DNA Damage Responses: Mechanisms and Roles in Human Disease

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Abstract

Significant progress has been made in recent years in elucidating the molecular controls of cellular responses to DNA damage in mammalian cells. Much of our understanding of the mechanisms involved in cellular DNA damage response pathways has come from studies of human cancer susceptibility syndromes that are altered in DNA damage responses. *Ataxia-telangiectasia mutated* (*ATM*), the gene mutated in the disorder ataxia-telangiectasia, codes for a protein kinase that is a central mediator of responses to DNA double-strand breaks (DSB) in cells. Once activated, ATM phosphorylates numerous substrates in the cell that modulate the response of the cell to the DNA damage. We recently developed a novel system to create DNA DSBs at defined endogenous sites in the human genome and used this system to detect protein recruitment and loss at and around these breaks by chromatin immunoprecipitation. Results from this system showed the functional importance of ATM kinase activity and phosphorylation in the response to DSBs and supported a model in which ordered chromatin structure changes that occur after DNA breakage and that depend on functional NBS1 and ATM facilitate DNA DSB repair. Insights about these pathways provide us with opportunities to develop new approaches to benefit patients. Examples and opportunities for developing inhibitors that act as sensitizers to chemotherapy or radiation therapy or activators that could improve responses to cellular stresses, such as oxidative damage, are discussed. Relevant to the latter, we have shown benefits of an ATM activator in disease settings ranging from metabolic syndrome to cancer prevention. (Mol Cancer Res 2008;6(4):517–24)

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Introduction

Why should we study DNA damage responses? From a cancer perspective, it is easy to make the argument that DNA damage responses are one of the most important pathways to understand in cancer biology. First, DNA damage causes cancer. We know this from animal models and, unfortunately, we know this from human experiences including radiation exposures and less drastic environmental exposures that are causally linked to cancer development. We also link DNA damage responses to cancer by the fact that many familial cancer syndromes are caused by inheritance of mutation in genes involved in DNA damage responses. DNA damage responses are also relevant to cancer because of current approaches to therapy. Radiation therapy and many chemotherapeutic agents target the DNA and we will likely be using such therapeutic modalities for the foreseeable future, even with the addition of targeted therapies to our armamentarium for treating cancer. Finally, DNA damage contributes to many of the side effects of cancer therapy, such as hair loss, nausea and vomiting, and bone marrow suppression. So, DNA damage causes cancer, is used to treat cancer, and contributes to the side effects of treating cancer.

It should also be noted that there are many physiologic settings other than cancer in which DNA damage responses and other cellular stress responses are important. For example, in hypoxia-reperfusion injury, such as occurs in myocardial infarction or stroke, oxidative stress contributes to tissue damage. Several neurodegenerative disorders have been linked to increased reactive oxygen species that contribute to the death of neuronal cells. Many of the manifestations of aging have been attributed to alterations in the DNA, some of which occur because of chronic exposure to oxidative damage. Finally, environmental exposures to radiation or chemicals, whether inadvertent or purposeful, and relevant for biodefense issues, engage DNA damage response pathways.

Much of what we have learned about the molecular steps involved in cellular stress responses has actually come from the study of inherited human diseases (Table 1). For example, Fanconi's anemia, a disorder where individuals are prone to development of myeloid leukemias, results from inheritance of one of several different mutated genes involved in repair of DNA cross-links. Ataxia-telangiectasia, a syndrome where children have a high incidence of leukemias and lymphomas, is due to inheritance of a mutation in a single gene, called *ataxia-telangiectasia mutated* (*ATM*), which is involved in helping cells respond to DNA strand breaks. Xeroderma pigmentosum, a syndrome where patients have a very high incidence of skin cancer, results from inheritance of one of several genes

Table 1. Cancer Susceptibility Linked to Altered DNA Damage Responses

Cancer Susceptibility Syndrome	Functional Defect	Tumor Susceptibility
Fanconi's anemia	Cross-link repair	Acute myeloid leukemias
Ataxia-telangiectasia	DSB repair, checkpoints	Acute leukemias, lymphomas
Xeroderma pigmentosum	Excision repair	Skin cancers
Nijmegen breakage syndrome	DSB repair, checkpoints	Acute leukemias, lymphomas
Li-Fraumeni (*p53* heterozygosity)	Cell cycle, apoptosis	Many different tumors
HNPCC	Mismatch repair	Colon, uterine, others
BRCA1, BRCA2 heterozygosity	DSB and other DNA repair	Breast, ovary

Abbreviation: HNPCC, hereditary nonpolyposis colon cancer.

involved in DNA excision repair. For these syndromes, an individual must inherit a mutated gene from both parents. However, several familial cancer syndromes result from inheritance of a mutated DNA damage response gene from only one parent. These devastating diseases include Li-Fraumeni syndrome, which is caused by inherited mutations in the *p53* gene and in which individuals have a very high risk (close to 100%) of developing many different cancers in their lifetime. The hereditary nonpolyposis colon cancer syndromes result from inheritance of one of several genes involved in mismatch repair, and these patients have a high incidence of colon cancer and uterine cancers. Inheritance of a single mutated copy of either the *BRCA1* or *BRCA2* genes, both of which participate in DNA damage signaling, markedly increases the risk of women for developing breast or ovarian cancers. The insights gained in DNA damage response pathways from the studies of these human diseases are great examples of "bedside to bench" research and have led to better understanding of these pathways. The ultimate payback will be that these insights will lead us back to development of new therapies and prevention strategies for cancer. This lecture will provide examples of how these insights are leading back to new approaches to treatment and prevention.

The ATM Damage Response Pathway

ATM and p53

In order for cellular responses to damage to be optimal, the repair has to be coordinated with other events that are going on in the cell, such as cell cycle progression or programmed cell death. Thus, there are many events that the cell has to coordinate after DNA damage. With this question in mind, 16 years ago, my laboratory was studying how cell cycle progression is altered following exposure to DNA damaging agents. We made the observation that there is a rapid increase in p53 protein levels following DNA damage and showed that this increase of p53 causes cells to arrest in the G_1 phase of the cycle. There were also other cell cycle arrests that occurred in human cells following ionizing radiation. Throughout S phase, initiation of new replicons was inhibited and cells arrested in G_2, ~30 min before visible condensation of chromosomes. Initiation of both the intra-S-phase arrests and the G_2 arrest was independent of p53 (1, 2). Shortly thereafter, we showed that the induction of p53 did not occur normally in cells from patients with the hereditary syndrome ataxia-telangiectasia. Although we had no idea what gene or genes were defective in this syndrome, this information told us that whatever the gene was, it somehow signaled the p53 after DNA damage. In collaboration with Al Fornace and Bert Vogelstein, we identified the first bona fide downstream target of p53, GADD45 (3). These insights showed that a signal transduction pathway, not dissimilar from what happens when a growth factor binds to growth factor receptor, is initiated in response to DNA damage, in this case involving p53, GADD45, and the as-yet-unidentified ataxia-telangiectasia gene.

In the ensuing years, many other downstream transcriptional targets of p53 were identified, perhaps the best known of which is the p21 cyclin-dependent kinase inhibitor discovered by the Vogelstein and Elledge laboratories (4, 5). This gene product turned out to be responsible for the arrest of cells in the G_1 phase of the cycle. My laboratory continued to focus on molecular mechanisms involved in the induction of p53 and identified an ATM-dependent, DNA damage–induced phosphorylation event in p53 protein (Ser^{15}; ref. 6). A major breakthrough occurred when Yossi Shiloh and his colleagues cloned the gene mutated in ataxia-telangiectasia, *ATM* (7). The predicted protein coded by this gene was very large (~350 kDa) and contained a phosphatidylinositol 3-kinase–like sequence in its COOH terminus. Once the gene was cloned, we were able to make a mammalian expression vector, manipulate *ATM* in cells, and show that ATM was a protein kinase (8). The activity of the kinase increased in cells after DNA damage and the first identified bona fide substrate of this protein kinase turned out to be p53 (8, 9).

We immediately knew that there had to be much more to the story because whereas cells that lack p53 are abnormal in G_1 arrest or in apoptosis, cells that lack ATM are also defective in the S-phase and G_2 checkpoints and are hypersensitive to ionizing radiation. Thus, there had to be substrates of the ATM protein kinase other than p53. We took a straightforward biochemical approach to first determine the consensus target sequence of the ATM protein kinase and then began to identify substrates. The ATM kinase phosphorylates serine or threonine residues as long as they are next to a glutamine and there were certain preferences in amino acids in the surrounding sequences (10). Using this information, we began to look for proteins that had these consensus target sequences and would test them first *in vitro* and then in cells. Once the proteins were identified, the functional roles of the phosphorylation events could be quickly determined because the phosphorylation sites were identified by this approach and could be mutated. Over a period of about 2 to 3 years, multiple substrates of the ATM protein kinase were identified. In addition to substrates that interacted with p53 and contributed to G_1 arrest or apoptosis, such as chk2 or mdm2, numerous substrates that were involved in the S-phase or G_2 arrests were identified (Fig. 1; ref. 11). Interestingly, many of these ATM targets also turned out to be important cancer susceptibility genes, including *BRCA1, FANCD2*, and *NBS1*, thus further emphasizing the importance of these damage signaling pathways in cancer development.

ATM Activation and Other Events at the Double-Strand Break

Although ATM targets were being discovered quickly, it remained unclear how this signaling response to DNA damage pathway was initiated. In other words, the link between the introduction of DNA alterations and the activation of ATM kinase activity remained to be clarified. A major breakthrough in this regard occurred with the identification of an ionizing radiation–induced autophosphorylation event in the ATM protein and the functional characterization of this phosphorylation event (12). In unstressed cells, ATM was found to exist as an inactive homodimer with the COOH-terminal kinase domain of each monomer blocked by tight binding to an internal domain of the partner ATM protein in the dimer. Following DNA damage, an intermolecular autophosphorylation occurred on Ser[1981] and this phosphorylation event disrupted the inactive dimer. The kinase domain in the resulting monomeric ATM protein was then unencumbered and was free to phosphorylate substrates containing the ATM consensus target sequence. Surprisingly, we found that ATM could also be activated in the absence of double-strand breaks (DSB). This observation, combined with other kinetic and quantitative observations, led to the suggested model that the initiation of ATM activation did not require the direct binding of ATM dimers to DSBs but rather resulted from structural changes in the nucleus that could be "sensed" by the ATM dimer at a distance from the DSB (12).

If the cellular stress failed to cause DNA breaks, then a low level of ATM activation would occur and this small amount of activated ATM could phosphorylate nucleoplasmic substrates, such as p53. However, if the stress induced a DSB, such as would occur after ionizing irradiation, then many other things began to happen. Several different proteins get recruited to that break, such as the MRN complex, BRCA1, and 53BP1. These proteins are recruited to the DSB independently of ATM. At this point, ATM activation had also occurred independent of these proteins moving to the DSB. But then, these two separate pathway components come together and the activated ATM protein is recruited to the DSB, apparently via binding to the NBS1 protein in the MRN complex (13-15). Now, this activated ATM kinase is located at the DSB and can phosphorylate substrates there, including histone H2AX, BRCA1, 53BP1, and NBS1 (Fig. 2). By mechanisms remaining to be elucidated, these phosphorylation events contribute to the initiation of cell cycle checkpoints and repair of the DNA.

More recently, the story has gotten even more complex. Kozlov et al. (16) identified two additional ATM phosphorylation sites and suggested that all three phosphorylation sites were important in the activation of ATM. In addition, several laboratories, including my own, reported that abnormalities in

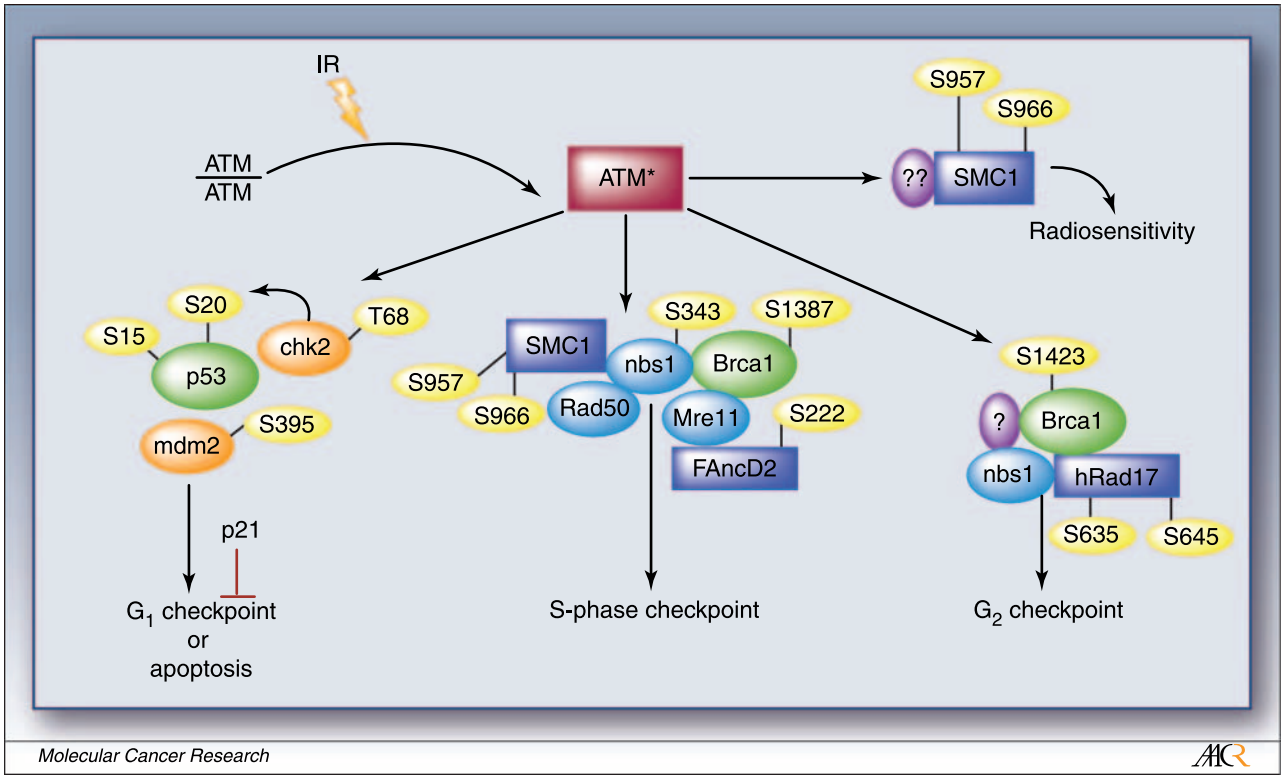

FIGURE 1. Schematic representation of the signal transduction pathways initiated by ATM following ionizing irradiation (*IR*) and the functional roles of the ATM targets. Following ionizing radiation, the ATM kinase is activated via intermolecular autophosphorylation of Ser[1981] and subsequent dissociation of the ATM homodimer. The ATM kinase is then free to circulate in the cell and subsequently phosphorylate chk2, p53, and mdm2 to initiate the G_1 arrest; NBS1, FANCD2, BRCA1, and SMC1 to initiate the S-phase arrest; and BRCA1 and hRad17 to cause a G_2 arrest. SMC1 is the only target of ATM where mutation of the ATM phosphorylation sites affects radiosensitivity. BRCA1 and NBS1 seem to be required for SMC1 phosphorylation; thus, absence of either of these proteins results in radiosensitivity.

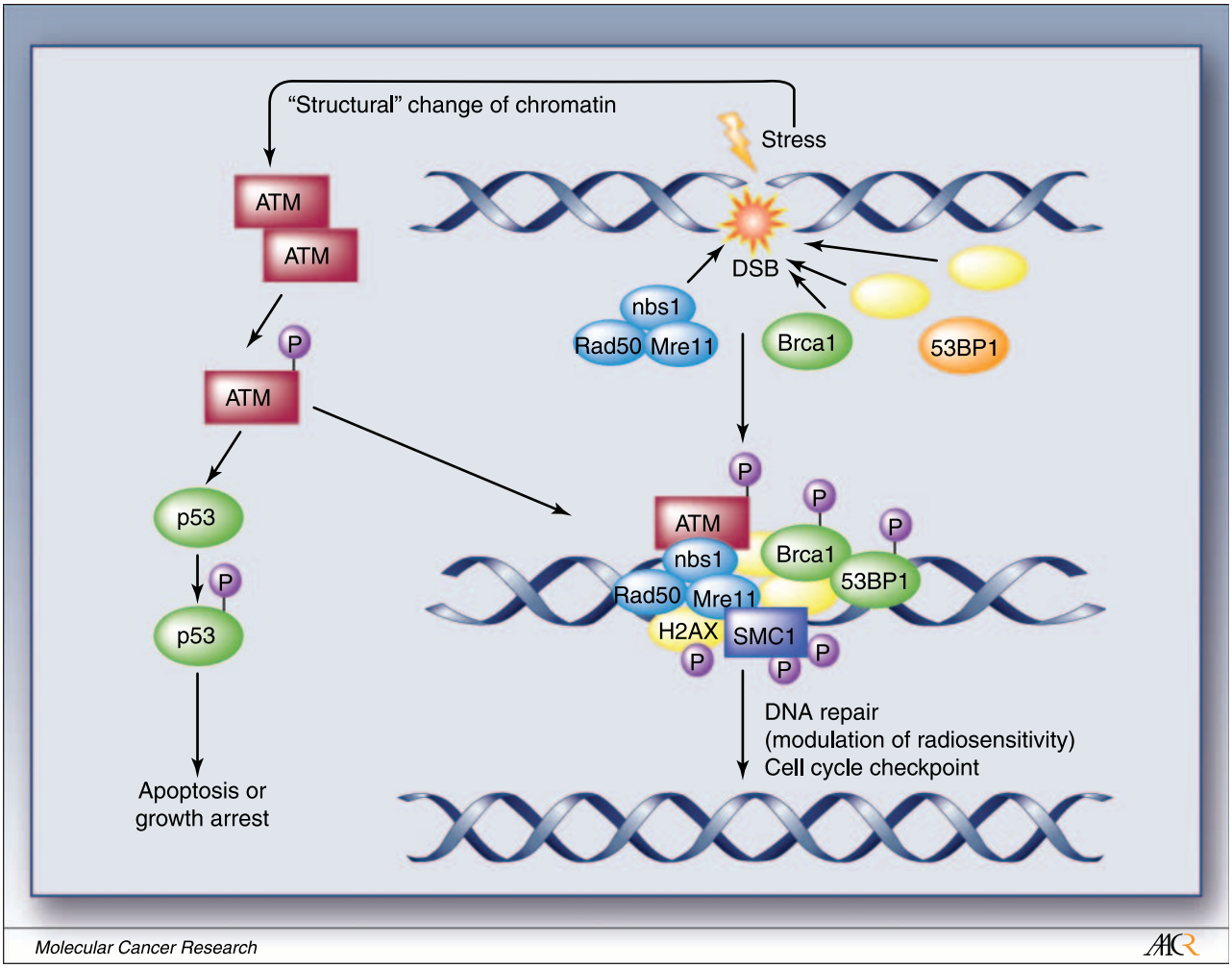

FIGURE 2. Proposed model for an ionizing radiation–induced signaling pathway. Chromatin or nuclear structure changes caused by DNA breakage or other mechanisms lead to intermolecular autophosphorylation of ATM dimers, resulting in release of phosphorylated and active ATM monomers. If DNA strand breaks are present, several proteins, including NBS1 and BRCA1, are recruited to the sites of the breaks independent of the ATM activation process. After activation, monomeric ATM can phosphorylate nucleoplasmic substrates, such as p53, and if NBS1 and BRCA1 have localized to DNA breaks, activated ATM is recruited to the break. At the DNA break, activated ATM can phosphorylate substrates, including SMC1, NBS1, and BRCA1. The phosphorylation of SMC1 reduces chromosomal breakage and enhances cell survival.

the MRN complex reduced the level of ATM activation, thus suggesting that somehow the MRN complex influences the ATM activation mechanism (15, 17-19). In contrast, some other results raised questions about the critical nature of ATM phosphorylation and questioned the model that ATM activation occurred at a distance from the DSB. *In vitro* studies suggested that the binding of ATM directly to DSBs can contribute to its activation (20-22) and mouse studies suggested that Ser^{1981} phosphorylation might not be required for ATM activation *in vivo* (23). These latter results basically raised the question of whether an inactive ATM dimer must go to the DSB to get activated or whether the process of ATM activation begins and then the activated ATM monomer is recruited to the DSB.

To begin to further probe the steps in this signaling pathway further, we needed a much more sophisticated set of assays. Many insights into the responses of mammalian cells to DNA damage have come from focus formation assays using immunofluorescence as the detector of protein binding to DNA DSBs. Although this approach has been useful, there are many limitations to such assays. To improve on this approach, we developed a novel system to create DSBs at defined endogenous sites in the human genome and used this system to detect protein recruitment and loss at and around these breaks by chromatin immunoprecipitation (24). As predicted by results from focus formation assays, the detection of human ATM protein at site-specific DSBs required functional NBS1 protein. However, we also were able to definitively show that both ATM kinase activity and ATM autophosphorylation on Ser^{1981} were required for the detection of ATM protein at DSBs. These results confirmed the biological importance of ATM autophosphorylation on Ser^{1981} and supported our proposed model whereby active ATM monomers, but not inactive ATM dimers, bound effectively to DSBs.

This assay led to many other important insights into the molecular events occurring at and around DSBs in mammalian cells. For example, it has been known for many years that nucleosomes are disrupted during the process of DNA excision

repair (25) and recent work in yeast had suggested that nucleosomes were also disrupted during the process of DSB repair (26). With this novel assay, we were able to show for the first time that nucleosomes were transiently disrupted at DSB sites in mammalian cells. In yeast, this process was dependent on the MRX complex (26) and we found that nucleosome disruption after DSBs was dependent on the analogous MRN complex in mammalian cells. Surprisingly, however, we also found that this process depended on the kinase activity of ATM. This suggested the intriguing possibility that the activated ATM protein kinase was phosphorylating a protein that could modify chromatin structure and, in particular, cause disruption of the nucleosome.

Using this assay, we could also assess the recruitment of repair enzymes to the DSB in human cells. We found that both NBS1 and active ATM were required for efficient recruitment of the repair cofactor XRCC4 to DSBs. The most straightforward explanation for this observation is that the nucleosome must be disrupted at the DSB in order for the recruitment of the repair enzyme(s) and both NBS1 and ATM are required for the nucleosome disruption. Further, aberrant recruitment of repair enzymes to DSBs in the absence of NBS1 or ATM predicted that DSB repair would be altered in cells lacking either of these gene products. Although both NBS1 and ATM have been well documented to be required for DNA damage–induced cell cycle checkpoints (11), whether their absence leads to true DSB repair deficiencies has been long debated (27-29). Because this assay created DSBs at defined sites in the genome, it was possible to directly measure repair (religation) of DSBs in mammalian cells for the first time. Using both PCR analysis and Southern blotting, we were able to quantitatively document accumulation of DNA breaks at the I-PpoI sites in cells lacking either NBS1 or ATM. Thus, for the first time, it was possible to state that both of these gene products are required for normal DSB repair.

In addition to assessing recruitment of protein to DSBs with this assay, it was also possible to assess recruitment of proteins to DNA sequences surrounding the DSB. Similar to results reported in yeast (30), by using primers specific for surrounding DNA sequences, we found that NBS1 is located only directly at the DSB site, whereas γ-H2AX is not found at the DSB but rather is located only at surrounding sequences. Thus, although focus formation assays suggest that these two proteins colocalize, our more informative assays showed that they are not located in exactly the same spot. Interestingly, ATM protein was bound both directly at the DSB and in the surrounding region. By assessing the appearance and disappearance of these proteins at and around the DSB at various times after I-PpoI induction, we further found a very dynamic process occurring. ATM initially binds to the DSB and in surrounding sequences; however, shortly thereafter, ATM leaves the DSB and the repair enzymes appear. Even after leaving the DSB, ATM remains bound to the surrounding sequence, suggesting that it is phosphorylating proteins in surrounding chromatin to facilitate the total DSB response. After the DNA is repaired, the histones can once again be found at the DSB, suggesting a restoration of the nucleosome at the site. In summary, this new assay provided many novel insights into the dynamic molecular events occurring during the process of DSB repair in human cells. In coming months to years, it is anticipated that the use of this assay will uncover many more molecular details involved with DSB responses.

Potential Clinical Benefits of Understanding the Steps in the Atm-p53 Pathway

Because DNA damage contributes to the genesis of cancer and other human diseases, there should be potential clinical benefits from understanding these signaling pathways. One obvious possibility is that one could think about developing inhibitors of ATM kinase or other steps in the pathway that would make tumor cells more sensitive to radiation therapy or chemotherapy. Efforts in many different laboratories are under way to identify and test such inhibitors. In addition to sensitizing tumors to radiation therapy or chemotherapy, such agents might have antitumor activity on their own. The argument for this possibility arises from the fact that most tumor masses exist in a very harsh microenvironment, exposed to intermittent hypoxia, oxidative stress, acidic pH, and intermittent glucose deprivation. In addition, it seems that tumor cells have activated DNA damage and stress response pathways during the transformation process (31, 32), suggesting that these stress response pathways are important for tumor development and growth. Thus, inhibiting these stress response pathways may be selectively toxic for tumor cells, providing a beneficial therapeutic index even in the absence of combining them with standard cytotoxic therapies.

Cancer Prevention from Activation of the Atm-p53 Pathway

In contrast to inhibitors of these stress response pathways, the development of pathway activators seems daunting. However, if loss of these signaling pathways leads to sensitization to radiation or chemotherapies and leads to cancer development, then perhaps stimulating these pathways could be protective from the toxicities of cytotoxic agents or could lead to novel mechanisms of cancer prevention. Screening small-molecule libraries for pathway activators is challenging. However, we were fortunate that during our studies of the mechanisms of Atm activation, we found that the antimalarial drug chloroquine was capable of activating the Atm-p53 pathway without causing any detectable DNA damage (12, 15). With this drug in hand, we could use it for proof-of-principle studies to ask whether activation of the pathway could have clinical benefits. We found that chloroquine preferentially enhanced death of cells overexpressing the *Myc* oncogene and impaired *Myc*-induced lymphomagenesis in mouse models. Chloroquine-induced cell death *in vitro* was dependent on p53 but not on Atm or Arf. Accordingly, chloroquine impaired spontaneous lymphoma development *in vivo* in *Atm*-deficient, but not in *p53*-deficient, mice (33).

As we explored the mechanisms by which chloroquine treatment worked as a cancer prevention agent, we showed that its use enhanced markers of both macroautophagy and apoptosis but ultimately impaired protein degradation in autophagolysosomes. Interestingly, chloroquine-induced cell death was not abrogated by overexpression of Bcl-2 or deletion of *Bax* and *Bak* and thus did not require caspase-mediated apoptosis. However, when both apoptosis and autophagic pathways were blocked simultaneously, chloroquine-induced killing of Myc-overexpressing cells was blunted. Thus, chloroquine induces lysosomal stress and provokes a p53-dependent cell death that does not require caspase-mediated

apoptosis. These findings suggested that agents targeting lysosomal-mediated degradation may be effective in cancer prevention and specifically showed efficacy for intermittent chloroquine use in mouse models of two genetically distinct human cancer syndromes: Burkitt lymphoma and ataxia-telangiectasia. Chloroquine was also tested in a rodent breast cancer model and found to markedly reduce mammary carcinomas in rats and mice treated with a potent chemical carcinogen (34). Interestingly, intermittent (weekly) chloroquine administration seemed effective in both of these tumor models and oral administration is effective with the compound. Thus, these rodent model systems open up new possibilities to consider for novel and practical approaches to cancer prevention in humans.

Atm, Chloroquine, and Metabolic Syndrome

Based on observations that ataxia-telangiectasia patients, who totally lack ATM protein, can develop a type of insulin-resistant diabetes, we explored potential roles of Atm in insulin signaling and reported several years ago that insulin can activate ATM kinase activity in certain cell types and that a lack of Atm in those cell types negatively affected insulin-stimulated pathways (35). Because insulin activated Atm, and cells lacking Atm could have abnormal responses to insulin, and ataxia-telangiectasia patients could develop a type of insulin-resistant diabetes (36, 37), and epidemiologic studies had suggested that carriers of ATM mutations (i.e., parents of ataxia-telangiectasia patients) had increased cardiovascular disease (38), we investigated whether ATM deficiency promoted insulin resistance and cardiovascular disease. In a close collaboration with the laboratory of Dr. Clay Semenkovich, the role of Atm in metabolic syndrome and the ability of chloroquine, as an Atm activator, to reverse the symptoms of the syndrome were explored.

Metabolic syndrome is a common disorder associated with insulin resistance and atherosclerosis. We found that deficiency

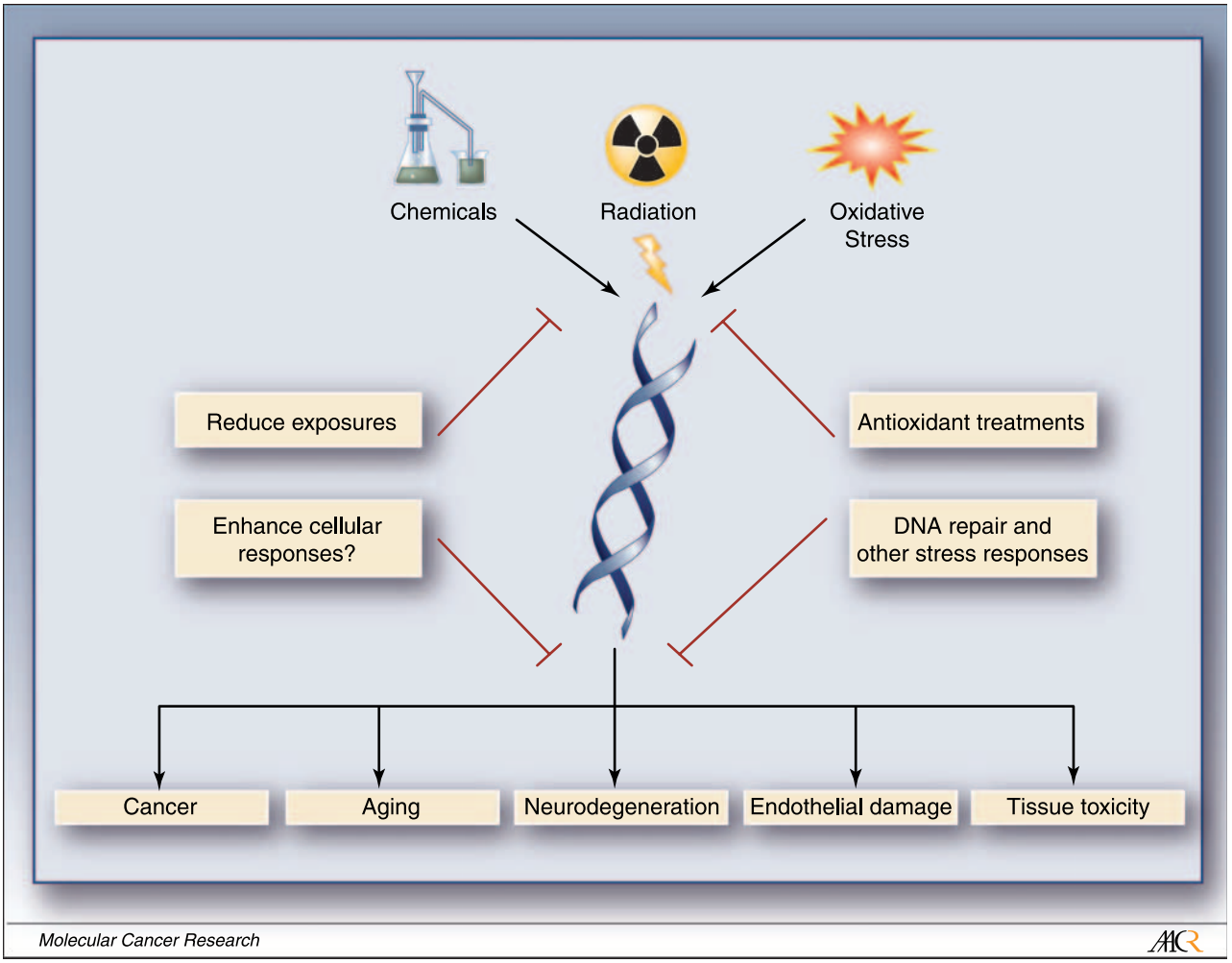

FIGURE 3. Cellular DNA is constantly challenged by damaging exposures, including chemicals, radiation, and even natural metabolic products, such as reactive oxygen species. Damage to DNA can lead to many different acute and chronic pathologic conditions, ranging from cancer to neurodegeneration to endothelial damage. Reducing exposures and damage to the DNA when feasible makes sense, but protection cannot reach 100%. The ability of our cells to repair the damage reduces the morbidity resulting from the exposures. It seems reasonable to now suggest that we have learned enough about the molecular controls of these cellular stress responses to begin to manipulate them to further reduce the risk of disease.

of one or two alleles of ATM worsened all features of the metabolic syndrome and accelerated atherosclerosis in apoE$^{-/-}$ mice when fed a high-fat diet (39). Treatment of *Atm*$^{+/+}$*ApoE*$^{-/-}$ mice with low-dose, weekly chloroquine decreased atherosclerosis. In an Atm-dependent manner, chloroquine decreased macrophage lipoprotein lipase activity, decreased blood pressure, and improved glucose tolerance. Chloroquine also improved metabolic abnormalities in *ob/ob* and *db/db* mice. These results suggest that Atm-dependent stress pathways mediate susceptibility to the metabolic syndrome and that chloroquine or related agents promoting ATM activity could represent a novel approach for modulating insulin resistance and decreasing vascular disease. As with the cancer prevention studies, these results showed that chloroquine, given in low dose once a week as an Atm activator, reverses several of the abnormalities seen in metabolic syndrome. Human clinical trials are already under way exploring the potential benefits of Atm activation with chloroquine in this clinical syndrome that affects up to 10% to 20% of the U.S. population.

Conclusion

Each of the over 12 trillion cells in our bodies contains over 3 billion nucleotides in the DNA, the sequence and composition of which determines our physiology and our disease status. With such importance attached to that DNA sequence and composition, one might expect nature to do everything it could to protect the DNA. However, our DNA is constantly challenged by natural and man-made chemicals, natural and man-made forms of radiation, and even by endogenous metabolism. It has been estimated that there are many thousand damaging events per day per cell in our bodies. We know that DNA damage can lead to many different disease processes, including cancer, aging, neurodegeneration, cardiovascular disease, and other tissue toxicities (Fig. 3). With all this damage occurring, why are we not full of pathologies? One reason is because we have these elegant stress response and repair pathways that help our cells deal with the ongoing damage. In addition, we do not rely just on these elegant pathways, but we also do everything we can to reduce exposures: we have laws about what chemicals can be put into the water and we have rules about exposures to radioactive isotopes. In addition, a multibillion-dollar antioxidant industry exists that basically tries to target the endogenous sources of DNA damage. Our studies have led us to consider another approach to dealing with these damaging processes, namely, trying to intervene with the molecular and cellular responses to DNA damage to try to have a significant effect on human disease.

Acknowledgments

Gratitude for the success of my laboratory over the years has to go to many people. I have had wonderful students and postdoctoral fellows who have worked extraordinarily hard to make the little and the big discoveries. I tried to mention many of them specifically as I gave the Clowes lecture. I have also had wonderful colleagues at both Johns Hopkins and St. Jude Children's Research Hospital, which has made the journey even more enjoyable. I also specifically talked about my collaborators on the most recent and exciting projects described in this text: Clay Semenkovich, Ray Monnat, and John Cleveland. I would like to give specific thanks to Margaret Hall, my Executive Associate, for all that she does for me every day and specifically for her help in assembling this manuscript. I should note that my patients and ataxia-telangiectasia kids have been an incredible inspiration because of their constant hope and their courage. I also thank the AACR and Eli Lilly for this recognition.

References

1. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. Cancer Res 1991;51:6304–11.

2. Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci U S A 1992;89:7491–5.

3. 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;71:587–97.

4. El-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. Cell 1993;75:817–25.

5. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G_1 cyclin-dependent kinases. Cell 1993;75:805–16.

6. Siliciano JD, Canman CE, Taya Y, Sakaguchi K, Appella E, Kastan MB. DNA damage induces phosphorylation of the amino terminus of p53. Genes Dev 1997;11:3471–81.

7. Savitsky K, Bar-Shira A, Gilad S, et al. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 1995;268:1749–53.

8. Canman CE, Lim D-S, Cimprich KA, et al. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science 1998;281:1677–9.

9. Banin S, Moyal L, Shieh S-Y, et al. Enhanced phosphorylation of p53 by ATM in response to DNA damage. Science 1998;281:1674–7.

10. Kim S-T, Lim D-S, Canman CE, Kastan MB. Substrate specificities and identification of putative substrates of ATM kinase family members. J Biol Chem 1999;274:37538–43.

11. Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. Nature 2004;432:316–23.

12. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 2003;421:499–506.

13. Lee JH, Xu B, Lee CH, et al. Distinct functions of Nijmegen breakage syndrome in ataxia telangiectasia mutated-dependent responses to DNA damage. Mol Cancer Res 2003;1:674–81.

14. Falck J, Coates J, Jackson SP. Conserved modes of recruitment of ATM, ATR and DNA-PKcs to sites of DNA damage. Nature 2005;434:605–11.

15. Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes Dev 2004;18:1423–38.

16. Kozlov SV, Graham ME, Peng C, Chen P, Robinson PJ, Lavin MF. Involvement of novel autophosphorylation sites in ATM activation. EMBO J 2006;25:3504–14.

17. Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. EMBO J 2003;22:5612–21.

18. Carson CT, Schwartz RA, Stracker TH, Lilley CE, Lee DV, Weitzman MD. The Mre11 complex is required for ATM activation and the G_2/M checkpoint. EMBO J 2003;22:6610–20.

19. You Z, Chahwan C, Bailis J, Hunter T, Russell P. ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. Mol Cell Biol 2005;25:5363–79.

20. Lee JH, Paull TT. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. Science 2004;304:93–6.

21. Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science 2005;308:551–4.

22. Dupre A, Boyer-Chatenet L, Gautier J. Two-step activation of ATM by DNA and the Mre11-Rad50-Nbs1 complex. Nat Struct Mol Biol 2006;13:451–7.

23. Pellegrini M, Celeste A, Difilippantonio S, et al. Autophosphorylation at serine 1987 is dispensable for murine Atm activation *in vivo*. Nature 2006;443:222–5.

24. Berkovich E, Monnat RJ Jr., Kastan MB. Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. Nat Cell Biol 2007;9:683–90.

25. Smerdon MJ, Lieberman MW. Nucleosome rearrangement in human chromatin during UV-induced DNA repair synthesis. Proc Natl Acad Sci U S A 1978;75:4238–41.

26. Tsukuda T, Fleming AB, Nickoloff JA, Osley MA. Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. Nature 2005;438:379–83.

27. Pandita TK, Hittelman WN. The contribution of DNA and chromosome

repair deficiencies to the radiosensitivity of ataxia-telangiectasia. Radiat Res 1992;131:214–23.

28. Pandita TK, Hittelman WN. Initial chromosome damage but not DNA damage is greater in ataxia telangiectasia cells. Radiat Res 1992;130:94–103.

29. Morgan SE, Lovly C, Pandita TK, Shiloh Y, Kastan MB. Fragments of ATM which have dominant-negative or complementing activity. Mol Cell Biol 1997;17:2020–39.

30. Shroff R, Arbel-Eden A, Pilch D, et al. Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr Biol 2004;14:1703–11.

31. Gorgoulis VG, Vassiliou LV, Karakaidos P, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005;434:907–13.

32. Bartkova J, Rezaei N, Liontos M, et al. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 2006;444:633–7.

33. MacLean KH, Dorsey FC, Cleveland JL, Kastan MB. Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. J Clin Invest 2007;118:79–88.

34. Loehberg CR, Thompson T, Kastan MB, et al. Ataxia telangiectasia-mutated and p53 are potential mediators of chloroquine-induced resistance to mammary carcinogenesis. Cancer Res 2007;67:12026–33.

35. Yang D, Kastan MB. Participation of ATM in insulin signalling through phosphorylation of eIF-4E binding protein 1 (4E-BP1). Nat Cell Biol 2000;2:893–8.

36. Schalch DS, McFarlin DE, Barlow MH. An unusual form of diabetes mellitus in ataxia telangiectasia. N Engl J Med 1970;282:1396–402.

37. Bar RS, Levis WR, Rechler MM, et al. Extreme insulin resistance in ataxia telangiectasia. N Engl J Med 1978;298:1164–71.

38. Su Y, Swift M. Mortality rates among carriers of ataxia-telangiectasia mutant alleles. Ann Intern Med 2000;133:770–8.

39. Schneider JG, Finck BN, Ren J, et al. ATM-dependent suppression of stress signaling reduces vascular disease in metabolic syndrome. Cell Metab 2006;4:377–89.