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OUR CELLS GET STRESSED TOO! IMPLICATIONS FOR HUMAN DISEASE

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Abstract

Significant progress has been made in recent years elucidating the molecular controls of cellular responses to DNA damage in mammalian cells. Many of the insights that we have gained into the mechanisms involved in cellular DNA damage response pathways have come from studies of human cancer susceptibility syndromes that are altered in DNA damage responses. ATM, the gene mutated in the cancer-prone disorder, Ataxia-telangiectasia, is a protein kinase that is a central mediator of responses to DNA double strand breaks in cells. Such insights provide us with opportunities to develop new approaches to benefit patients. For example, inhibitors of the ATM pathway have the potential to act as sensitizers to chemotherapy or radiation therapy and could even have anti-neoplastic effects on their own. Conversely, activators of ATM could improve responses to cellular stresses such as oxidative damage. The potential benefits of ATM modulation in disease settings ranging from metabolic syndrome to cancer will be discussed.

The composition and sequence of the three billion bases in the DNA of our cells are major determinants of cellular function and individual physiology. Unfortunately, our DNA is constantly being challenged by agents that either arise as a consequence of normal metabolism or result from exposures to natural or man-made products in the environment. These agents, which range from sunlight to chemicals to natural or manmade forms of ionizing radiation to metabolically-produced oxygen radicals, can either directly damage bases or can break the phosphodiester backbone on which the bases reside. Though we can work hard to reduce our exposures to DNA damaging agents, we cannot totally eliminate exposure. Thus, we must rely on the elegant mechanisms that our cells have developed to repair DNA damage. The observation that individuals who inherit mutations in DNA damage response genes can exhibit many clinical problems, including cancer predisposition, neurodegeneration, increased cardiovascular disease, and premature aging (1), speaks to the broad range of physiologic processes dependent on cellular responses to DNA damage.

DNA DAMAGE RESPONSE PATHWAYS

Cellular DNA can be damaged in several different ways: nucleotide bases can be covalently altered, the DNA phosphodiester backbone can be broken on one strand (single strand break) or on both strands (double strand break), or chemical interstrand cross-links can be introduced. Predictably, different mechanisms must be utilized to repair these broadly differing types of DNA damage. Nucleotide excision repair, base excision repair, O⁶-alkyltransferase, and mismatch repair are among the mechanisms that help cells deal with base damage. Single strand DNA breaks are easily fixed, but the complex mechanisms of nonhomologous end-joining and

homologous recombination are involved in the repair of DNA double strand breaks. Further, the latter mechanism can only be effectively used in the late S, G₂ or M phases of the cell cycle, when homologous chromosomes are present in the cell. Though the following discussion will focus on responses to DNA double strand breaks, similar comments could be made about responses to these other types of DNA damage.

Many of the insights that we have gained into the mechanisms involved in cellular DNA damage response pathways have come from studies of human cancer susceptibility syndromes that are altered in DNA damage responses. For example, the genes mutated in cancer-prone diseases such as Fanconi's Anemia, Ataxia-telangiectasia, Xeroderma Pigmentosum, LiFraumeni syndrome, hereditary breast and ovarian cancers, and Hereditary Non-Polyposis Colon Cancer are all involved in DNA damage responses. One of these disorders, Ataxiatelangiectasia (A-T), is characterized by multiple physiologic abnormalities, including neurodegeneration, immunologic abnormalities, cancer predisposition, sterility, and metabolic abnormalities. The gene mutated in this disorder, *Atm*, is a protein kinase that is activated by the introduction of DNA double strand breaks in cells. *Atm* activity is required for cell cycle arrests induced by ionizing irradiation (IR) in G₁, S, and G₂ phases of the cell cycle. Several targets of the *Atm* kinase have been identified that participate in these IR-induced cell cycle arrests. For example, phosphorylation of p53, mdm2, and Chk2 participate in the G₁ checkpoint; Nbs1, Brca1, FancD2, and Smc1 participate in the transient IR-induced S-phase arrest; and Brca1 and hRad17 have been implicated in the G₂/M checkpoint. Though *Atm* is critical for cellular responses to IR, related kinases, like *Atr*, appear to be important for responses to other cellular stresses (2). Some substrates appear to be shared by the two kinases with the major difference being which stimulus is present and which kinase is used to initiate the signaling pathway.

Characterization of these *Atm* substrates permitted us to manipulate these proteins in cell lines and selectively abrogate single or multiple checkpoints. Using this approach, we demonstrated that abrogation of checkpoints does not by itself result in radiosensitivity (3-5). Though this has been known for several years in regards to the S-phase checkpoint, it was a surprising finding that abrogation of the G₂/M checkpoint did not cause radiosensitivity. This observation suggested that some other function of *Atm*, other than checkpoint control, was important for cellular survival following ionizing irradiation. In characterizing targets of the *Atm* kinase, the only substrate whose phosphorylation seems to impact on radiosensitivity is Smc1 (6;7). We previously demonstrated that the phosphorylation of Smc1 by ATM required the presence of both Nbs1 and Brca1 proteins. We found that this dependence results from the role that these two proteins play in recruiting both Smc1 protein and activated *Atm* to the sites of DNA breaks. We generated mice in which the two *Atm* phosphorylation sites in the Smc1 protein are mutated; cells from these mice demonstrate normal ATM activation, normal phosphorylation of both Nbs1 and Brca1 after IR, and normal migration of these proteins to DNA breaks (7). Despite these normal activities of *Atm*, Nbs1 and Brca1, these cells exhibit a defective S-phase checkpoint, radiosensitivity, and increased chromosomal breakage after IR similar to that seen in cells lacking *Atm*. These results suggest that the phosphorylation of Smc1 is the critical target of this signaling pathway for these endpoints and that the reason that cells lacking Nbs1 and Brca1 are radiosensitive and exhibit chromosomal breakage is due to a failure to recruit Smc1 to the sites of DNA breaks where it gets phosphorylated by previously activated *Atm*.

Recent studies also elucidated the mechanism by which DNA damage activates the *Atm* kinase and initiates these critical cellular signaling pathways (8). *Atm* normally exists as an inactive homodimer bound to nuclear chromatin in unperturbed cells and introduction of DNA damage induces intermolecular autophosphorylation on serine 1981 in both *Atm* molecules. This phosphorylation causes a dissociation of the *Atm* molecules and frees it up to now circulate around the cell and phosphorylate the substrates that regulate cell cycle progression and DNA

repair processes. This regulation of Atm activity in the cell represents a novel mechanism of protein kinase regulation and appears to result from alterations in higher order chromatin structure rather than direct binding of Atm to DNA strand breaks. Though Nbs1 and Brca1 are not required for the initial activation of Atm after IR, these two proteins are required for the migration of activated Atm to the sites of DNA breaks. It is through this process of recruitment of activated Atm along with Smc1 recruitment to the DNA breaks that leads to Smc1 phosphorylation by Atm and presumably initiation of some repair process(es) that reduce chromosomal breakage and enhance cell survival.

BROAD CLINICAL RELEVANCE: CANCER

Cellular responses to DNA damage impact many aspects of cancer biology. First, damage to cellular DNA causes cancer. We know this from epidemiologic studies, from animal models, and from the observation that many human cancer susceptibility syndromes arise from mutations in genes involved in DNA damage responses. Second, DNA damage is used to cure cancer. The majority of the therapeutic modalities that we currently use to treat malignancies target the DNA, including radiation therapy and many chemotherapeutic agents. Third, DNA damage is responsible for the majority of the side effects of therapy. Bone marrow suppression, GI toxicities, and hair loss are all attributable to DNA damage-induced cellular apoptosis of proliferating progenitor cells in these tissues. Thus, DNA damage causes the disease, is used to treat the disease, and is responsible for the toxicity of therapies for the disease.

Recent work has demonstrated that DNA damage pathways are activated very early in the process of tumor development (9;10) and elegant epidemiologic studies demonstrated long ago that exposure to environmental agents contributes to the development of the vast majority of human cancers (11). Thus, enhancement of damage response pathways could be a powerful approach to cancer prevention. The observations that individuals inheriting mutations in such genes have such a high risk of cancer and since mice carrying extra copies of genes like p53 appear to be relatively resistant to cancer development (12) provide credibility for this approach.

The flip side of this cancer coin is that blocking these damage response pathways could be used to enhance the effectiveness of cancer therapies by making tumor cells more sensitive to DNA damaging therapies like radiation therapy and cytotoxic chemotherapies. Since many of the proteins involved in these signaling pathways are kinases, they represent good targets for generation of small molecule inhibitors. Though normal tissues might also be sensitized by such inhibitors, it is possible to circumvent this problem by delivering the radiation directly to the tumor by physical or biological targeting, such as with isotopes conjugated to tumor-directed antibodies. In addition, it is conceivable that the somatic mutations in tumors might make them inherently more sensitive to these inhibitions than normal cells. Such a paradigm was recently suggested with the increased sensitivity of Brca2-mutant tumor cells to inhibition by PARP inhibitors (13;14). The harsh microenvironment of tumor cells, including hypoxia, nutrient deprivation, acid pH, etc, might even make tumor cells *in vivo* selectively susceptible to inhibition of cellular stress response pathways without having to add chemotherapy or radiation therapy. Finally, blockade of stress-induced apoptotic pathways may help protect normal tissues from the toxicities of chemotherapy and radiation therapy. Reducing bone marrow suppression and damage to gastrointestinal mucosa are prime candidates for such interventions.

BROAD CLINICAL RELEVANCE: OTHER DISEASE PROCESSES

Metabolic syndrome is a common disorder associated with insulin resistance and atherosclerosis. AT patients exhibit unusual glucose intolerance and insulin resistance (1) and we found that insulin treatment can activate the ATM kinase and that insulin signaling in some cell types is altered by loss of ATM (15). Exploring this link further, we found that heterozygous

or homozygous deficiency of ATM enhances the metabolic syndrome and accelerates atherosclerosis in high fat-fed apoE^{-/-} mice (16). Hyperinsulinemic-euglycemic clamps showed these animals to have hepatic insulin resistance, confirmed by finding decreased IRS-2-associated PI 3-kinase activity and decreased Akt activity in liver. Treatment of ATM^{+/+}apoE^{-/-} mice with low dose chloroquine, an ATM activator, decreased atherosclerosis. In an ATM-dependent manner, chloroquine also decreased macrophage JNK activity, decreased macrophage lipoprotein lipase activity (a proatherogenic consequence of JNK activation), decreased blood pressure, and improved glucose tolerance (16). These results suggest that ATM-dependent stress pathways mediate susceptibility to the metabolic syndrome and that chloroquine could represent a novel therapy to decrease vascular disease in this disorder. Further, the results suggest that carriers of ATM mutations could represent a reasonable fraction of the general population who develop insulin resistance and metabolic syndrome.

Though cellular suicide mechanisms may protect the organism in some physiologic settings, such as by preventing cancer, the double-edged sword is that these same DNA damage response pathways that help prevent cancer can also contribute to debilitating disease processes. For example, neuronal cell death after stroke or in several neurodegenerative disorders likely occurs via programmed cell death responding to cellular stress signals. A recent link of the p53 tumor suppressor gene to Huntington's Disease and potentially other neurodegenerative diseases (17), supports this notion. A similar problem may occur in ischemia-reperfusion injuries, such as occur in heart attack and stroke, and modulation of p53-mediated cellular suicide activity may impact on the amount of tissue injury (18). Conversely, p53 induction by oxidative damage may help reduce the development of atherosclerosis, perhaps by suppressing the growth or enhancing the death of cells involved in causing atherosclerotic lesions (19;20). These examples illustrate the spectrum of clinical settings in which stress response signaling pathways participate. If DNA damage response pathways contribute to the pathogenesis of these and other disorders associated with oxidative stress, then modulation of these pathways has the potential to intervene with these disease processes.

In summary, modulation of DNA damage signaling pathways has the potential to impact on some of the most common and debilitating diseases affecting mankind. We would be remiss not to actively study these pathways and try to develop drugs or biologics that can manipulate one or more of the many molecular events that determine cellular outcome following stressful exposures. The opportunity to impact on cancer, cardiovascular disease and neurologic disorders is too great to pass up.

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