damage. For this reason, ATM and ATR seem to be the main decision makers, informing effectors such as p53 how to function. The increased resistance of tumours carrying mutations in ATM[29] demonstrates the importance of ATM in initiating cell death pathways. It is important to note that inactivation of p53 in cancer cells can lead to either drug sensitization or resistance, depending on the genotoxic agent used. Thus, bulky adducts induce a p53-triggered upregulation of DNA damage repair and tolerance genes (*XPC*, *DDB2* and DNA polymerase η (*POLH*)), which is a dominant survival trait[81,184], but upon small adducts, such as O^6MeG, upregulated apoptosis genes play a key role[138]. Although much work has focused on how different modifications of p53 dictate differential promoter activation of pro-survival and pro-death genes, it is not entirely clear how p53-deficient cells undergo cell death following DNA damage. Thus, the p53-independent pathways that are activated by DNA damage through MKP1, JNK, caspase 2 and possibly other factors need further exploration. The importance of DDR-triggered pro-survival factors (for example, DNA repair and damage tolerance), as well as thresholds that need to be overcome before cell death pathways can be initiated, have been recognized. Inhibitors that specifically target components in these pathways are in active development and in clinical trials (for examples see Supplementary Information S1 (Table)). Thus, inhibitors targeting the ATM/ATR–CHK1/CHK2 axis, cell cycle effectors and DNA repair show promise in combination with genotoxin-based chemotherapeutics, but might also have benefits per se in cancers that exhibit replication stress and extensive endogenous ROS production (for example, inhibitors of PARP1 and MTH1). By targeting anti-apoptosis proteins and pathways it is conceivable that the threshold for death is lowered for genotoxic- and biological-based therapies. In the future, it needs to be explored in more detail how specific DNA lesions activate and coordinate the complex interplay between survival and death. A deep understanding of the repair and processing of specific DNA lesions during replication and transcription and how they activate survival and death pathways is of fundamental importance for cancer therapy. The ultimate goal is to apply this knowledge to protect normal tissue during therapy with classic genotoxic anticancer drugs while simultaneously sensitizing cancer cells to die. The protection of normal tissue has far-reaching implications for stem cells, as they have been shown to activate DNA damage-triggered apoptosis easily[185], and for the elimination of genomically compromised cells from the healthy population as a cancer prevention strategy.

1. Voulgaridou, G. P., Anestopoulos, I., Franco, R., Panayiotidis, M. I. & Pappa, A. DNA damage induced by endogenous aldehydes: current state of knowledge. *Mutat. Res.* **711**, 13–27 (2011).
2. Fialkow, L., Wang, Y. & Downey, G. P. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. *Free Radic. Biol. Med.* **42**, 153–164 (2007).
3. Kielbassa, C., Roza, L. & Epe, B. Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis* **18**, 811–816 (1997).
4. Cadet, J., Ravanat, J. L., TavernaPorro, M., Menoni, H. & Angelov, D. Oxidatively generated complex DNA damage: tandem and clustered lesions. *Cancer Lett.* **327**, 5–15 (2012).
5. Roos, W. P. & Kaina, B. DNA damage-induced cell death: from specific DNA lesions to the DNA damage response and apoptosis. *Cancer Lett.* **332**, 237–248 (2013).
6. Dipple, A. DNA adducts of chemical carcinogens. *Carcinogenesis* **16**, 437–441 (1995).
7. Miller, E. C. & Miller, J. A. Mechanisms of chemical carcinogenesis. *Cancer* **47**, 1055–1064 (1981).
8. Hoeijmakers, J. H. Genome maintenance mechanisms for preventing cancer. *Nature* **411**, 366–374 (2001).
9. Roos, W. P. & Kaina, B. DNA damage-induced cell death by apoptosis. *Trends Mol. Med.* **12**, 440–450 (2006).
10. Lowe, S. W. & Lin, A. W. Apoptosis in cancer. *Carcinogenesis* **21**, 485–495 (2000).
11. Fulda, S. Cell death and survival signaling in oncogenesis. *Klin. Padiatr.* **222**, 340–344 (2010).
12. Helleday, T., Petermann, E., Lundin, C., Hodgson, B. & Sharma, R. A. DNA repair pathways as targets for cancer therapy. *Nat. Rev. Cancer* **8**, 193–204 (2008).
13. Fu, D., Calvo, J. A. & Samson, L. D. Balancing repair and tolerance of DNA damage caused by alkylating agents. *Nat. Rev. Cancer* **12**, 104–120 (2012).
14. Kaina, B., Christmann, M., Naumann, S. & Roos, W. P. MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. *DNA Repair (Amst.)* **6**, 1079–1099 (2007).
15. Ensminger, M. *et al.* DNA breaks and chromosomal aberrations arise when replication meets base excision repair. *J. Cell Biol.* **206**, 29–43 (2014).
16. Zeman, M. K. & Cimprich, K. A. Causes and consequences of replication stress. *Nat. Cell Biol.* **16**, 2–9 (2014).
17. Halazonetis, T. D., Gorgoulis, V. G. & Bartek, J. An oncogene-induced DNA damage model for cancer development. *Science* **319**, 1352–1355 (2008).
18. Ando, K. *et al.* PIDD death-domain phosphorylation by ATM controls prodeath versus prosurvival PIDDosome signaling. *Mol. Cell* **47**, 681–693 (2012).
19. Lips, J. & Kaina, B. DNA double-strand breaks trigger apoptosis in p53-deficient fibroblasts. *Carcinogenesis* **22**, 579–585 (2001).
20. Virag, L., Robaszkiewicz, A., Rodriguez-Vargas, J. M. & Oliver, F. J. Poly(ADP-ribose) signaling in cell death. *Mol. Aspects Med.* **34**, 1153–1167 (2013).
21. Chiu, L. Y., Ho, F. M., Shiah, S. G., Chang, Y. & Lin, W. W. Oxidative stress initiates DNA damager MNNG-induced poly(ADP-ribose) polymerase-1-dependent parthanatos cell death. *Biochem. Pharmacol.* **81**, 459–470 (2011).
22. Zhou, Z. D., Chan, C. H., Xiao, Z. C. & Tan, E. K. Ring finger protein 146/Iduna is a poly(ADP-ribose) polymer binding and PARsylation dependent E3 ubiquitin ligase. *Cell Adh. Migr.* **5**, 463–471 (2011).
23. Roos, W. P. *et al.* The translesion polymerase Rev3L in the tolerance of alkylating anticancer drugs. *Mol. Pharmacol.* **76**, 927–934 (2009).
24. Ashour, M. E., Atteya, R. & El-Khamisy, S. F. Topoisomerase-mediated chromosomal break repair: an emerging player in many games. *Nat. Rev. Cancer* **15**, 137–151 (2015).
25. Stingele, J., Habermann, B. & Jentsch, S. DNA–protein crosslink repair: proteases as DNA repair enzymes. *Trends Biochem. Sci.* **40**, 67–71 (2015).
26. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. & Bonner, W. M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* **273**, 5858–5868 (1998).
Identification of a specific marker of DSBs, namely, the phosphorylated form of H2AX (γH2AX), greatly stimulated research on DNA damage. γH2AX is currently the most sensitive marker for DSBs and blocked replication forks.
27. Eich, M., Roos, W. P., Nikolova, T. & Kaina, B. Contribution of ATM and ATR to the resistance of glioblastoma and malignant melanoma cells to the methylating anticancer drug temozolomide. *Mol. Cancer Ther.* **12**, 2529–2540 (2013).
28. Stankovic, T. *et al.* ATM mutations in sporadic lymphoid tumours. *Leuk. Lymphoma* **43**, 1563–1571 (2002).
29. Kim, H. *et al.* Having pancreatic cancer with tumoral loss of ATM and normal TP53 protein expression is associated with a poorer prognosis. *Clin. Cancer Res.* **20**, 1865–1872 (2014).
30. Pusapati, R. V. *et al.* ATM promotes apoptosis and suppresses tumorigenesis in response to Myc. *Proc. Natl Acad. Sci. USA* **103**, 1446–1451 (2006).
31. Bitomsky, N. & Hofmann, T. G. Apoptosis and autophagy: regulation of apoptosis by DNA damage signalling — roles of p53, 73 and HIPK2. *FEBS J.* **276**, 6074–6083 (2009).
32. Dahal, G. R. *et al.* Caspase-2 cleaves DNA fragmentation factor (DFF45)/inhibitor of caspase-activated DNase (ICAD). *Arch. Biochem. Biophys.* **468**, 134–139 (2007).
Here, the authors demonstrated a direct link between nuclear caspase 2 and the apoptosis-triggering nuclease CAD.
33. Bernstein, C., Bernstein, H., Payne, C. M. & Garewal, H. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat. Res.* **511**, 145–178 (2002).
34. Xu, Y. & Baltimore, D. Dual roles of *ATM* in the cellular response to radiation and in cell growth control. *Genes Dev.* **10**, 2401–2410 (1996).
In this paper it is shown, using *Atm*-knockout mice, that ATM plays a role in the DDR to ionizing radiation.
35. Swift, M., Morrell, D., Massey, R. B. & Chase, C. L. Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N. Engl. J. Med.* **325**, 1831–1836 (1991).
36. Thompson, D. *et al.* Cancer risks and mortality in heterozygous ATM mutation carriers. *J. Natl Cancer Inst.* **97**, 813–822 (2005).
37. Tanaka, A. *et al.* Germline mutation in ATR in autosomal- dominant oropharyngeal cancer syndrome. *Am. J. Hum. Genet.* **90**, 511–517 (2012).
In references 35–37, evidence is provided that mutations in *ATM* and *ATR* can predispose to cancer development.
38. Khanna, K. K. Cancer risk and the ATM gene: a continuing debate. *J. Natl Cancer Inst.* **92**, 795–802 (2000).
39. Galluzzi, L., Vitale, I., Vacchelli, E. & Kroemer, G. Cell death signaling and anticancer therapy. *Front. Oncol.* **1**, 5 (2011).
40. Helleday, T. Homologous recombination in cancer development, treatment and development of drug resistance. *Carcinogenesis* **31**, 955–960 (2010).
41. Sale, J. E. Competition, collaboration and coordination—determining how cells bypass DNA damage. *J. Cell Sci.* **125**, 1633–1643 (2012).
42. Vandenabeele, P., Galluzzi, L., Vanden Berghe, T. & Kroemer, G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat. Rev. Mol. Cell Biol.* **11**, 700–714 (2010).
43. Bartek, J. & Lukas, J. DNA damage checkpoints: from initiation to recovery or adaptation. *Curr. Opin. Cell Biol.* **19**, 238–245 (2007).
44. Vakifahmetoglu, H., Olsson, M. & Zhivotovsky, B. Death through a tragedy: mitotic catastrophe. *Cell Death Differ.* **15**, 1153–1162 (2008).
45. Lahav, G. *et al.* Dynamics of the p53-Mdm2 feedback loop in individual cells. *Nat. Genet.* **36**, 147–150 (2004).
46. Zhang, X. P., Liu, F., Cheng, Z. & Wang, W. Cell fate decision mediated by p53 pulses. *Proc. Natl Acad. Sci. USA* **106**, 12245–12250 (2009).
47. Tian, X. J., Liu, F., Zhang, X. P., Li, J. & Wang, W. A two-step mechanism for cell fate decision by coordination of nuclear and mitochondrial p53 activities. *PLoS ONE* **7**, e38164 (2012).
48. Inga, A., Storici, F., Darden, T. A. & Resnick, M. A. Differential transactivation by the p53 transcription factor is highly dependent on p53 level and promoter target sequence. *Mol. Cell. Biol.* **22**, 8612–8625 (2002).
The differential binding affinity of p53 for target promoters and its contribution to the different functions of p53 was demonstrated in this work.
49. Nicol, S. M. *et al.* The RNA helicase p68 (DDX5) is selectively required for the induction of p53-dependent p21 expression and cell-cycle arrest after DNA damage. *Oncogene* **32**, 3461–3469 (2013).
50. Tanaka, T. Ohkubo, S., Tatsuno, I. & Prives, C. hCAS/CSE1L associates with chromatin and regulates expression of select p53 target genes. *Cell* **130**, 638–650 (2007).
51. Sullivan, A. & Lu, X. ASPP: a new family of oncogenes and tumour suppressor genes. *Br. J. Cancer* **96**, 196–200 (2007).
52. Lettre, G. *et al.* Genome-wide RNAi identifies p53-dependent and -independent regulators of germ cell apoptosis in *C. elegans*. *Cell Death Differ.* **11**, 1198–1203 (2004).
53. Loughery, J., Cox, M., Smith, L. M. & Meek, D. W. Critical role for p53-serine 15 phosphorylation in stimulating transactivation at p53-responsive promoters. *Nucleic Acids Res.* **42**, 7666–7680 (2014).
54. Jabbur, J. R., Huang, P. & Zhang, W. DNA damage-induced phosphorylation of p53 at serine 20 correlates with p21 and Mdm-2 induction *in vivo*. *Oncogene* **19**, 6203–6208 (2000).
References 53 and 54 show that p53 phosphorylated at Ser15 and Ser20 displays different roles in auto-regulation and cell cycle checkpoint activation.
55. Pietsch, E. C., Sykes, S. M., McMahon, S. B. & Murphy, M. E. The p53 family and programmed cell death. *Oncogene* **27**, 6507–6521 (2008).
56. Ichwan, S. J. *et al.* Defect in serine 46 phosphorylation of p53 contributes to acquisition of p53 resistance in oral squamous cell carcinoma cells. *Oncogene* **25**, 1216–1224 (2006).
57. Mayo, L. D. *et al.* Phosphorylation of human p53 at serine 46 determines promoter selection and whether apoptosis is attenuated or amplified. *J. Biol. Chem.* **280**, 25953–25959 (2005).
In this paper it is shown that p53 phosphorylated at Ser46 specifically activates pro-apoptotic genes.
58. Oda, K. *et al.* p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell* **102**, 849–862 (2000).
59. Hofmann, T. G. *et al.* Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. *Nat. Cell Biol.* **4**, 1–10 (2002).
In this paper, it is shown for the first time that HIPK2 is responsible for phosphorylating p53 at Ser46 in response to DNA damage.
60. Bulavin, D. V. *et al.* Phosphorylation of human p53 by p38 kinase coordinates N-terminal phosphorylation and apoptosis in response to UV radiation. *EMBO J.* **18**, 6845–6854 (1999).
61. Yoshida, K., Liu, H. & Miki, Y. Protein kinase Cδ regulates Ser^{46} phosphorylation of p53 tumor suppressor in the apoptotic response to DNA damage. *J. Biol. Chem.* **281**, 5734–5740 (2006).
62. Okamura, S. *et al. p53DINP1*, a p53-inducible gene, regulates p53-dependent apoptosis. *Mol. Cell* **8**, 85–94 (2001).
63. Taira, N., Nihira, K., Yamaguchi, T., Miki, Y. & Yoshida, K. DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. *Mol. Cell* **25**, 725–738 (2007).
64. Lee, M. G. *et al.* XAF1 directs apoptotic switch of p53 signaling through activation of HIPK2 and ZNF313. *Proc. Natl Acad. Sci. USA* **111**, 15532–15537 (2014).
65. Winter, M. *et al.* Control of HIPK2 stability by ubiquitin ligase Siah-1 and checkpoint kinases ATM and ATR. *Nat. Cell Biol.* **10**, 812–824 (2008).
This paper shows that HIPK2 is part of the DDR.
66. Guo, A. *et al.* The function of PML in p53-dependent apoptosis. *Nat. Cell Biol.* **2**, 730–736 (2000).
67. Takekawa, M. *et al.* p53-inducible Wip1 phosphatase mediates a negative feedback regulation of p38 MAPK-p53 signaling in response to UV radiation. *EMBO J.* **19**, 6517–6526 (2000).
68. Nakayama, K., Qi, J. & Ronai, Z. The ubiquitin ligase Siah2 and the hypoxia response. *Mol. Cancer Res.* **7**, 443–451 (2009).
69. Zhao, S. *et al.* Glioma-derived mutations in *IDH1* dominantly inhibit IDH1 catalytic activity and induce HIF-1α. *Science* **324**, 261–265 (2009).
70. Bulavin, D. V. *et al.* Amplification of *PPM1D* in human tumors abrogates p53 tumor-suppressor activity. *Nat. Genet.* **31**, 210–215 (2002).
71. Yip, K. W. & Reed, J. C. Bcl-2 family proteins and cancer. *Oncogene* **27**, 6398–6406 (2008).
72. Tamm, I. *et al.* IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res.* **58**, 5315–5320 (1998).

73. Ambrosini, G., Adida, C. & Altieri, D. C. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat. Med.* **3**, 917–921 (1997).
Here, it was demonstrated that survivin, which was thought to only have a role during fetal development, is overexpressed in tumours, having a role in apoptosis regulation.
74. Knauer, S. K., Mahendrarajah, N., Roos, W. P. & Kramer, O. H. The inducible E3 ubiquitin ligases SIAH1 and SIAH2 perform critical roles in breast and prostate cancers. *Cytokine Growth Factor Rev.* **26**, 405–413 (2015).
75. Andera, L. & Wasylyk, B. Transcription abnormalities potentiate apoptosis of normal human fibroblasts. *Mol. Med.* **3**, 852–863 (1997).
76. De Carvalho, D. D. *et al.* DNA methylation screening identifies driver epigenetic events of cancer cell survival. *Cancer Cell* **21**, 655–667 (2012).
77. Ljungman, M. & Lane, D. P. Transcription — guarding the genome by sensing DNA damage. *Nat. Rev. Cancer* **4**, 727–737 (2004).
78. Derheimer, F. A. *et al.* RPA and ATR link transcriptional stress to p53. *Proc. Natl Acad. Sci. USA* **104**, 12778–12783 (2007).
This paper showed convincingly that DNA lesions blocking transcription activate ATR and consequently the DDR.
79. Conforti, G., Nardo, T., D'Incalci, M. & Stefanini, M. Proneness to UV-induced apoptosis in human fibroblasts defective in transcription coupled repair is associated with the lack of *Mdm2* transactivation. *Oncogene* **19**, 2714–2720 (2000).
80. Hwang, B. J., Ford, J. M., Hanawalt, P. C. & Chu, G. Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. *Proc. Natl Acad. Sci. USA* **96**, 424–428 (1999).
81. Barckhausen, C., Roos, W. P., Naumann, S. C. & Kaina, B. Malignant melanoma cells acquire resistance to DNA interstrand cross-linking chemotherapeutics by p53-triggered upregulation of DDB2/XPC-mediated DNA repair. *Oncogene* **33**, 1964–1974 (2014).
82. Batista, L. F., Roos, W. P., Christmann, M., Menck, C. F. & Kaina, B. Differential sensitivity of malignant glioma cells to methylating and chloroethylating anticancer drugs: 53 determines the switch by regulating *xpc*, *ddb2*, and DNA double-strand breaks. *Cancer Res.* **67**, 11886–11895 (2007).
Here, the dependence on the anticancer drug to activate the dual function of p53 in regulating apoptosis or DNA repair is demonstrated.
83. Proietti De Santis, L. *et al.* Transcription coupled repair efficiency determines the cell cycle progression and apoptosis after UV exposure in hamster cells. *DNA Repair (Amst.)* **1**, 209–223 (2002).
84. Ljungman, M., O'Hagan, H. M. & Paulsen, M. T. Induction of ser15 and lys382 modifications of p53 by blockage of transcription elongation. *Oncogene* **20**, 5964–5971 (2001).
85. Herrlich, P. *et al.* The mammalian UV response: mechanism of DNA damage induced gene expression. *Adv. Enzyme Regul.* **34**, 381–395 (1994).
86. Tomicic, M. T. *et al.* Delayed c-Fos activation in human cells triggers XPF induction and an adaptive response to UVC-induced DNA damage and cytotoxicity. *Cell. Mol. Life Sci.* **68**, 1785–1798 (2011).
87. Haas, S. & Kaina, B. c-Fos is involved in the cellular defence against the genotoxic effect of UV radiation. *Carcinogenesis* **16**, 985–991 (1995).
88. Kaina, B., Haas, S. & Kappes, H. A general role for c-Fos in cellular protection against DNA-damaging carcinogens and cytostatic drugs. *Cancer Res.* **57**, 2721–2731 (1997).
89. Christmann, M., Tomicic, M. T., Origer, J., Aasland, D. & Kaina, B. c-Fos is required for excision repair of UV-light induced DNA lesions by triggering the re-synthesis of XPF. *Nucleic Acids Res.* **34**, 6530–6539 (2006).
In references 86–89, evidence is provided that FOS has a dual role in regulating DNA repair and, if repair is saturated, apoptosis.
90. Christmann, M. & Kaina, B. Transcriptional regulation of human DNA repair genes following genotoxic stress: trigger mechanisms, inducible responses and genotoxic adaptation. *Nucleic Acids Res.* **41**, 8403–8420 (2013).
91. Marteijn, J. A., Lans, H., Vermeulen, W. & Hoeijmakers, J. H. Understanding nucleotide excision repair and its roles in cancer and ageing. *Nat. Rev. Mol. Cell Biol.* **15**, 465–481 (2014).
92. Hamdi, M. *et al.* DNA damage in transcribed genes induces apoptosis via the JNK pathway and the JNK-phosphatase MKP-1. *Oncogene* **24**, 7135–7144 (2005).
The fundamental relationship between NER, JNK, MKP1 and apoptosis was elucidated in this article.
93. Brozovic, A. *et al.* Long-term activation of SAPK/JNK, 38 kinase and fas-L expression by cisplatin is attenuated in human carcinoma cells that acquired drug resistance. *Int. J. Cancer* **112**, 974–985 (2004).
94. Hirsch, D. D. & Stork, P. J. Mitogen-activated protein kinase phosphatases inactivate stress-activated protein kinase pathways *in vivo*. *J. Biol. Chem.* **272**, 4568–4575 (1997).
95. Christmann, M., Tomicic, M. T., Aasland, D. & Kaina, B. A role for UV-light-induced c-Fos: stimulation of nucleotide excision repair and protection against sustained JNK activation and apoptosis. *Carcinogenesis* **28**, 183–190 (2007).
96. Roos, W. P. *et al.* Intrinsic anticancer drug resistance of malignant melanoma cells is abrogated by IFN-β and valproic acid. *Cancer Res.* **71**, 4150–4160 (2011).
97. Christmann, M., Verbeek, B., Roos, W. P. & Kaina, B. O^6-Methylguanine-DNA methyltransferase (MGMT) in normal tissues and tumors: enzyme activity, promoter methylation and immunohistochemistry. *Biochim. Biophys. Acta* **1816**, 179–190 (2011).
98. Weller, M. *et al.* MGMT promoter methylation in malignant gliomas: ready for personalized medicine? *Nat. Rev. Neurol.* **6**, 39–51 (2010).
99. Smith, J. Human Sir2 and the 'silencing' of p53 activity. *Trends Cell Biol.* **12**, 404–406 (2002).
100. Chen, W. Y. *et al.* Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. *Cell* **123**, 437–448 (2005).
In this paper, the feedback loop between p53, HIC1 and the deacetylase SIRT1 was described.
101. Soengas, M. S. *et al.* Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* **409**, 207–211 (2001).
102. von Zglinicki, T., Saretzki, G., Ladhoff, J., d'Adda di Fagagna, F. & Jackson, S. P. Human cell senescence as a DNA damage response. *Mech. Ageing Dev.* **126**, 111–117 (2005).
103. Stevenson, A. F. & Cremer, T. Senescence *in vitro* and ionising radiations—the human diploid fibroblast model. *Mech. Ageing Dev.* **15**, 51–63 (1981).
104. Rodier, F. *et al.* Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat. Cell Biol.* **11**, 973–979 (2009).
105. Mirzayans, R., Andrais, B., Hansen, G. & Murray, D. Role of $p16^{INK4A}$ in replicative senescence and DNA damage-induced premature senescence in p53-deficient human cells. *Biochem. Res. Int.* **2012**, 951574 (2012).
106. Broccoli, D., Smogorzewska, A., Chong, L. & de Lange, T. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat. Genet.* **17**, 231–235 (1997).
107. Fumagalli, M. *et al.* Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. *Nat. Cell Biol.* **14**, 355–365 (2012).
The authors demonstrated that the shelterin protein TRF2 prevents the completion of DSB repair by NHEJ in telomeres by inhibiting ligase IV, thereby causing persistent ATM-mediated DDR signalling, leading to senescence.
108. van Steensel, B., Smogorzewska, A. & de Lange, T. TRF2 protects human telomeres from end-to-end fusions. *Cell* **92**, 401–413 (1998).
109. Brunori, M. *et al.* TRF2 inhibition promotes anchorage-independent growth of telomerase-positive human fibroblasts. *Oncogene* **25**, 990–997 (2006).
110. Hundley, J. E. *et al.* Increased tumor proliferation and genomic instability without decreased apoptosis in MMTV-ras mice deficient in p53. *Mol. Cell. Biol.* **17**, 723–731 (1997).
111. Knizhnik, A. V. *et al.* Survival and death strategies in glioma cells: autophagy, senescence and apoptosis triggered by a single type of temozolomide-induced DNA damage. *PLoS ONE* **8**, e55665 (2013).
112. Stambolic, V. *et al.* Regulation of PTEN transcription by p53. *Mol. Cell* **8**, 317–325 (2001).
113. Crighton, D. *et al.* DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* **126**, 121–134 (2006).
114. Ravikumar, B. *et al.* Raised intracellular glucose concentrations reduce aggregation and cell death caused by mutant Huntingtin exon 1 by decreasing mTOR phosphorylation and inducing autophagy. *Hum. Mol. Genet.* **12**, 985–994 (2003).
115. Cao, C. *et al.* Inhibition of mammalian target of rapamycin or apoptotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells. *Cancer Res.* **66**, 10040–10047 (2006).
116. Mah, L. Y., O'Prey, J., Baudot, A. D., Hoekstra, A. & Ryan, K. M. *DRAM-1* encodes multiple isoforms that regulate autophagy. *Autophagy* **8**, 18–28 (2012).
117. de Murcia, J. M. *et al.* Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells. *Proc. Natl Acad. Sci. USA* **94**, 7303–7307 (1997).
118. Olson, R. D., Boerth, R. C., Gerber, J. G. & Nies, A. S. Mechanism of adriamycin cardiotoxicity: evidence for oxidative stress. *Life Sci.* **29**, 1393–1401 (1981).
119. Munoz-Gamez, J. A. *et al.* PARP-1 is involved in autophagy induced by DNA damage. *Autophagy* **5**, 61–74 (2009).
120. Rodriguez-Vargas, J. M. *et al.* ROS-induced DNA damage and PARP-1 are required for optimal induction of starvation-induced autophagy. *Cell Res.* **22**, 1181–1198 (2012).
References 117, 119 and 120 describe the fundamental role of PARP1 for survival and regulation of autophagy following DNA damage.
121. Li, L., Ishdorj, G. & Gibson, S. B. Reactive oxygen species regulation of autophagy in cancer: implications for cancer treatment. *Free Radic. Biol. Med.* **53**, 1499–1410 (2012).
122. Lamore, S. D. & Wondrak, G. T. Autophagic-lysosomal dysregulation downstream of cathepsin B inactivation in human skin fibroblasts exposed to UVA. *Photochem. Photobiol. Sci.* **11**, 163–172 (2012).
123. Suzuki, A. *et al.* IGF-1 phosphorylates AMPK-α subunit in ATM-dependent and LKB1-independent manner. *Biochem. Biophys. Res. Commun.* **324**, 986–992 (2004).
124. Ochs, K. & Kaina, B. Apoptosis induced by DNA damage O^6-methylguanine is Bcl-2 and caspase-9/3 regulated and Fas/caspase-8 independent. *Cancer Res.* **60**, 5815–5824 (2000).
125. Caporali, S. *et al.* DNA damage induced by temozolomide signals to both ATM and ATR: role of the mismatch repair system. *Mol. Pharmacol.* **66**, 478–491 (2004).
126. Alexander, A., Kim, J. & Walker, C. L. ATM engages the TSC2/mTORC1 signaling node to regulate autophagy. *Autophagy* **6**, 672–673 (2010).
127. Alexander, A. *et al.* ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. *Proc. Natl Acad. Sci. USA* **107**, 4153–4158 (2010).
This paper describes the link between ATM and mTOR.
128. Park, C., Suh, Y. & Cuervo, A. M. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. *Nat. Commun.* **6**, 6823 (2015).
129. Pattingre, S. *et al.* Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* **122**, 927–939 (2005).
130. Wei, Y., Sinha, S. & Levine, B. Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation. *Autophagy* **4**, 949–951 (2008).
131. Tsujimoto, Y. & Shimizu, S. Another way to die: autophagic programmed cell death. *Cell Death Differ.* **12** (Suppl. 2), 1528–1534 (2005).
132. Tsai, W. B., Chung, Y. M., Takahashi, Y., Xu, Z. & Hu, M. C. Functional interaction between FOXO3a and ATM regulates DNA damage response. *Nat. Cell Biol.* **10**, 460–467 (2008).
133. Kanzawa, T., Kondo, Y., Ito, H., Kondo, S. & Germano, I. Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. *Cancer Res.* **63**, 2103–2108 (2003).
134. Isakson, P., Bjoras, M., Boe, S. O. & Simonsen, A. Autophagy contributes to therapy-induced degradation of the PML/RARA oncoprotein. *Blood* **116**, 2324–2331 (2010).
135. Rez, G., Toth, S. & Palfia, Z. Cellular autophagic capacity is highly increased in azaserine-induced premalignant atypical acinar nodule cells. *Carcinogenesis* **20**, 1893–1898 (1999).
136. Yang, P. M. & Chen, C. C. Life or death? Autophagy in anticancer therapies with statins and histone deacetylase inhibitors. *Autophagy* **7**, 107–108 (2011).
137. Gozuacik, D. & Kimchi, A. Autophagy as a cell death and tumor suppressor mechanism. *Oncogene* **23**, 2891–2906 (2004).

138. Roos, W. P. *et al.* Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O^6-methylguanine. *Oncogene* **26**, 186–197 (2007).
139. Altieri, D. C. Survivin and IAP proteins in cell-death mechanisms. *Biochem. J.* **430**, 199–205 (2010).
140. Adida, C. *et al.* Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. *Am. J. Pathol.* **152**, 43–49 (1998).
141. Santa Cruz Guindalini, R., Mathias Machado, M. C. & Garicochea, B. Monitoring survivin expression in cancer: implications for prognosis and therapy. *Mol. Diagn. Ther.* **17**, 331–342 (2013).
142. Greve, B. *et al.* Survivin, a target to modulate the radiosensitivity of Ewing's sarcoma. *Strahlenther. Onkol.* **188**, 1038–1047 (2012).
143. Wallace, M. D., Southard, T. L., Schimenti, K. J. & Schimenti, J. C. Role of DNA damage response pathways in preventing carcinogenesis caused by intrinsic replication stress. *Oncogene* **33**, 3688–3695 (2014).
144. Wu, Y. K. *et al.* Nuclear survivin expression: a prognostic factor for the response to taxane-platinum chemotherapy in patients with advanced non-small cell lung cancer. *Med. Oncol.* **31**, 79 (2014).
145. Sedlak, T. W. *et al.* Multiple Bcl-2 family members demonstrate selective dimerizations with Bax. *Proc. Natl Acad. Sci. USA* **92**, 7834–7838 (1995).
146. Boehme, K. A., Kulikov, R. & Blattner, C. p53 stabilization in response to DNA damage requires Akt/PKB and DNA-PK. *Proc. Natl Acad. Sci. USA* **105**, 7785–7790 (2008).
147. Li, Y., Xiong, H. & Yang, D. Q. Functional switching of ATM: sensor of DNA damage in proliferating cells and mediator of Akt survival signal in post-mitotic human neuron-like cells. *Chin. J. Cancer* **31**, 364–372 (2012).
148. Caporali, S. *et al.* AKT is activated in an ataxia-telangiectasia and Rad3-related-dependent manner in response to temozolomide and confers protection against drug-induced cell growth inhibition. *Mol. Pharmacol.* **74**, 173–183 (2008).
149. Fraser, M. *et al.* MRE11 promotes AKT phosphorylation in direct response to DNA double-strand breaks. *Cell Cycle* **10**, 2218–2232 (2011).
150. Datta, S. R. *et al.* Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* **91**, 231–241 (1997).
151. Kim, A. H., Khursigara, G., Sun, X., Franke, T. F. & Chao, M. V. Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. *Mol. Cell. Biol.* **21**, 893–901 (2001).
152. Cardone, M. H. *et al.* Regulation of cell death protease caspase-9 by phosphorylation. *Science* **282**, 1318–1321 (1998).
153. Mayo, L. D. & Donner, D. B. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. *Proc. Natl Acad. Sci. USA* **98**, 11598–11603 (2001).
154. Wirawan, E. *et al.* Caspase-mediated cleavage of Beclin-1 inactivates Beclin-1-induced autophagy and enhances apoptosis by promoting the release of proapoptotic factors from mitochondria. *Cell Death Dis.* **1**, e18 (2010).
The importance of caspase cleavage of Beclin 1 in the switch between autophagy and apoptosis is demonstrated in this paper.
155. Trichonas, G. *et al.* Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis. *Proc. Natl Acad. Sci. USA* **107**, 21695–21700 (2010).
156. Osborn, S. L. *et al.* Fas-associated death domain (FADD) is a negative regulator of T-cell receptor-mediated necroptosis. *Proc. Natl Acad. Sci. USA* **107**, 13034–13039 (2010).
157. Vanlangenakker, N., Bertrand, M. J., Bogaert, P., Vandenabeele, P. & Vanden Berghe, T. TNF-induced necroptosis in L929 cells is tightly regulated by multiple TNFR1 complex I and II members. *Cell Death Dis.* **2**, e230 (2011).
158. Chan, F. K. *et al.* A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. *J. Biol. Chem.* **278**, 51613–51621 (2003).
159. Hu, X., Han, W. & Li, L. Targeting the weak point of cancer by induction of necroptosis. *Autophagy* **3**, 490–492 (2007).
160. Skulachev, V. P. Bioenergetic aspects of apoptosis, necrosis and mitoptosis. *Apoptosis* **11**, 473–485 (2006).
161. Los, M. *et al.* Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. *Mol. Biol. Cell* **13**, 978–988 (2002).
162. Newton, K. *et al.* Activity of protein kinase RIPK3 determines whether cells die by necroptosis or apoptosis. *Science* **343**, 1357–1360 (2014).
163. Duprez, L. *et al.* Intermediate domain of receptor-interacting protein kinase 1 (RIPK1) determines switch between necroptosis and RIPK1 kinase-dependent apoptosis. *J. Biol. Chem.* **287**, 14863–14872 (2012).
In references 161–163, the role of PARP1 and RIPK3–RIPK1 was demonstrated in the switch between necroptosis and apoptosis.
164. Upton, J. W., Kaiser, W. J. & Mocarski, E. S. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. *Cell Host Microbe* **11**, 290–297 (2012).
165. Kaiser, W. J. & Offermann, M. K. Apoptosis induced by the toll-like receptor adaptor TRIF is dependent on its receptor interacting protein homotypic interaction motif. *J. Immunol.* **174**, 4942–4952 (2005).
166. O'Donnell, M. A. *et al.* Caspase 8 inhibits programmed necrosis by processing CYLD. *Nat. Cell Biol.* **13**, 1437–1442 (2011).
167. He, M. X. & He, Y. W. A role for c-FLIP(L) in the regulation of apoptosis, autophagy, and necroptosis in T lymphocytes. *Cell Death Differ.* **20**, 188–197 (2013).
168. McComb, S. *et al.* cIAP1 and cIAP2 limit macrophage necroptosis by inhibiting Rip1 and Rip3 activation. *Cell Death Differ.* **19**, 1791–1801 (2012).
169. Cai, Z. *et al.* Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. *Nat. Cell Biol.* **16**, 55–65 (2014).
170. Andrabi, S. A., Dawson, T. M. & Dawson, V. L. Mitochondrial and nuclear cross talk in cell death: parthanatos. *Ann. NY Acad. Sci.* **1147**, 233–241 (2008).
171. Burkle, A. Poly(ADP-ribose). The most elaborate metabolite of NAD^+. *FEBS J.* **272**, 4576–4589 (2005).
172. Szabo, C. & Dawson, V. L. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends Pharmacol. Sci.* **19**, 287–298 (1998).
173. Ying, W., Garnier, P. & Swanson, R. A. NAD^+ repletion prevents PARP-1-induced glycolytic blockade and cell death in cultured mouse astrocytes. *Biochem. Biophys. Res. Commun.* **308**, 809–813 (2003).
174. Osato, K. *et al.* Apoptosis-inducing factor deficiency decreases the proliferation rate and protects the subventricular zone against ionizing radiation. *Cell Death Dis.* **1**, e84 (2010).
175. Leist, M., Single, B., Castoldi, A. F., Kuhnle, S. & Nicotera, P. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J. Exp. Med.* **185**, 1481–1486 (1997).
This paper shows for the first time that the ATP level determines the switch between apoptosis and necrosis.
176. Huang, C. T., Huang, D. Y., Hu, C. J., Wu, D. & Lin, W. W. Energy adaptive response during parthanatos is enhanced by PD98059 and involves mitochondrial function but not autophagy induction. *Biochim. Biophys. Acta* **1843**, 531–543 (2013).
177. Kerr, J. F., Wyllie, A. H. & Currie, A. R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26**, 239–257 (1972).
These authors described for the first time that apoptosis is an important cell death mechanism.
178. Kroemer, G. *et al.* Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* **16**, 3–11 (2009).
179. Tang, H. L., Yuen, K. L., Tang, H. M. & Fung, M. C. Reversibility of apoptosis in cancer cells. *Br. J. Cancer* **100**, 118–122 (2009).
180. Maynard, S. *et al.* Human embryonic stem cells have enhanced repair of multiple forms of DNA damage. *Stem Cells* **26**, 2266–2274 (2008).
181. Bauer, M. *et al.* Human monocytes are severely impaired in base and DNA double-strand break repair that renders them vulnerable to oxidative stress. *Proc. Natl Acad. Sci. USA* **108**, 21105–21110 (2011).
182. Narciso, L. *et al.* Terminally differentiated muscle cells are defective in base excision DNA repair and hypersensitive to oxygen injury. *Proc. Natl Acad. Sci. USA* **104**, 17010–17015 (2007).
183. Kauffmann, A. *et al.* High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene* **27**, 565–573 (2008).
184. Tomicic, M. T. *et al.* Translesion polymerase eta is upregulated by cancer therapeutics and confers anticancer drug resistance. *Cancer Res.* **74**, 5585–5596 (2014).
185. Roos, W. P., Christmann, M., Fraser, S. T. & Kaina, B. Mouse embryonic stem cells are hypersensitive to apoptosis triggered by the DNA damage O^6-methylguanine due to high E2F1 regulated mismatch repair. *Cell Death Differ.* **14**, 1422–1432 (2007).
186. Volcic, M. *et al.* NF-κB regulates DNA double-strand break repair in conjunction with BRCA1-CtIP complexes. *Nucleic Acids Res.* **40**, 181–195 (2012).
187. Huang, T. T., Wuerzberger-Davis, S. M., Wu, Z. H. & Miyamoto, S. Sequential modification of NEMO/IKKγ by SUMO-1 and ubiquitin mediates NF-κB activation by genotoxic stress. *Cell* **115**, 565–576 (2003).
188. Cai, Q. & Robertson, E. S. Ubiquitin/SUMO modification regulates VHL protein stability and nucleocytoplasmic localization. *PLoS ONE* **5**, e12636 (2010).
189. Hur, G. M. *et al.* The death domain kinase RIP has an essential role in DNA damage-induced NF-κB activation. *Genes Dev.* **17**, 873–882 (2003).
190. Brzoska, K. & Szumiel, I. Signalling loops and linear pathways: NF-κB activation in response to genotoxic stress. *Mutagenesis* **24**, 1–8 (2009).
191. Lee, H. H., Dadgostar, H., Cheng, Q., Shu, J. & Cheng, G. NF-κB-mediated up-regulation of Bcl-x and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes. *Proc. Natl Acad. Sci. USA* **96**, 9136–9141 (1999).
192. Chu, Z. L. *et al.* Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-kappaB control. *Proc. Natl Acad. Sci. USA* **94**, 10057–10062 (1997).
193. Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D. V. & Baldwin, A. S. Jr. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**, 1680–1683 (1998).
194. Zhou, A., Scoggin, S., Gaynor, R. B. & Williams, N. S. Identification of NF-κB-regulated genes induced by TNFα utilizing expression profiling and RNA interference. *Oncogene* **22**, 2054–2064 (2003).
195. Cusack, J. C. Jr *et al.* Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-κB inhibition. *Cancer Res.* **61**, 3535–3540 (2001).
196. Yao, R. & Cooper, G. M. Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science* **267**, 2003–2006 (1995).
197. Wendel, H. G. *et al.* Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* **428**, 332–337 (2004).
198. Toulany, M. *et al.* Akt promotes post-irradiation survival of human tumor cells through initiation, progression, and termination of DNA-PKcs-dependent DNA double-strand break repair. *Mol. Cancer Res.* **10**, 945–957 (2012).

Acknowledgements
The authors' work is supported by Deutsche Forschungsgemeinschaft (DFG KA724 and RO3617) and Deutsche Krebshilfe.

Competing interests statement
The authors declare no competing interests.

SUPPLEMENTARY INFORMATION
See online article: S1 (table)
ALL LINKS ARE ACTIVE IN THE ONLINE PDF