Pergamon

European Journal of Cancer, Vol. 35, No. 4, pp. 540–548, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(98)00414-6

Review

Cellular Responses to Radiation and Risk of Breast Cancer

W. Jongmans* and J. Hall

Unit of Mechanisms of Carcinogenesis, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372, Lyon Cedex 08, France

Mutations in the ataxia telangiectasia gene (*ATM*) result in an abnormal p53-mediated cellular response to DNA damage produced by ionising radiation. This deficiency is believed to contribute to the radiosensitivity and high cancer risk seen in ataxia telangiectasia (AT) patients and AT heterozygotes. Epidemiological studies have demonstrated that relatives of AT patients are particularly predisposed to breast cancer. This observation, together with the finding that a relatively high proportion of breast cancer patients display an abnormal severe reaction of normal tissues following radiotherapy, has led to the suggestion that AT heterozygosity plays a role in radiosensitivity and breast cancer development. The cloning of the *ATM* gene has allowed this possibility to be examined at the molecular level. The studies reported to date remain inconclusive, with the number of AT heterozygotes being found in radiosensitive breast cancer patients being less than would be expected based on the family studies. The potential role of several other recently identified genes which are involved in the cellular DNA damage response to ionising radiation and which could also play a role in radiosensitivity and breast cancer development are reviewed. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: ataxia telangiectasia, Nijmegen breakage syndrome, p53, DNA-dependent protein kinase, breast cancer, radiosensitivity
Eur J Cancer, Vol. 35, No. 4, pp. 540–548, 1999

INTRODUCTION

MAMMALIAN CELLS proliferate and divide through an orderly sequence of four phases that compose the cell cycle. A cell that is destined to proliferate first enters the G1 phase, a period during which the cell size increases and preparations are made to enable DNA replication in the subsequent S phase. In the following G2 phase preparations are made for the onset of the M phase in which the duplicated DNA is equally separated between two daughter cells. As a cell progresses through these phases, control is exerted at various checkpoints to ensure the completion of all biochemical events in one phase before initiating the next.

Correspondence to J. Hall; e-mail: hall@iarc.fr
*Present address: Department of Molecular Genetics, Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, Leiden, The Netherlands.
This review is based on the EACR Award Lecture given by W. Jongmans as part of the European Cancer Conference, held on 14–18 Sep. 1997 in Hamburg, Germany.
Received 16 Nov. 1998; accepted 19 Nov. 1998.

The tumour suppressor protein p53 plays an important role in the DNA damage cell cycle checkpoints [1]. In normal cells activation of the p53-mediated DNA damage response pathway has unquestionably been connected with G1 cell cycle arrest. The p53 protein has also been shown to be involved in G2 cell cycle arrest [2], but this role remains less clear. Cell cycle arrest following exposure to radiation damage is believed to play an essential role in maintaining genomic stability, as it allows a prolonged period for DNA damage to be repaired before DNA replication or cellular division. Indeed, genomic instability is commonly observed in tumour cells and impaired control of cell cycle progression might explain this instability [3]. The important role for *p53* in tumorigenesis is further demonstrated by the finding that this gene is mutated in approximately 50% of human cancers [4].

The p53 protein has been found to play a separate role in DNA damage-induced programmed cell death (apoptosis) [1]. Whether a cell undergoes cell cycle arrest or apoptosis following p53 activation appears to depend on a variety of

factors, such as the extent of DNA damage, level of p53 expression and cell type, but also on the presence of several other gene products [5, 6]. The products of the ataxia telangiectasia gene (*ATM*) and the Nijmegen breakage syndrome gene (*NBS1*) have been implicated upstream of the p53-mediated cellular DNA damage response to ionising radiation [7–9]. Mutations in these genes confer sensitivity to ionising radiation and predisposition to cancer [10]. However, several other genes have also been shown to be involved in the cellular DNA damage response to ionising radiation [11].

It is well known that cells derived from normal individuals show variability in their radiosensitivity [12, 13]. This may partly be due to different expression levels of the genes that affect the radiosensitivity of cells. Given the increased incidence of breast cancer in the human population following exposure to ionising radiation [14], this would suggest that the radiosensitive subgroup that exists among the general population might face special hazards from routine diagnostic or therapeutic procedures involving radiation. Therefore, a better understanding of the possible relationship between genes that influence radiosensitivity and breast cancer development is clearly warranted.

ATAXIA TELANGIECTASIA HETEROZYGOSITY AND BREAST CANCER

Breast cancer is one of the most common malignancies in the world, affecting 1 in 10 women in the U.K. and 1 in 9 in the U.S.A. Linkage analysis of certain families with a high risk of breast cancer has identified two important susceptibility genes, *BRCA1* and *BRCA2*, on chromosome 17q21 and 13q, respectively [15]. In addition, germ-line mutations in the *p53* gene on chromosome 17p cause a high risk of premenopausal breast cancer as part of the Li–Fraumeni syndrome. However, epidemiological studies have shown that mutations in these genes are relatively rare in the general population and each is likely to account for less than 2% of all breast cancer cases [15]. In principle, a greater proportion of breast cancer cases within the population could be accounted for by more common genes that may have relatively low penetrance with regard to breast cancer.

The *ATM* gene mutated in ataxia telangiectasia (AT) patients is one candidate for such a susceptibility gene for breast cancer [16]. AT is an autosomal recessive syndrome displaying cerebellar ataxia, oculocutaneous telangiectasia, immunodeficiency, chromosomal instability, radiosensitivity and a predisposition to lymphoid cancer in childhood [17]. However, epithelial cancers, including breast cancer, have been reported for older AT patients who show a milder AT phenotype [17, 18]. AT is a relatively rare disorder and has been estimated to occur in 1 in 40,000 to 1 in 300,000 of the population in the U.S.A. and Europe [19]. It has been estimated that approximately 1% of the general population is heterozygous for *ATM* gene mutations, with some variation in this frequency in different ethnic populations [19–22]. AT heterozygotes are clinically asymptomatic. However, there is consistent evidence that they may be at increased risk of epithelial cancers, especially breast cancer [19, 20]. The relative risk of breast cancer for female AT heterozygotes was estimated to be 3.9-fold higher than that of the general population and would suggest that AT heterozygotes could account for about 4% of all breast cancer cases in the general population [19]. In these studies there was little suggestion that breast cancer risk among AT heterozygotes is age dependent. However, a recent study of AT heterozygotes in the U.S.A. showed that breast cancer risk appears to increase from the age of 60 years or older [20]. In this age group, breast cancer risk was found to be 6.6-fold higher than in those less than 60 years of age and, if these figures are correct, up to 8% of all breast cancers arising in the U.S.A. may occur in AT heterozygotes [20].

The rare autosomal recessive disorder called the Nijmegen breakage syndrome (NBS) was considered as a variant form of AT based on biological similarities between the syndromes [23]. NBS is characterised by microcephaly, growth and mental retardation, immunodeficiency, chromosomal instability, radiosensitivity and a high incidence of lymphoid cancers in childhood [17]. However, NBS patients show neither the cerebellar ataxia nor the ocular telangiectasia seen in AT patients, while AT patients do not show microcephaly or mental retardation. With the recent identification of several additional NBS patients in eastern and central Europe [24], linkage analysis has enabled the mapping of the *NBS* gene on chromosome 8q21 [25, 26]. This chromosomal localisation is different to that of the *ATM* gene on chromosome 11q23.1 and demonstrates that AT and NBS are genetically distinct diseases [16]. Fine mapping of the chromosomal region 8q21 has recently resulted in the cloning of the *NBS1* gene, which was shown to be mutated in NBS patients [27, 28]. A higher risk for epithelial cancers has been found among NBS heterozygotes of Czech and Slovakian origin than among the normal population in these regions [29].

ATM *mutation analysis in breast cancer patients*

The *ATM* gene spans approximately 150 kb of genomic DNA, which is composed of 66 small exons [30]. Most of the mutations in the *ATM* gene that have been described in more than 200 different AT families are unique and they are distributed uniformly along the length of the *ATM* gene [16, 18, 21, 31–36]. Approximately 70% of the reported *ATM* mutations would result in a truncated and unstable ATM protein [36]. The remaining *ATM* mutations are in-frame insertions/deletions and missense mutations. Two websites detail the spectrum of *ATM* mutations reported to date (http://www.vmmc.org/vmrc/atm.htm and http://www.uwcm.ac.uk//uwcm/mg/hgmd0.html).

The cloning of the *ATM* gene has provided the possibility of studying the role of *ATM* heterozygosity in breast cancer development by germ-line mutational analysis of breast cancer patients. In the first study, described by Vorechovsky and coworkers in 1996, three AT heterozygotes were identified among 88 selected breast cancer cases of Swedish families with a history of breast cancer, leukaemia and lymphoma [37]. In this study, blood DNA samples were screened using single-strand conformation polymorphism (SSCP) mutation analysis, which detects all types of *ATM* mutation. The proportion of 3.4% of primary breast cancer patients being AT heterozygotes would support the increased risk of breast cancer among AT heterozygotes. However, all the breast cancer cases in this study had a family history of cancers typically seen in AT families and may not be representative of the situation in sporadic unselected breast cancer cases. That the cases in this study may possibly have been enriched for *ATM* mutations is also suggested by the finding of a clustering of missense *ATM* mutations in non-AT patients with sporadic T-cell prolymphocytic leukaemia [38]. This type of malignancy is frequently seen in AT patients [39].

In another study described by FitzGerald and colleagues in 1997, only two AT heterozygotes were found among 401 selected breast cancer cases diagnosed under the age of 40 years and with or without a family history of breast or other epithelial cancers [22]. This AT heterozygote frequency of 0.5% was even lower than that found in the control group of healthy individuals, 1% in this study [22]. Although this may suggest that the *ATM* gene is not a susceptibility gene for breast cancer, it should be noted that, in this study, protein truncation testing (PTT) mutation analysis was used, which detects ATM protein truncating mutations. Based on current knowledge of the *ATM* mutation profile seen in AT patients, it might be expected that approximately 30% of all *ATM* mutations, which would not truncate the ATM protein, will not be detected by this technique. In addition, recent studies have indicated that the *ATM* mutation spectrum may vary among AT patients from a single ethnic population [18, 40]. For instance, 40% of all *ATM* mutations found among the German AT patients appear to be missense mutations [40]. Many of the in-frame insertions/deletions and missense *ATM* mutations that do not truncate the ATM protein are associated with some degree of expression of mutant ATM protein [18, 41]. The presence, within the cell, of mutant ATM protein with residual function may lead to less severe clinical and cellular phenotypes of AT [18, 42–44]. Moreover, it appears that these types of *ATM* mutation could be more relevant in tumour development [18, 37, 38]. This seems to be supported by a more recent German study of 200 unselected breast cancer patients, in which several patients carrying a heterozygote missense *ATM* mutation have been found with SSCP analysis of genomic DNA [45]. In contrast, in this study no *ATM* mutations could be found with PTT mutation analysis.

RADIOSENSITIVE BREAST CANCER PATIENTS

It is well known from clinical observations that a range of normal tissue reactions can be seen in cancer patients following treatment with radiotherapy, varying from very mild to severe and occasionally lethal. Several studies have shown that approximately 5% of all breast cancer patients show severe (e.g. EORTC grade 3 or 4) early or late normal tissues reaction following radiotherapy [46–48]. The most dramatic examples of severe normal tissue reaction to radiotherapy have been seen in AT patients who sustained devastating, life-threatening normal tissue necrosis [49, 50]. Cultured cells derived from AT patients retain their extreme sensitivity to ionising radiation *in vitro* [51]. AT heterozygotes also display *in vitro* radiosensitivity when compared with normal individuals, although not as pronounced as in AT patients [51].

Epidemiological studies have shown that radiation of the breast increases the risk of breast cancer, especially among young women [14]. Increased incidences of breast cancer were reported in women who were irradiated for childhood Hodgkin's disease, exposed during atomic bombing or occupationally exposed to ionising radiation. Considering the *in vitro* radiosensitivity and increased risk for breast cancer among AT heterozygotes [19, 20], these findings suggest that AT heterozygotes may face special hazards from routine diagnostic or therapeutic procedures involving radiation. Accordingly, it may be expected that a substantial proportion of the radiosensitive breast cancer patients are AT heterozygotes.

The *in vitro* radiosensitivity as seen in AT heterozygotes has been extensively studied in radiosensitive cancer patients in order to determine whether this could predict their treatment response to radiotherapy. Several studies have shown a correlation between the *in vitro* radiosensitivity of breast cancer patients and the clinical occurrence of late (e.g. fibrosis, telangiectasia) normal tissue reaction to radiotherapy [48, 52, 53]. This may suggest that a germ-line mutation could underlie the radiosensitivity of these breast cancer patients. However, no clear correlation has been found between the *in vitro* radiosensitivity of cancer patients and the clinical occurrence of an early (e.g. erythema, moist desquamation) normal tissue reaction following radiotherapy [52, 54, 55]. Remarkably, only mild reactions of normal tissue following radiotherapy have been reported in breast cancer patients who were found to be AT heterozygotes [22, 56]. It should be noted that the number of cases examined was small and individual variation in the degree of *in vitro* radiosensitivity has been observed among AT heterozygotes and even AT homozygotes [10]. Therefore, it may be expected that such individual variations will become more apparent when studying individual cases.

ATM *mutation analysis in radiosensitive breast cancer patients*

The cloning of the *ATM* gene in 1995 enabled the study of the role of *ATM* heterozygosity in the radiosensitivity of certain breast cancer patients at a molecular level. In the first study, no AT heterozygotes were detected among 16 British breast cancer patients showing mainly severe acute normal tissue reaction to radiotherapy [57]. In this study, SSCP-based restriction endonuclease fingerprinting (REF) mutation analysis was used, which detects all types of *ATM* mutation in AT patients. These results strongly suggest that AT heterozygotes do not represent a substantial proportion of the radiosensitive breast cancer patients with severe early normal tissue reaction to radiotherapy. As mentioned previously, it appears that the *in vitro* radiosensitivity seen in breast cancer patients correlates better with late than with early normal tissue reactions to radiotherapy. Therefore, a more substantial proportion of AT heterozygotes may be found among breast cancer patients with a severe late normal tissue reaction to radiotherapy. However, in a similar study among 15 Australian breast cancer patients showing a severe late normal tissue reaction to radiotherapy, no AT heterozygotes were found with PTT mutation analysis [58]. As already mentioned, PTT analysis detects only 60–70% of all *ATM* mutations in AT patients. In another Dutch study among 11 breast cancer cases who developed breast cancer after irradiation for Hodgkin's disease, one AT heterozygote was detected using PTT mutation analysis [59].

Although these studies do not exclude a causative role for *ATM* heterozygosity in the radiosensitivity of certain breast cancer patients and the risk of developing breast cancer, it would appear that the proportion of AT heterozygotes among the radiosensitive breast cancer patients is less than the expected 4% of breast cancer patients being AT heterozygotes.

THE p53-MEDIATED CELLULAR DNA DAMAGE RESPONSE TO IONISING RADIATION

Mammalian cells respond to ionising radiation by inhibiting cell cycle progression and activating the processing of induced DNA damage or inducing programmed cell death

(apoptosis). The signal transduction pathways that determine these cellular responses to ionising radiation are generally triggered by DNA damage, although oxidative damage to plasma membrane lipids may also stimulate stress response pathways that induce apoptosis in certain cell types [60]. The tumour suppressor protein p53 has been shown to play a central role in controlling the cellular DNA damage response following exposure to DNA damaging agents such as ionising radiation, ultraviolet (UV) light and DNA alkylating agents [1]. In normal cells following exposure to such DNA damaging agents the cellular content of the p53 protein increases rapidly. This increase in the p53 protein level results from enhanced protein stability [61]. The p53 protein is a transcription factor that binds to sequence-specific sites in the promotor region of several genes, such as *WAF1/CIP1*, *mdm2*, *WIP1*, *GADD45*, *bax* and *IGF-BP3*. The transcriptional activation of these p53 target genes is associated with either cell cycle arrest, DNA repair or apoptosis [1, 62]. The p53 protein also represses transcription of a number of cellular genes with promotors lacking p53-binding sites, such as *c-fos*, *c-jun*, *Rb* and *bcl-2* [1]. The potency of p53 as a broad-range repressor may be an important component of its tumour suppressor function.

Recent evidence has shown that the *mdm2* gene product functions as a negative feedback regulator of p53 [63, 64]. Expression of the *mdm2* gene is regulated by p53, while the mdm2 protein stimulates the proteolytic degradation of p53. This mechanism normally maintains p53 at very low levels. However, phosphorylation of p53 following ionising radiation has been shown to inhibit the mdm2-induced proteolytic degradation of p53 and results in the increase of p53 protein levels [65]. In approximately 50% of all human tumours, the *p53* gene has been found to be mutated [4]. The function of p53 may be inactivated owing to *mdm2* gene amplification in some of the remaining tumours containing wild-type p53 [66].

Inhibition of cell cycle progression following exposure to ionising radiation

The *WAF1/CIP1* gene encoding p21 is one of the best-documented genes transcriptionally activated by p53 following DNA damage [67]. The p21 protein plays an essential role in G1 cell cycle arrest in response to radiation damage, because of its role as an inhibitor of G1 cyclin-dependent kinases [68]. Several of the G1 cyclin-dependent kinases target the retinoblastoma protein, pRb, for phosphorylation. Hyperphosphorylation of pRb is required to advance G1 to S phase transition. The p53-mediated G1 cell cycle arrest following exposure to ionising radiation appears to differ among different cell types. In tumour-derived cells expressing wild-type p53, exposure to ionising radiation was originally found to result in a transient G1 cell cycle arrest [69]. In normal human fibroblasts, ionising radiation appears to result in a permanent G1 cell cycle arrest with prolonged induction of p21, resembling senescence [70]. Although these differences in the G1 cell cycle arrest could be due to the inactivation of components upstream or downstream of p53 during tumour formation, cell-type specific differences in the cell cycle checkpoint response have recently also been shown in normal human cells [71].

The p21 protein appears to have a second, separate function in the inhibition of DNA replication during the S-phase by acting as an inhibitor of the human proliferating cell nuclear protein (PCNA), an auxiliary protein for DNA polymerases delta and epsilon [72].

DNA repair of ionising radiation-induced DNA damage

Ionising radiation is known to induce a broad variety of DNA lesions, such as single-strand breaks, double-strand breaks, base damages and protein–DNA cross-links. Among these different lesions, DNA double-strand breaks are thought to be the most important lesions with respect to chromosomal aberrations and cell death observed following the exposure of cells to ionising radiation.

At least two distinct pathways (e.g. non-homologous and homologous recombinational repair) have been shown to facilitate the repair of DNA double-strand breaks induced by ionising radiation [73]. The DNA-dependent protein kinase (DNA-PK) plays an important role in the non-homologous recombinational repair of DNA double-strand breaks and cells defective in any one of the subunits of DNA-PK are extremely sensitive to ionising radiation [11]. DNA-PK is a multiprotein complex composed of a DNA-binding subunit, Ku, and a catalytic subunit, DNA-PKcs, which is a nuclear serine–threonine protein kinase. DNA-PKcs is active when bound to DNA ends by Ku, a heterodimeric protein of 70 000 (Ku70) and 86 000 mol. wt (Ku86) subunits. Once bound to the DNA ends by Ku, DNA-PKcs may activate signalling pathways that facilitate the processing of DNA double-strand breaks induced either by ionising radiation or during diverse non-homologous DNA recombination processes, such as V(D)J recombination [74].

The p53 protein is found to regulate the expression of the KARP-1 protein following ionising radiation. This Ku86-related KARP-1 protein is expressed from a promotor upstream of the human Ku86 locus and appears to function as a regulator of DNA-PK activity [75]. This suggests a regulatory role for p53 in the repair of ionising radiation-induced DNA double-strand breaks. Other indications that p53 is involved indirectly in the repair of DNA damage is suggested by the interaction of the proteins of two p53 target genes, *WAF1/CIP*(p21) and *GADD45*, with PCNA [72, 76]. The binding of p21 to PCNA inhibits the function of PCNA in DNA replication, whereas it appears not to inhibit its function in DNA repair [77]. The interaction of p53 with DNA repair proteins, such as the replication-repair protein RPA, the redox-repair protein Ref-1 and subunits of the transcription-repair factor TFIIH (e.g. XPB, XPD and p62), may also suggest that p53 is directly involved in the repair of DNA damage and cell cycle arrest [1, 78]. However, the interaction of p53 with the subunits of TFIIH may also be linked to p53-mediated apoptosis [79].

DNA damage sensors activating p53

DNA damage cell cycle checkpoints are postulated to act in a signal-transduction system that communicates information between a DNA lesion and components of the cell cycle. In recent years, exciting progress has been made in understanding how mammalian cells detect ionising radiation-induced DNA damage and transduce signals mediated by p53 to the regulatory machinery of the cell. The genes defective in AT and NBS patients have been implicated upstream of p53 in the cellular response to DNA damage induced by ionising radiation. p53 induction is found to be delayed and reduced following exposure to ionising radiation in AT and NBS cell lines [7, 9, 80]. Subsequently, the

increase in the level of the transcripts of genes that are activated by p53, such as the *WAF1/CIP1*(p21) gene, is reduced in AT and NBS cells following exposure to ionising radiation [8, 9]. Accordingly, AT and NBS cells are impaired in the regulation of cell cycle arrest following exposure to ionising radiation [9, 23, 81, 82]. In contrast, the increase in p53 protein and *WAF1/CIP1*(p21) mRNA expression following exposure to UV light or DNA alkylating agents is similar in AT, NBS and normal cells [8, 9, 83]. These results suggest that the *ATM* and *NBS1* gene products are involved in the signalling of some form of DNA strand breaks induced by ionising radiation.

The carboxyl-terminal region of the ATM protein shows similarities with the catalytic domains of phosphatidylinositol 3-kinases [16]. This similarity is shared with a family of ATM-related proteins which includes Mec1p and Tel1p in budding yeast, Rad3p in fission yeast, *Drosophila melanogaster mei-41*, mammalian DNA-PKcs and ATR [84, 85]. Interestingly, these proteins are involved in the control of cell cycle progression, processing of DNA damage and maintenance of genome stability, all processes found to be defective in AT as well as NBS cells [10]. The phosphatidylinositol 3-kinase domain of the ATM protein functions as a protein kinase [86, 87], as appears to be the case for most ATM-related proteins. The similarity between DNA-PKcs and the ATM protein strongly supports the hypothesis that the ATM protein is involved specifically in the signalling of DNA double-strand breaks induced by ionising radiation [88].

The *NBS1* gene mutated in NBS patients has recently been cloned [27, 28]. It spans a chromosomal region of 50 kb on chromosome 8q21 and is composed of 16 exons. This gene encodes for a novel 95 000 mol. wt protein, nibrin. Sequence comparisons of nibrin with other proteins revealed two domains in the amino-terminal region, a fork-head-associated domain and a breast cancer carboxy-terminal domain. Notably, both domains have been found separately in proteins that function in the DNA damage cell cycle checkpoints. Interestingly, nibrin has been identified as a member of the hRAD50/hMRE11 double-strand break repair complex [28] which together with DNA-PK is implicated in the non-homologous recombinational DNA double-strand break repair pathway [89]. Thus, these new findings may suggest the following model for the activation of the p53-mediated cellular response to ionising radiation (Figure 1). In this model, DNA double-strand breaks induced by ionising radiation are directly sensed and stabilised by the Ku proteins. The DNA-Ku protein complex then activates DNA-PKcs to facilitate the non-homologous recombinational rejoining of the DNA double-strand breaks. The non-homologous recombinational DNA double-strand break repair pathway comprises several successive steps [74]. The exact involvement of the hRAD50/hMRE11/nibrin complex and the XRCC4/ligase IV complex in these steps needs to be elucidated. Analogous to the DNA-Ku protein complex activating DNA-PKcs, the hRAD50/hMRE11/nibrin complex may activate ATM to act as a protein kinase. Subsequently, activated ATM may phosphorylate p53 and activate the cellular DNA damage response to ionising radiation.

The DNA-PK protein has also been implicated in the p53-mediated DNA damage response to ionising radiation, since it was found to phosphorylate p53 in the amino-terminal transactivation domain *in vitro* [90]. However, several studies have shown that cells lacking DNA-PK function display normal induction of p53 protein levels and *WAF1/CIP1* gene expression, and subsequently arrest at G1-S and G2-M following exposure to ionising radiation [91, 92]. These results suggest that DNA-PK is not essential for activating the p53-mediated cell cycle arrest in response to ionising radiation [see also Ref. 93]. However, DNA-PK might be involved in p53-mediated apoptosis which occurs without p53 stabilisation [94], e.g. when irreparable DNA damage is induced by ionising radiation.

Induction of apoptosis following exposure to ionising radiation

Apoptosis, also known as programmed cell death, is a genetically controlled cellular process that results in cell death [95]. Apoptosis occurs normally during embryonic development of the limbs, immune regulation and normal cell turnover. Therefore, decreased apoptosis may contribute to neoplastic transformation. It is becoming increasingly clear that apoptosis also functions as a cancer prevention mechanism that eliminates cells with severe DNA damage induced by ionising radiation, UV light or a variety of cytotoxic drugs [96]. An early event during DNA damage-induced apoptosis of most mammalian cells is the cleavage of a number of substrates such as poly(ADP-ribose)polymerase and DNA-PKcs by interleukin-1β-converting enzyme-related proteases (caspases) [97]. The role of these DNA-repair proteins in apoptosis is still not clear, but their cleavage may reduce the efficiency of DNA-repair processes.

The p53 protein has been found to play a separate role in DNA damage-induced apoptosis in certain cell types, but

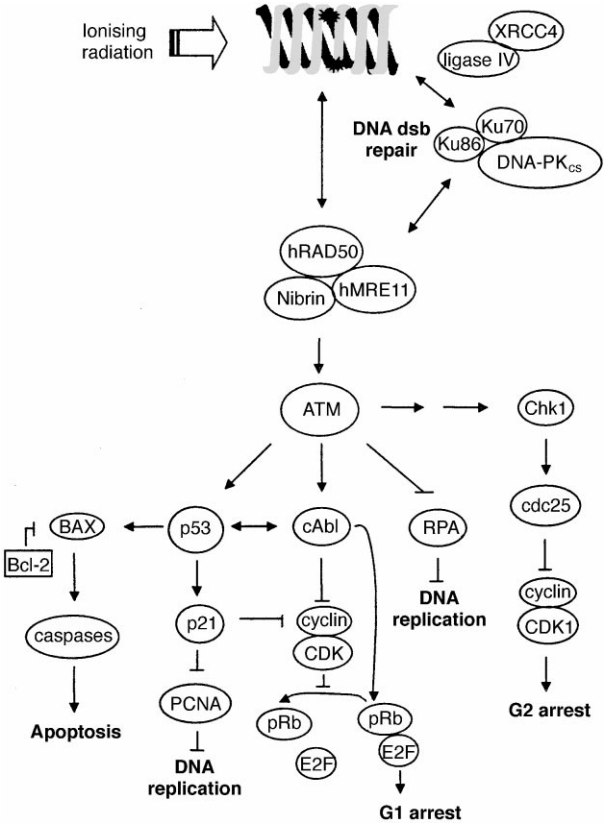

Figure 1. Simplified model for the cellular response to ionising radiation-induced DNA double-strand breaks. Recent studies have indicated that the ATM protein could additionally regulate G2 cell cycle arrest through the activation of Chk1 [117]. ⊥, Inhibition.

how this role is linked to its cell cycle function is not yet clear. The p53 protein can induce apoptosis by transcriptional activation of the *BAX* gene [98] and the insulin-like growth factor binding protein 3 (IGF-BP3) gene [99]. IGF-BP3 is an antagonist of insulin-like growth factor-1 (IGF-1) and downregulation of IGF-1 is correlated with the apoptotic response [100]. The pro-apoptotic Bax protein prevents the ability of the Bcl-2 protein to suppress apoptosis by forming heterodimers with the Bcl-2 protein. The intracellular ratio of the Bax protein to the Bcl-2 protein has been proposed to regulate apoptosis through the formation of homodimers and heterodimers [98].

The regulatory role of ATM in p53 activation following exposure to ionising radiation suggests that the ATM protein could control radiation-induced apoptosis [101]. However, it remains unclear whether the increased cell killing of AT cells following irradiation is due to a deregulated radiation-induced apoptotic response [102], or whether this is a consequence of the prolonged block of AT cells in G2 phase owing to the irreparable DNA damage inflicted in G1 or S phase.

Regulation of p53-mediated cellular DNA damage responses

The mechanisms that govern the p53-mediated cellular DNA damage response still remain elusive. However, several variables, such as the extent of DNA damage and the levels of p53 in cells, may influence the balance between p53-mediated cell cycle arrest and repair of DNA damage or induction of apoptosis following exposure to ionising radiation [5]. Phosphorylation of p53 at several sites by multiple protein kinases such as ATM, DNA-PK, mitogen-activated protein kinase, cyclin-dependent kinase and Jun amino-terminal kinase, has also been proposed as a mechanism to modulate the relative efficiency of activation or repression of the different p53 target genes [1, 86, 87, 103].

The p53-mediated cellular DNA damage response also depends on several gene products that co-operate with p53 [6]. Interferon regulatory factor (IRF)-1 is another gene product that has recently been implicated in the DNA damage response to ionising radiation. The cellular IRF-1 protein levels increase following ionising radiation by post-transcriptional stabilisation [104]. Although the expression of IRF-1 and p53 is independent of one another, the transcriptional induction of the *WAF1/CIP1*(p21) gene and subsequent G1 cell cycle arrest following ionising radiation is found to be dependent on both p53 and IRF-1 [104]. However, IRF-1 and p53 are required for distinct pathways in DNA damage-induced apoptosis [105]. The way in which IRF-1 participates in co-operation with p53 in the DNA damage response to ionising radiation remains unknown.

c-Abl tyrosine kinase is another protein implicated in the DNA damage response to ionising radiation. c-Abl activation following ionising radiation is associated with G1 cell cycle arrest or induction of apoptosis [106, 107]. The c-Abl mediated G1 cell cycle arrest is induced by a p53-dependent, but *WAF1/CIP1*(p21)-independent mechanism that inhibits the G1 cyclin-dependent kinase, CDK2. However, the stabilisation of p53 does not seem to require c-Abl, nor is p53 needed for activation of c-Abl following ionising radiation [106–108]. The additional interaction of c-Abl with the retinoblastoma protein, pRb, another key regulator of the G1 to S phase transition, may suggest an alternative pathway regulating the cellular DNA damage response to ionising radiation [109]. Functional pRb is necessary for the p53-mediated G1 cell cycle arrest following ionising radiation. Loss of functional pRb may contribute to p53-mediated apoptosis due to inappropriate cell proliferation signals by entering into S phase [110]. Recent work has shown that DNA-PK as well as ATM may activate c-Abl in response to ionising radiation [108, 111, 112]. Assuming that DNA-PK is involved in the induction of apoptosis and ATM in cell cycle regulation in response to ionising radiation, the balance between the activation of c-Abl and p53 by these protein kinases could determine the fate of cells, with repair of DNA damage and survival or the induction of apoptosis.

The novel *ING1*(p33) gene product has also been shown to interact with p53 and modulates the ability of p53 to act as a transcriptional activator [113]. The growth-suppressive activity of p53 by the activation of *WAF1/CIP1*(p21) gene transcription depends on the expression of *ING1*(p33). Ectopic expression of the *ING1* gene leads to G1 cell cycle arrest or induces apoptosis in several cell types [114, 115]. Reduced expression of *ING1*(p33) has been found in several breast tumour cell lines [115].

CONCLUSIONS

The large size of the *ATM* gene and the numerous distinct *ATM* mutations that have been reported among AT patients make identification of *ATM* mutations in the general population for clinical diagnosis laborious and impractical. Initial studies indicated that approximately 90% of all *ATM* mutations found in AT patients would truncate the ATM protein and should be detectable by PTT mutation analysis [33]. However, recent studies have shown that this figure may be as low as 60–70% of all *ATM* mutations, depending on the ethnic population [18, 36, 40]. Moreover, certain missense *ATM* mutations that do not truncate the ATM protein appear to be more associated with breast tumour development [18]. Such associations may also explain the differences in breast cancer risk in AT families from different ethnic populations [19]. Unfortunately, many of the studies concerning the role of *ATM* heterozygosity in breast cancer have used PTT mutation analysis, which will only detect ATM protein truncating mutations. In addition, many of these studies have statistical margins that would not allow accurate risk estimates to be made [116]. Therefore, large-scale population-based studies, which pay attention to all possible *ATM* mutations, as well as the selection of breast cancer cases and matched controls, will be required to answer the precise role of *ATM* heterozygosity in breast cancer.

From the available data it would appear that *ATM* heterozygosity plays only a partial role in the radiosensitivity of certain breast cancer patients and subsequently in the risk of developing radiation-induced breast cancer. Several other gene products, such as NBS1, DNA-PK, c-Abl, IRF-1 and ING1(p33), have recently been shown to be involved in the p53-mediated DNA damage response to ionising radiation. Mutations in these genes confer sensitivity to ionising radiation or predisposition to cancer, or both. Therefore, several of these genes may also be the underlying cause of a proportion of the radiosensitive breast cancer patients and need to be investigated in the near future. In light of the similarities between the NBS and AT syndrome and the finding that the *NBS1* gene product co-operates with ATM in activating the p53-mediated DNA damage response, it may be expected that female relatives of NBS patients might also have an

increased risk for breast cancer. The recent cloning of the *NBS1* gene should now allow the investigation of a possible similar increased risk for breast cancer among NBS heterozygotes.

1. Ko LJ, Prives C. p53: puzzle and paradigm. *Genes Dev* 1996, **10**, 1054–1072.
2. Pellegata NS, Antoniono RJ, Redpath JL, Stanbridge EJ. DNA damage and p53-mediated cell cycle arrest: a reevaluation. *Proc Natl Acad Sci USA* 1996, **93**, 15209–15214.
3. Hartwell LH, Kastan MB. Cell cycle control and cancer. *Science* 1994, **266**, 1821–1828.
4. Hainaut P, Hernandez T, Robinson A, *et al*. IARC Database of p53 mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. *Nucl Acid Res* 1998, **26**, 205–213.
5. Chen X, Ko LJ, Jayaraman L, Prives C. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev* 1996, **10**, 2438–2451.
6. Polyak K, Waldman T, He TC, Kinzler KW, Vogelstein B. Genetic determinants of p53-induced apoptosis and growth arrest. *Genes Dev* 1996, **10**, 1945–1952.
7. Kastan MB, Zhan Q, El-Deiry WS, *et al*. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* 1992, **71**, 587–597.
8. Artuso M, Esteve A, Brésil H, Vuillaume M, Hall J. The role of the ataxia-telangiectasia gene in the p53, WAF/CIP1(p21) and GADD45-mediated response to ionising radiation. *Oncogene* 1995, **11**, 1427–1435.
9. Jongmans W, Vuillaume M, Chrzanowska K, Smeets D, Sperling K, Hall J. Nijmegen Breakage Syndrome cells fail to induce the p53-mediated DNA damage response following exposure to ionising radiation. *Mol Cell Biol* 1997, **17**, 5016–5022.
10. Shiloh Y. Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. *Annu Rev Genet* 1997, **31**, 635–662.
11. Zdzienicka MZ. Mammalian mutants defective in the response to ionising radiation-induced DNA damage. *Mutat Res* 1995, **336**, 203–213.
12. Cox R, Masson WK. Radiosensitivity in cultured human fibroblasts. *Int J Radiat Biol* 1980, **38**, 575–576.
13. Little JB, Nove J, Strong LC, Nichols WW. Survival of human diploid skin fibroblasts from normal individuals after X-irradiation. *Int J Radiat Res* 1988, **54**, 899–910.
14. Goss PE, Sierra S. Current perspectives on radiation-induced breast cancer. *J Clin Oncol* 1998, **16**, 338–347.
15. Ford D, Easton DF. The genetics of breast and ovarian cancer. *Br J Cancer* 1995, **72**, 805–812.
16. Savitsky K, Bar-Shira A, Gilard S, *et al*. A single ataxia-telangiectasia gene with a product similar to PI 3-kinase. *Science* 1995, **268**, 1749–1753.
17. Sedgwick RP, Boder E. Ataxia-telangiectasia. In De Jong JMBV, ed. *Hereditary Neuropathies and Spinocerebellar Atrophies*, Handbook of Clinical Neurology, Vol. 16. Amsterdam, Elsevier Science Publishers, 1991, 347–423.
18. Stankovic T, Kidd AMJ, Sutcliffe A, *et al*. ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. *Am J Hum Genet* 1998, **62**, 334–345.
19. Easton DF. Cancer risks in AT heterozygotes. *Int J Radiat Biol* 1994, **66**, S177–S182.
20. Athma P, Rappaport R, Swift M. Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. *Cancer Genet Cytogenet* 1996, **92**, 130–134.
21. Gilad S, Bar-shira A, Harnik R, *et al*. Ataxia-telangiectasia: founder effect among North African Jews. *Hum Mol Genet* 1996, **5**, 2033–2037.
22. FitzGerald MG, Bean JM, Hegde SR, *et al*. Heterozygous ATM mutations do not contribute to early onset of breast cancer. *Nature Genet* 1997, **15**, 307–310.
23. Jaspers NG, Taalman RD, Baan C. Patients with an inherited syndrome characterised by immunodeficiency, microcephaly, and chromosomal instability: genetic relationship to ataxia-telangiectasia. *Am J Hum Genet* 1988, **42**, 66–73.
24. Van der Burgt I, Chrzanowska KH, Smeets D, Weemaes C. Nijmegen Breakage Syndrome. *J Med Genet* 1996, **33**, 153–156.
25. Saar K, Chrzanowska KH, Stumm M, *et al*. The gene for ataxia telangiectasia-variant (Nijmegen breakage syndrome) maps to a 1cM interval on chromosome 8q21. *Am J Hum Genet* 1997, **60**, 605–610.
26. Matsuura S, Weemaes C, Smeets D, *et al*. Genetic mapping using microcell-mediated chromosome transfer suggests a locus for Nijmegen Breakage Syndrome at chromosome 8q21-24. *Am J Hum Genet* 1997, **60**, 1487–1494.
27. Varon R, Vissinga C, Platzer M, *et al*. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen Breakage Syndrome. *Cell* 1998, **93**, 467–476.
28. Carney JP, Maser RS, Olivares H, *et al*. The hMre11/hRad50 protein complex and Nijmegen Breakage Syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell* 1998, **93**, 477–486.
29. Seemanova E. An increased risk for malignant neoplasms in heterozygotes for a syndrome of microcephaly, normal intelligence, growth retardation, remarkable facies, immunodeficiency and chromosomal instability. *Mutat Res* 1990, **238**, 321–324.
30. Uziel T, Savitsky K, Platzer M, *et al*. Genomic organization of the ATM gene. *Genomics* 1996, **33**, 317–320.
31. Byrd PJ, McConville CM, Cooper P, *et al*. Mutations revealed by sequencing the 5′ half of the gene for ataxia telangiectasia. *Hum Mol Genet* 1996, **5**, 145–149.
32. Baumer A, Bernthaler U, Wolz W, Hoehn H, Schindler D. New mutations in the ataxia telangiectasia gene. *Hum Genet* 1996, **98**, 246–249.
33. Gilad S, Khosravi R, Shkedy D, *et al*. Predominance of null mutations in ataxia-telangiectasia. *Hum Mol Genet* 1996, **5**, 433–439.
34. Telatar M, Wang Z, Udar N, *et al*. Ataxia-telangiectasia: mutations in ATM cDNA detected by protein truncation screening. *Am J Hum Genet* 1996, **59**, 40–44.
35. Wright J, Teraoka S, Onengut S, *et al*. A high frequency of distinct ATM gene mutations in ataxia-telangiectasia. *Am J Hum Genet* 1996, **59**, 839–846.
36. Telatar M, Teraoka S, Wang Z, *et al*. Ataxia-telangiectasia: identification and detection of founder-effect mutations in the ATM gene in ethnic populations. *Am J Hum Genet* 1998, **62**, 86–97.
37. Vorechovsky I, Luo L, Lindblom A, *et al*. ATM mutations in cancer families. *Cancer Res* 1996, **56**, 4130–4133.
38. Vorechovsky I, Luo L, Dyer MJS, *et al*. Clustering of missense mutations in the ataxia-telangiectasia gene in a sporadic T-cell leukaemia. *Nature Genet* 1997, **17**, 96–99.
39. Taylor AM, Metcalfe JA, Thick J, Mak YF. Leukemia and lymphoma in ataxia telangiectasia. *Blood* 1996, **87**, 423–438.
40. Dörk T, Bendix R, Skawran B, *et al*. Mutation analysis of the ATM gene in German ataxia telangiectasia families. *Dis Markers* 1998, **14**, 35–36.
41. Watters D, Khanna KK, Beamish H, *et al*. Cellular localisation of the ataxia-telangiectasia (ATM) gene product and discrimination between mutated and normal forms. *Oncogene* 1997, **14**, 1911–1921.
42. McConville CM, Stankovic T, Byrd PJ, *et al*. Mutations associated with variant phenotypes in ataxia telangiectasia. *Am J Hum Genet* 1996, **59**, 320–330.
43. Lakin ND, Weber P, Stankovic T, Rottinghaus ST, Taylor AMR, Jackson SP. Analysis of the *ATM* protein in wild-type and ataxia telangiectasia cells. *Oncogene* 1996, **13**, 2707–2716.
44. Gilad S, Chessa L, Khosravi R, *et al*. Genotype–phenotype relationships in ataxia-telangiectasia and variants. *Am J Hum Genet* 1998, **62**, 551–561.
45. Bendix R, Skawran B, Dörk T, *et al*. Mutation analysis of the ATM gene in unselected breast cancer patients. *Dis Markers* 1998, **14**, 24.
46. Norman A, Kagan AR, Chan SL. The importance of genetics for the optimalisation of radiation therapy. *Am J Clin Oncol* 1988, **11**, 84–88.
47. Riberio GG, Magee B, Swindell R, Harris M, Banerjee SS. The Christie Hospital breast conservation trial: an update at 8 years from inception. *Clin Oncol* 1993, **5**, 278–283.
48. Ramsay J, Birrell G. Normal tissue radiosensitivity in breast cancer patients. *Int J Radiat Oncol Biol Phys* 1995, **31**, 339–344.

49. Gotoff SP, Amirnokri E, Liebnor EJ. Ataxia-telangiectasia, neoplasma, untoward response to X-irradiation and tuberous sclerosis. *Am J Dis Child* 1967, **114**, 617–627.
50. Cunliffe PN, Mann JR, Cameron AH, Roberts KD. Radiosensitivity in ataxia-telangiectasia. *Br J Radiol* 1975, **48**, 374–376.
51. Murnane JP, Kapp LN. A critical look at the association of human genetic syndromes with sensitivity to ionizing radiation. *Semin Cancer Biol* 1993, **4**, 93–104.
52. Brock WA, Tucker SL, Geara FB, *et al.* Fibroblast radiosensitivity versus acute and late normal skin responses in patients treated for breast cancer. *Int J Radiat Oncol Biol Phys* 1995, **32**, 1371–1379.
53. West CML, Elyan SAG, Berry P, Cowan R, Scott DA. Comparison of the radiosensitivity of lymphocytes from normal donors, cancer patients, individuals with ataxia-telangiectasia (A-T) and A-T heterozygotes. *Int J Radiat Biol* 1995, **68**, 197–203.
54. Begg AC, Russell NS, Knaken H, Lebesque JV. Lack of correlation of human fibroblast radiosensitivity *in vitro* with early skin reactions in patients undergoing radiotherapy. *Int J Radiat Biol* 1993, **64**, 393–405.
55. Johansen J, Bentzen SM, Overgaard J, Overgaard M. Relationship between the *in vitro* radiosensitivity of skin fibroblasts and the expression of subcutaneous fibrosis, telangiectasia, and skin erythema after radiotherapy. *Radiother Oncol* 1996, **40**, 101–109.
56. Ramsay J, Birrell G, Lavin M. Breast cancer and radiotherapy in ataxia-telangiectasia heterozygote. *Lancet* 1996, **347**, 1627.
57. Appleby JM, Barber JBP, Levine E, *et al.* Absence of mutations in the ATM gene in breast cancer patients with severe responses to radiotherapy. *Br J Cancer* 1997, **76**, 1546–1549.
58. Ramsay J, Birrell G, Lavin M. Testing for mutations of the ataxia telangiectasia gene in radiosensitive breast cancer patients. *Radiother Oncol* 1998, **47**, 125–128.
59. Broeks A, Floore A, Russell N, *et al.* The ATM mutation spectrum in the Dutch population. *Dis Markers* 1998, **14**, 25–26.
60. Hannun YA. Functions of ceramide in coordinating cellular responses to stress. *Science* 1996, **274**, 1855–1859.
61. Maltzman W, Czyzyk L. UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. *Mol Cell Biol* 1984, **4**, 1689–1694.
62. Fiscella M, Zhang HL, Fan S, *et al.* WIP1, a novel human protein phosphatase that is induced in response to ionizing radiation in a p53-dependent manner. *Proc Natl Acad Sci USA* **94**, 6048–6053.
63. Haupt Y, Maya R, Kaza A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997, **387**, 296–299.
64. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by mdm2. *Nature* 1997, **387**, 299–303.
65. Shieh S-Y, Ikeda M, Taya Y, Prives C. DNA damage-induced phosphorylation of p53 alleviates inhibition by mdm2. *Cell* 1997, **91**, 325–334.
66. Chen L, Argrawal S, Zhou W, Zhang RW, Chen J. Synergistic activation of p53 by inhibition of Mdm2 expression and DNA damage. *Proc Natl Acad Sci USA* 1998, **95**, 195–200.
67. El-Deiry WS, Harper JW, O'Connor PM, *et al.* WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Res* 1994, **54**, 1169–1174.
68. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 1993, **75**, 805–816.
69. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 1991, **51**, 6304–6311.
70. Di Leonardo A, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev* 1994, **8**, 2540–2551.
71. Flatt PM, Price JO, Shaw A, Pietenpol JA. Differential cell cycle checkpoint response in normal human keratinocytes and fibroblasts. *Cell Growth Differ* 1998, **9**, 535–545.
72. Waga S, Hannon GJ, Beach D, Stillman B. The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA. *Nature* 1994, **369**, 574–578.
73. Hendrickson EA. Insights from model systems: cell-cycle regulation of mammalian DNA double-strand-break repair. *Am J Hum Genet* 1997, **61**, 795–800.
74. Lieber MR, Grawunder U, Wu X, Yaneva M. Tying loose ends: roles of Ku and DNA-dependent protein kinase in the repair of double-strand breaks. *Curr Opin Genet Dev* 1997, **7**, 99–104.
75. Myung K, Braastad C, He DM, Hendrickson EA. KARP-1 is induced by DNA damage in a p53- and ataxia telangiectasia mutated-dependent fashion. *Proc Natl Acad Sci USA* 1998, **95**, 7664–7669.
76. Smith ML, Chen I-T, Zhan Q, *et al.* Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science* 1994, **266**, 1376–1380.
77. Li R, Waga S, Hannon GJ, Beach D, Stillman B. Differential effects by the p21 CDK inhibitor on PCNA-dependent DNA replication and repair. *Nature* 1994, **371**, 534–537.
78. Jayaraman L, Murthy KGK, Zhu C, Curran T, Xantkhoudakis S, Prives C. Identification of redox/repair protein Ref-1 as a potent activator of p53. *Genes Dev* 1997, **11**, 558–570.
79. Warbrick E. Apoptosis: a new twist to the tale? *Curr Biol* 1996, **6**, 1057–1059.
80. Matsuura K, Balmukhanov T, Tauchi H, *et al.* Radiation induction of p53 in cells from Nijmegen Breakage Syndrome is defective but not similar to ataxia-telangiectasia. *Biochem Biophys Res Commun* 1998, **242**, 602–607.
81. Khanna KK, Beamish H, Yan J, *et al.* Nature of G1/S cell cycle checkpoint defect in ataxia-telangiectasia. *Oncogene* 1995, **11**, 609–681.
82. Sullivan KE, Veksler E, Lederman H, Lees-Miller SP. Cell cycle checkpoints and DNA repair in Nijmegen Breakage Syndrome. *Clin Immunol Immunopathol* 1997, **82**, 43–48.
83. Khanna KK, Lavin MF. Ionizing radiation and UV induction of p53 protein by different pathways in ataxia-telangiectasia cells. *Oncogene* 1993, **8**, 3307–3312.
84. Zakian VA. ATM-related genes: what do they tell us about functions of the human gene? *Cell* 1995, **82**, 685–687.
85. Bentley NJ, Holtzman DA, Keegan KS, *et al.* The *Schizosaccharomyces pombe* rad3 checkpoint gene. *EMBO J* 1996, **15**, 6641–6651.
86. Banin S, Moyal L, Shieh S-Y, *et al.* Enhanced phosphorylation of p53 by ATM in response to DNA damage. *Science* 1998, **281**, 1674–1677.
87. Canman CE, Lim D-S, Cimprich KA, *et al.* Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science* 1998, **281**, 1677–1679.
88. Hartley KO, Gell D, Smith GCM, *et al.* DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. *Cell* 1995, **82**, 849–856.
89. Milne GT, Jin S, Shannon KB, Weaver DT. Mutations in two Ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1996, **16**, 4189–4198.
90. Lees-Miller SP, Sakaguchi K, Ullrich SJ, Appella E, Anderson CW. Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. *Mol Cell Biol* 1992, **12**, 5041–5049.
91. Fried LM, Koumenis C, Peterson SR, *et al.* The DNA damage response in DNA-dependent protein kinase-deficient SCID mouse cells: replication protein A hyperphosphorylation and p53 induction. *Proc Natl Acad Sci USA* 1996, **93**, 13825–13830.
92. Rathmell WK, Kaufmann WK, Hurt JC, Byrd LL, Chu G. DNA-dependent protein kinase is not required for accumulation of p53 or cell cycle arrest after DNA damage. *Cancer Res* 1997, **57**, 68–74.
93. Jongmans W, Artuso M, Vuillaume M, Brésil H, Jackson SP, Hall J. The role of ataxia telangiectasia and the DNA-dependent protein kinase in the p53-mediated cellular response to ionising radiation. *Oncogene* 1996, **13**, 1133–1138.
94. Barlow C, Brown KD, Deng C-X, Tagle DA, Wynshaw-Boris A. Atm selectively regulates distinct p53-dependent cell-cycle checkpoint and apoptotic pathways. *Nature Genet* 1997, **17**, 453–456.
95. Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 1980, **68**, 251–306.

96. Harrington EA, Fanidi A, Evan GI. Oncogenes and cell death. *Curr Opin Genet Dev* 1994, **4**, 120–129.
97. Kumar S, Lavin MF. The ICE family of cysteine proteases as effectors of cell death. *Cell Death Differ* 1996, **3**, 255–267.
98. Korsmeyer SJ. Regulators of cell death. *Trends Genet* 1995, **11**, 101–105.
99. Buckbinder L, Talbott R, Velasco-Miguel S, *et al*. Induction of the growth inhibitor IGF-binding protein 3 by p53. *Nature* 1995, **377**, 646–649.
100. Baserga R. Oncogenes and strategy of growth factors. *Cell* 1994, **79**, 927–930.
101. Meyn MS. Ataxia telangiectasia and cellular responses to DNA damage. *Cancer Res* 1995, **55**, 5991–6001.
102. Duchaud E, Ridet A, Stoppa-Lyonnet D, Janin N, Moustacchi E, Rosselli F. Deregulated apoptosis in ataxia telangiectasia: association with clinical stigmata and radiosensitivity. *Canc Res* 1996, **56**, 1400–1404.
103. Ko LJ, Shieh S-Y, Chen X, *et al*. p53 is phosphorylated by CDK7-cyclin H in a p36(MAT1)-dependent manner. *Mol Cell Biol* 1997, **17**, 7220–7229.
104. Tanaka N, Ishihara M, Lamphier MS, *et al*. Cooperation of the tumor suppressors IRF-1 and p53 in response to DNA damage. *Nature* 1996, **382**, 816–818.
105. Tamura T, Ishihar M, Lamphier MS, *et al*. An IRF-1 dependent pathway of DNA damage-induced apoptosis in mitogen-activated T lymphocytes. *Nature* 1995, **376**, 596–599.
106. Yuan ZM, Huang Y, Whang Y, *et al*. Role for c-Abl tyrosine kinase in growth arrest response to DNA damage. *Nature* 1996, **382**, 272–275.
107. Yuan ZM, Huang Y, Ishiko T, Kharbanda S, Weichselbaum R, Kufe D. Regulation of DNA damage-induced apoptosis by the c-Abl tyrosine kinase. *Proc Natl Acad Sci USA* 1996, **94**, 1437–1440.
108. Baskaran R, Wiid LD, Whitaker LL, *et al*. Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. *Nature* 1997, **387**, 516–519.
109. Welch PJ, Wang JYJ. A C-terminal protein-binding domain in the retinoblastoma protein regulates nuclear c-Abl tyrosine kinase in the cell cycle. *Cell* 1993, **75**, 779–790.
110. Kasten MM, Giordano A. pRb and the Cdks in apoptosis and the cell cycle. *Cell Death Differ* 1998, **5**, 132–140.
111. Shafman T, Khanna KK, Kedar P, *et al*. Interaction between *ATM* protein and c-Abl in response to DNA damage. *Nature* 1997, **387**, 520–523.
112. Kharbanda S, Pandey P, Jin S, *et al*. Functional interaction between DNA-PK and c-Abl in response to DNA damage. *Nature* 1997, **386**, 732–735.
113. Garkavtes I, Grigorian IA, Ossovskaya VS, Chernov MV, Chumakov PM, Gudkov AV. The candiate tumor suppressor p33(ING1) cooperates with p53 in cell growth control. *Nature* 1998, **391**, 295–298.
114. Helbing CC, Veillette C, Riabowol K, Johnston RN, Gartavtsev I. A novel candidate tumor suppressor, ING1, is involved in the regulation of apoptosis. *Cancer Res* 1997, **57**, 1255–1258.
115. Garkavtes I, Kazarov AR, Gudkov AV, Riabowol K. Suppression of the novel growth inhibitor p33(ING1) promotes neoplastic transformation. *Nature Genet* 1996, **14**, 415–420.
116. Bishop DT, Hopper J. AT-tributable risk? *Nature Genet* 1997, **15**, 226.
117. Weinert T. A DNA damage checkpoint meets the cell cycle engine. *Science* 1997, **277**, 1450–1451.

Acknowledgements—We wish to thank Drs R. Montesano and M.Z. Zdzienicka for critically reading this manuscript. J.H. was partially supported by grants from the EC no. ERB CHRX-CT940581, the Association pour la Recherche contre le Cancer (ARC) and La Ligue Nationale contre le Cancer, Comité du Rhône. W.J. was supported by a Special Training Award from the International Agency for Research on Cancer.