DNA-PK suppresses a p53-independent apoptotic response to DNA damage

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p53 is required for DNA damage-induced apoptosis, which is central to its function as a tumour suppressor. Here, we show that the apoptotic defect of p53-deficient cells is nearly completely rescued by inactivation of any of the three subunits of the DNA repair holoenzyme DNA-dependent protein kinase (DNA-PK). Intestinal crypt cells from p53 nullizygous mice were resistant to radiation-induced apoptosis, whereas apoptosis in DNA-PK_{cs}/p53, Ku80/p53 and Ku70/p53 double-null mice was quantitatively equivalent to that seen in wild-type mice. This p53-independent apoptotic response was specific to the loss of DNA-PK, as it was not seen in ligase IV (Lig4)/p53 or ataxia telangiectasia mutated (Atm)/p53 double-null mice. Furthermore, it was associated with an increase in phospho-checkpoint kinase 2 (CHK2), and cleaved caspases 3 and 9, the latter indicating engagement of the intrinsic apoptotic pathway. This shows that there are two separate, but equally effective, apoptotic responses to DNA damage: one is p53 dependent and the other, engaged in the absence of DNA-PK, does not require p53.

Keywords: p53-independent apoptosis; DNA-PK; DNA damage; radiation

EMBO reports (2009) 10, 87–93. doi:10.1038/embor.2008.214

INTRODUCTION

The tumour suppressor p53 has a crucial function in the cellular response to DNA damage. After DNA damage, p53 is rapidly phosphorylated by the ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR) protein kinases leading to its stabilization, induction of its transcriptional targets and cell-cycle arrest or apoptosis (Vousden & Lu, 2002; Shiloh, 2003). p53-deficient cells fail to undergo G1 cell-cycle arrest or

apoptosis in response to DNA damage, emphasizing the crucial function of p53 in these responses. The apoptotic resistance of p53-deficient cells could have clinical implications, as p53 is frequently mutated in human tumours and p53-deficient tumour models have reduced sensitivity to radio- or chemotherapy (Johnstone *et al*, 2002). However, in some settings, p53-deficient cells can undergo apoptosis (Strasser *et al*, 1994; Merritt *et al*, 1997; Roos & Kaina, 2006), suggesting that there are alternative cell death pathways. Understanding p53-independent cell death pathways and using them to enhance the sensitivity of tumour cells to chemo- or radiotherapy could have therapeutic benefits (Brown & Attardi, 2005).

The DNA double-strand break (DSB) is the main DNA lesion that activates p53-dependent apoptosis (Nelson & Kastan, 1994). In addition to triggering programmed cell death, DSBs activate the repair machinery to rejoin the breaks through either homologous recombination or non-homologous end joining (NHEJ; Shrivastav *et al*, 2008). During NHEJ, two subunits, Ku70 and Ku80, form a heterodimer that binds to the broken DNA ends, which then recruit the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{cs}; Smith & Jackson, 1999). DNA breaks are synapsed together by DNA-PK_{cs} (DeFazio *et al*, 2002; Spagnolo *et al*, 2006) and processed to remove the overhanging 3' or 5' ends so that they can be rejoined by the DNA ligase IV (LIG4)–XRCC4 heterodimer (XRCC4 for X-ray repair complementing defective repair in Chinese hamster cells 4; Lees-Miller & Meek, 2003; Spagnolo *et al*, 2006).

Mutations in any one of these NHEJ components lead to impaired DSB repair and increased sensitivity to ionizing radiation (Zhu *et al*, 1996; Gu *et al*, 1997; Gao *et al*, 1998). Similar to ATM and ATR, DNA-PK_{cs} is a phosphoinositide-3 kinase-related protein kinase (PIKK). DNA-PK can phosphorylate p53 *in vitro* (Lees-Miller *et al*, 1992), but the function of p53 and apoptosis in the radiosensitive phenotype conferred by DNA-PK mutation is unclear. The finding of a synthetic lethal interaction between DNA-PK_{cs} and ATM provided a clue to the mechanism by which DNA-PK impinges on cell death pathways. Severe combined immunodeficient (SCID) mice, which lack DNA-PK activity owing to a mutation in DNA-PK_{cs} (Blunt *et al*, 1996) and *Atm*-null mice are born at normal frequencies, whereas *scid/scid Atm*^{−/−} compound mutant embryos die early in development (Gurley &

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Kemp, 2001). This shows that the function of ATM is required for the survival of cells with loss of NHEJ capacity. As p53 is a direct target of ATM signalling, we investigated whether simultaneous loss of DNA-PK_{cs} and p53 synergized to affect cell death pathways. *scid/scid p53*^{-/-} mice are viable (Gurley et al, 1998); however, here we report that although cells from *p53*-null mice are resistant to ionizing radiation-induced apoptosis, *scid/scid p53*^{-/-} compound mutant cells are highly sensitized to apoptosis. This identifies a role for DNA-PK in the suppression of p53-independent apoptosis and a cellular mechanism by which loss of DNA-PK sensitizes cells to the lethal effects of radiation.

RESULTS

As DNA-PK can phosphorylate p53 *in vitro*, we investigated first whether p53 induction or apoptosis was impaired in the absence of DNA-PK activity. Epithelial cells within the crypts of the small intestine from *Scid*, *DNA-PK_{cs}*^{-/-}, *Ku70*^{-/-} and *Ku80*^{-/-} null mice showed no apparent defects in ionizing radiation-induced p53 expression or apoptosis. Whole body radiation (4 Gy) induced apoptosis to a similar extent in both wild-type and all four DNA-PK-deficient strains (Fig 1A,D), and in some cases the apoptotic response in the absence of DNA-PK was enhanced compared with the wild-type response. *p53*^{-/-} mice showed little or no increase in this early wave of apoptosis, as reported previously (Merritt

Fig 1 | Elimination of DNA-PK rescues the apoptotic defect in p53-null mice. (A) H&E-stained sections of small intestinal crypts from unirradiated (no IR) or irradiated (4h post 4 Gy) mice. Mitotic cells (white arrowhead) and apoptotic cells (black arrowheads) are indicated. TUNEL assays and p53 immunostaining are also shown. Note the spatial coincidence of p53-expressing cells and apoptotic cells. (B) Spatial distribution of apoptotic cells within the crypt. Note that the distribution of apoptosis is similar between wild-type (WT), *DNA-PK*^{-/-} and *DNA-PK*^{-/-} *p53*^{-/-} mice, peaking at cell positions 4–7. (C) Time course of small intestinal crypt cell apoptosis after 4 Gy IR in *DNA-PK*- and *p53*-deficient mice (nd, not done). (D) Quantification of apoptotic cells in small intestinal crypts from irradiated mice (4h post 4 Gy; ten × 40 fields were counted per mouse, approximately 5 crypts per field and approximately 50 cells per crypt). Bars are the mean values plus standard deviations from 3–8 mice per genotype except for *Ku80*^{-/-} (*n* = 1). *Atm*, ataxia telangiectasia mutated; DNA-PK, DNA-dependent protein kinase; H&E, haematoxylin and eosin; IR, ionizing radiation; *Lig4*, ligase IV; *Scid*, severe combined immunodeficient; TUNEL, TdT-mediated dUTP nick-end labelling.

et al, 1994). The number, staining intensity and localization of cells that stained for nuclear p53 following radiation were also similar between wild-type and DNA-PK mutant mice (Fig 1A). Colonic crypt epithelial cells and hair follicle epithelial cells from the dorsal skin of *DNA-PK_{cs}*-null mice were also sensitive to ionizing radiation-induced apoptosis, whereas these same cell types from *p53*-null mice were resistant (data not shown). To determine whether DNA-PK was required for apoptosis in tumour cells, we crossed SCID mice with adenomatous polyposis coli (*Apc^{Min}*) mutant mice, which spontaneously develop intestinal adenomas (Su et al, 1992). Intestinal adenomas that developed in *scid/scid Apc^{Min}* mice also showed a rapid apoptotic response to ionizing radiation, comparable to that seen in tumours from *Apc^{Min}* mice with intact DNA-PK (Supplementary Fig 1 online). T-cell lymphomas that developed in SCID mice also showed a robust apoptotic response to DNA damage (Gurley et al, 1998). These *in vivo* results, obtained from several normal and neoplastic cell types using two mutant alleles of *DNA-PK_{cs}*, as well as all three components of the DNA-PK holoenzyme, show that DNA damage-induced p53 activation and apoptosis do not require DNA-PK activity.

DNA-PK-deficient cells are highly sensitive to radiation and apoptosis is one factor that can contribute to radiosensitivity. By contrast, p53-deficient cells are resistant to apoptosis and, to a certain extent, radioresistant (Gudkov & Komarova, 2003). To determine whether DNA-PK and p53 interact genetically in the radiation response, we examined apoptotic sensitivity in DNA-PK p53 compound mutant mice. Strikingly, the apoptotic response in these double mutants was quantitatively similar to wild type, indicating that the apoptotic defect of p53-null cells was nearly completely rescued by the concomitant mutation of DNA-PK. At 4 h post 4 Gy, the number of apoptotic cells per crypt in *scid/scid p53^{-/-}* mice was similar to that in *scid/scid* mice and substantially greater than that in *p53^{-/-}* mice or unirradiated wild-type mice (Fig 1; Supplementary Table I online). On a per cell basis, DNA-PK mutation increased the apoptotic sensitivity of p53-null cells around 40-fold. Apoptotic cells were identified by using the morphological criteria of cytoplasmic shrinkage, chromatin condensation and nuclear fragmentation, and confirmed by TdT-mediated dUTP nick-end labelling (Fig 1A) and active caspase 3 staining (Fig 2). *p53*-null mice remained resistant to rapid-onset apoptosis at doses as high as 20 Gy (data not shown). Time-course analysis showed that apoptosis was slightly delayed in *scid/scid p53^{-/-}* mice (compare the 2 h time point in Fig 1C) compared with wild-type mice and that p53-null mice showed a slight increase in apoptosis at later time points of 24 and 72 h.

To exclude the possibility that the mutant DNA-PK_{cs} protein in SCID mice acts in a gain-of-function manner to activate apoptosis, we examined *DNA-PK_{cs}* knockout mice. *DNA-PK_{cs}^{-/-} p53^{-/-}* compound null mice were also highly sensitized to apoptosis (Fig 1). Rescue of the apoptotic defect of p53-null cells by mutation of *DNA-PK_{cs}* was seen on the C57BL/6, 129 × C57BL/6 and C3H × C57BL/6 genetic backgrounds (data not shown), showing that this phenotype is independent of strain background. We also observed a *DNA-PK_{cs}* gene dosage effect on p53-independent apoptosis, as the number of apoptotic cells per crypt in irradiated *scid/+ p53^{-/-}* mice (0.65 ± 0.3) was sixfold greater than in littermate control *p53^{-/-}* mice (0.1 ± 0.01 ; Fig 1D).

In wild-type mice, ionizing radiation-induced apoptosis is not uniformly distributed through the small intestinal crypt, but is

localized between cell positions 4–10, which overlap with the stem cell and proliferative compartment of this tissue (Barker et al, 2008). It has been shown previously that the distribution of p53-positive cells overlaps precisely with the distribution of apoptotic cells (Merritt et al, 1994; Fig 1). This observation and the absence of apoptosis in p53-null mice suggested that the apoptotic sensitivity of these cells is due to p53 and its regulation. However, analysis of the spatial distribution of apoptotic cells in *DNA-PK^{-/-} p53^{-/-}* mice showed a nearly identical profile as in wild-type mice (Fig 1B), indicating that the apoptotic sensitivity of these cells is not solely due to p53.

Next, we used a genetic analysis to address the mechanism of this p53-independent apoptotic response. To determine whether this was unique to the loss of DNA-PK_{cs} or whether it could be triggered by deficiency in any component of the NHEJ pathway, we used a series of mouse mutants, all of which result in defective DNA DSB repair and radiosensitivity. Both *Ku70^{-/-} p53^{-/-}* and *Ku80^{-/-} p53^{-/-}* mice showed an apoptotic response virtually identical to wild-type mice and again much greater than their *p53^{-/-}* littermates (Fig 1A,D). Therefore, disabling any component of the DNA-PK holoenzyme, Ku70, Ku80 or DNA-PK_{cs}, equally and efficiently sensitizes cells to p53-independent apoptosis. Next, we tested the final component of the NHEJ pathway, LIG4. Similar to DNA-PK, deficiency in LIG4 results in the inefficient repair of DSBs, confirmed here by increased phosphorylated histone H2AX (p-H2AX) staining in irradiated crypt cells (Fig 2A). However, in marked contrast to *DNA-PK/p53*-deficient mice, intestinal crypt cells from *Lig4^{-/-} p53^{-/-}* mice were resistant to ionizing radiation-induced apoptosis (Figs 1,2). This shows that p53-independent apoptosis is not triggered by deficiencies in all NHEJ components, but is specific to DNA-PK.

Deficiency in the PIKK ATM also confers a radiosensitive phenotype. To determine whether the loss of ATM engaged p53-independent apoptosis, we compared the apoptotic response of *Atm^{-/-}* and *Atm^{-/-} p53^{-/-}* mice. Intestinal crypt cells from irradiated *Atm^{-/-}* mice showed robust, nearly wild-type levels of apoptosis, as reported previously (Gurley & Kemp, 2007), whereas those from *Atm^{-/-} p53^{-/-}* mice were as resistant as *p53^{-/-}* mice (Figs 1D,2). Therefore, the loss of Ku70, Ku80 and DNA-PK_{cs}, but not ATM or LIG4, sensitizes p53-null cells, indicating that p53-independent apoptosis is triggered specifically by the loss of DNA-PK.

Next, we examined possible mediators of this apoptotic response. The serine/threonine kinase, checkpoint kinase 2 (CHK2), is a crucial effector in the cellular response to DNA damage (Bartek & Lukas, 2003). CHK2 is phosphorylated at Thr 68 by ATM at the sites of DSBs (Shiloh, 2003) and can, in turn, phosphorylate p53, among other substrates. CHK2 has been shown to modulate apoptosis in p53-deficient cancer cell lines, suggesting it has p53-independent functions (Yang et al, 2002; Stevens et al, 2003; Urst et al, 2004). Radiation induced a rapid increase in phospho-CHK2 (pCHK2; Thr68) in crypt cells that peaked at 2 h and was already reduced by 4 h (Fig 2C). Irradiated CHK2-null tissue showed no staining, confirming antibody specificity (Supplementary Fig 2 online). Irradiated *Atm*-null crypts showed reduced levels of pCHK2, indicating that ATM regulates CHK2 in this setting (Fig 2). By contrast, high levels of pCHK2 staining persisted in *DNA-PK_{cs}^{-/-} p53^{-/-}*, *Ku70^{-/-} p53^{-/-}* and *Ku80^{-/-} p53^{-/-}* mice for 4 h and was still detected 24 h

Fig 2 | Mechanism of apoptosis in DNA-PK and p53 mutant mice. (A) H&E-stained sections and immunostaining for the indicated proteins from intestinal crypts at 4 h post 4 Gy. Note apoptotic cells in wild-type (WT) and *DNA-PK*^{-/-} *p53*^{-/-} mice but not in *Atm*^{-/-} *p53*^{-/-}, *Lig4*^{-/-} *p53*^{-/-} or *p53*^{-/-} mice. Increased p-H2AX is seen in both *DNA-PK*^{-/-} and *Lig4*^{-/-} crypts, but persistent pCHK2 staining is seen only in *DNA-PK*^{-/-} *p53*^{-/-} crypts, indicating that p53-independent apoptosis correlates with pCHK2 activation but not DNA damage *per se*. Arrowheads indicate positive stained cells. (B) Quantification of pCHK2 staining in intestinal crypts taken at 4 h post 4 Gy. (C) Time course of pCHK2 staining in crypts. Bars are the mean values plus standard deviations from 3–5 mice per genotype except for *Lig4*^{-/-} *p53*^{-/-} (*n* = 2) and *Atm*^{-/-} *p53*^{-/-} (*n* = 2). Note increased levels and persistent pCHK2 staining in *scid/scid* *p53*^{-/-} mutant mice compared with other genotypes. *Atm*, ataxia telangiectasia mutated; DNA-PK, DNA-dependent protein kinase; H&E, haematoxylin and eosin; IR, ionizing radiation; *Lig4*, Ligase IV; ND, not done, pCHK2, phosphorylated checkpoint kinase 2; p-H2AX, phosphorylated histone H2AX; *Scid*, severe combined immunodeficiency.

after irradiation. Prominent pCHK2 staining was not seen in *Lig4*^{-/-} *p53*^{-/-}, *Atm*^{-/-} *p53*^{-/-} or *p53*^{-/-} mice, genotypes that are resistant to apoptosis. E2F transcription factor 1 (E2F1), promyelocytic leukaemia (PML) and p73 have been proposed to be mediators of CHK2-regulated apoptosis; however, expression patterns of these proteins in irradiated crypts did not correlate with genotype or apoptosis (data not shown).

Next, we investigated whether p53-independent apoptosis is mediated through the intrinsic apoptotic pathway. This pathway is instigated by the activation of BH3-only proteins, leading to BCL2-associated X protein (BAX)- and BCL2-antagonist/killer 1 (BAK)-mediated release of cytochrome *c* from the mitochondria (Danial

& Korsmeyer, 2004). Cytosolic cytochrome *c* induces oligomerization of apoptotic peptidase activating factor 1 (APAF1) to form the apoptosome, which activates and cleaves pro-caspase 9, the initiator caspase in the intrinsic apoptosis pathway (Riedl & Salvesen, 2007). Active caspase 9, in turn, triggers the cleavage and activation of the executioner caspase 3, which is common to both intrinsic and extrinsic apoptotic pathways. Staining for cleaved caspases 9 and 3 was clearly detected in crypt cells undergoing apoptosis from both wild-type and DNA-PK p53-deficient mice at levels quantitatively similar to apoptosis, but was not detected in the apoptotic-resistant genotypes (Fig 2; Supplementary Table 1 online). This shows that p53-independent

Fig 3 | Role of DNA-PK in chemotherapy-induced apoptosis. (A) H&E-stained sections and immunostaining for p-H2AX of small intestinal crypts from irradiated (4h post 4 Gy), etoposide (4h) or 5-FU (24h)-treated mice. (B) Quantification of apoptotic cells in small intestinal crypts after treatment with etoposide or 5-FU. Bars are the mean values plus standard deviations from 3–8 mice per genotype. DNA-PK, DNA-dependent protein kinase; H&E, haematoxylin and eosin; 5-FU, 5-fluorouracil; IR, ionizing radiation; p-H2AX, phosphorylated histone H2AX.

apoptosis, engaged by the loss of DNA-PK, is executed through the intrinsic mitochondrial pathway.

The chemotherapy agents etoposide and 5-fluorouracil also induce p53-dependent apoptosis, so it was of clinical interest to determine whether the absence of DNA-PK sensitized *p53*-null cells to these agents. Treatment of mice with the type II topoisomerase inhibitor etoposide induced crypt cell apoptosis at 4 h in wild-type, *scid/scid* and *scid/scid p53*^{-/-} mice but not *p53*^{-/-} mice (Fig 3). By contrast, the antimetabolite 5-fluorouracil induced apoptosis (peaking at 24 h) in wild-type and *scid/scid* mutant mice, but not in *p53*^{-/-} or *scid/scid p53*^{-/-} mice. Radiation and etoposide are thought to induce apoptosis through the creation of DSBs, whereas 5-fluorouracil induces apoptosis through alterations in DNA replication or RNA metabolism (Pritchard *et al*, 1997). Consistent with this, p-H2AX was prominent in crypt cells from radiation- and etoposide-treated mice, but not 5-fluorouracil-treated mice (Fig 3). This shows that the loss of DNA-PK does not rescue all modes of p53-dependent apoptosis, but is specific to DNA damage-initiated apoptosis.

DISCUSSION

Here, we show that DNA-PK is not required for the canonical p53-mediated apoptotic response to DNA damage. By contrast,

DNA-PK has an anti-apoptotic function, suppressing a robust apoptotic response that is entirely p53 independent. By using a combination of genetic, chemical and immunohistochemical analyses, we characterized this DNA-PK-regulated apoptotic response. Previous studies have shown that p53 induction and apoptosis coincided precisely both spatially and temporally within the intestinal crypt, suggesting that the apoptotic sensitivity of these cells is attributed solely to p53 or its regulation. Apoptosis in *DNA-PK/p53*-null mice occurred in the same cell positions and, with the exception of a slight delay, with similar kinetics. This indicates that these cells are intrinsically sensitive and that apoptosis can be triggered either by p53-dependent or p53-independent pathways. This region contains the transit-amplifying compartment that is responsible for generating many of the differentiated cell types of the small intestine. The existence of redundant pathways to regulate apoptosis in these cells might act as a fail-safe mechanism to delete damaged cells and prevent neoplastic transformation.

Apoptosis in *DNA-PK/p53*-deficient mice was induced by ionizing radiation and etoposide, but not by 5-fluorouracil, implicating DNA damage as the initial apoptotic trigger. However, several lines of evidence indicate that DNA damage *per se* is insufficient to trigger rapid-onset p53-independent apoptosis. First, radiation doses as high as 20 Gy failed to induce rapid apoptosis in *p53*-null intestinal crypts. Second, the ionizing radiation-induced apoptosis that is seen in *p53*-null mice is modest, markedly delayed (>24 h after ionizing radiation) and coincides with the resumption of abundant mitotic activity. The apoptotic bodies at these late time points were larger and multinucleate, suggesting that they are undergoing G2/M cell death as an indirect consequence of the radiation damage (Merritt *et al*, 1997). By contrast, the rapid apoptosis (4 h after ionizing radiation) seen in *DNA-PK*^{-/-} *p53*^{-/-} mice is likely a direct response to ionizing radiation, arguing that the apoptotic responses in *DNA-PK*^{-/-} *p53*^{-/-} and *p53*^{-/-} mice are fundamentally different. Third, although LIG4 and ATM deficiency leads to defective DNA repair and radiosensitivity, crypt cells from *Lig4/p53* and *Atm/p53* double-null mice remained resistant to radiation-induced apoptosis. Finally, the fact that deletion of Ku70, Ku80 or DNA-PK_{cs} all showed a quantitatively equivalent and nearly complete rescue of apoptosis in a *p53*-null background implicate a direct role of the DNA-PK holoenzyme in the suppression of DNA damage-initiated p53-independent apoptosis. A common phenotype associated with the loss of Ku70, Ku80 and DNA-PK_{cs} is the inability to activate the holoenzyme DNA-PK and to process DNA DSBs. Deficiency of LIG4 also results in unrepaired breaks, but with one important distinction: the activity of DNA-PK would not be impaired, and therefore the breaks would be synapsed and processed. Unlike any of the DNA-PK subunits, LIG4 deficiency leads to embryonic lethality, attributed to massive p53-dependent apoptosis (Frank *et al*, 2000). This lethality is rescued by p53 deletion, as *Lig4*^{-/-} *p53*^{-/-} mice are viable. This crucial phenotypic distinction among components of the NHEJ pathway is extended by our studies to the differential regulation of p53-independent apoptosis.

p53-independent apoptosis was tightly associated with increased pCHK2 and caspase 9 activation, suggesting that the absence of DNA-PK, either directly through the loss of kinase activity or indirectly through the presence of unprocessed DSBs,

leads to hyperactivation of ATM signalling through CHK2 and/or other downstream targets resulting in engagement of the intrinsic apoptotic pathway.

Speculation

DNA DSBs activate two mutually exclusive responses: DNA repair, leading to cell survival, and apoptosis, leading to cell death; however, the regulation of the choice between these responses is poorly understood. It is now apparent that DNA-PK has a crucial function in both, in that it directly mediates DSB repair and suppresses apoptosis. Its role in tumour suppression (Kemp et al, 1999) or its usefulness as a target in cancer therapy (O'Connor et al, 2007) will likely depend on which of these responses predominate. Enhanced apoptosis in the absence of DNA-PK provides an explanation for the radiosensitive phenotype that has long been associated with DNA-PK. Importantly, p53 is not required for this cell death response, pointing to the NHEJ apparatus as a potential target to sensitize cancer cells.

METHODS

Mice. DNA-PK_{cs} knockout mice on a 129 × C57BL/6 background (Gao et al, 1998) or C57BL/6 (Prdkd^{scid}) mice (The Jackson Laboratory, Bar Harbor, ME, USA) were crossed with C57BL/6 p53 knockout mice (from L. Donehower) to generate DNA-PK_{cs}^{−/−} p53^{−/−} or scid/scid p53^{−/−} mice and control littermates. Atm^{−/−} (Barlow et al, 1996), Ku80^{−/−} (Zhu et al, 1996), Ku70^{−/−} (Gu et al, 1997) and Lig4^{+/−} (Frank et al, 2000) mice were similarly crossed with p53-null mice to generate Atm^{−/−} p53^{−/−}, Ku80^{−/−} p53^{−/−}, Ku70^{−/−} p53^{−/−} and Lig4^{−/−} p53^{−/−} mice. Genotyping was carried out using established PCR protocols and conditions, and are available on request. Here, 6- to 10-week-old mice were given 4 Gy of whole-body ionizing radiation using a Co⁶⁰ source and killed at given times post-irradiation. Unirradiated age-matched littermate controls were also killed for tissues. Tumour-bearing APC^{Min} mice (The Jackson Laboratory) were irradiated and tissues were taken 4 h post-ionizing radiation. For chemotherapy studies, mice were injected with etoposide (40 µg/g, i.p.) or 5-fluorouracil (40 µg/g, i.p.), and tissues were examined at 4 and 24 h, respectively.

Histology. Tissues were fixed, processed and stained for p53 (CM5; Novocastra Laboratories Ltd, Newcastle upon Tyne, UK), cleaved caspase 9 (Asp 353; ab52298; Abcam, Cambridge, MA, USA), cleaved caspase 3 (Asp175; Cell Signaling Technologies Inc, Danvers, MA, USA), pCHK2 (Thr68; Abcam) and phospho-histone H2AX (S139; Cell Signaling) using a three-step streptavidin technique as described previously (Gurley & Kemp, 2007). Numbers of apoptotic figures or positive-staining cells were counted in intestinal crypts of ten × 40 fields or in ten × 100 fields of intestinal adenomas.

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank Fred Alt for providing DNA-PK_{cs} and DNA ligase IV knockout mice, John Petrini and Noburo Motoyama for providing Chk2-null tissue, and S. Lawrence Bailey and Valerie Holcomb for technical assistance. This study was supported by grants from the National Institutes of Health (to C.J.K. and P.H.) and from the American Cancer Society (to Y.G.).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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