Identifying the missing link between UV and p53: The role of phosphatidyl inositol kinase–related kinases*

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Whether or not skin cancer develops after sun exposure depends on a carefully orchestrated response mounted by cells occurring after DNA damage by ultraviolet (UV) radiation. A major component of this response is mediated by the p53 tumor suppressor, a transcription factor that is activated by DNA damage. Increased transcriptional activity of p53 arrests the cell cycle via p21 induction,[1] augments DNA repair,[2] or, in the case of extensive damage, induces apoptosis.[3] This damage-responsive pathway is defective in 40% to 56% of basal cell carcinomas[4] and up to 90% of squamous cell carcinomas.[3] In both cases, loss of the p53/p21 pathway is inadequate for tumorigenesis, but markedly lowers the cell's defenses against further mutations.[5] Despite the importance of the UV response pathway in the prevention of skin cancers, the protein which senses UV-induced DNA damage and activates p53 is not known.

The response to DNA damage in yeast and mammals is mediated by members of the phosphatidyl inositol kinase (PIK)–related kinase family. There are 4 known PIK-related kinases in humans: (1) *ATM*, the gene which is mutated in ataxia-telangiectasia and necessary for the ionizing radiation DNA damage response. However, *ATM* is not required for the UV response because patients with ataxia-telangiectasia have a normal response to UV damage.[6] (2)DNA-dependent protein kinase (DNA-PK) is necessary for proper B- and T-lymphocyte antigen receptor recombination. DNA-PK-deficient mice suffer from severe combined immunodeficiency, in which neither B nor T lymphocytes can develop. DNA-PK is not needed for the UV response because these mice are unaffected in their response to UV-DNA damage.[7] (3) FK 506 binding protein–rapamycin-associated protein (FRAP) is the target of the immunosuppressant rapamycin. FRAP is responsible for G1/S arrest occurring after nutrient or mitogen deprivation.[8] Its role in the UV response has not been reported. (4) Ataxia-telangiectasia and rad-3–related (ATR)[9] is the human PIK-related kinase most closely related to Mec-1, which mediates the UV response in the yeast *Saccharomyces cerevisiae*. Thus FRAP or ATR is likely to be involved in the response to UV radiation in humans.

The activation of the UV response (induction of p53, p21, and cell cycle arrest) was evaluated by ribonuclease protection assays, fluorescence-activated cell sorter analysis of cell cycle, Western blotting, and immunofluorescence microscopy. Rapamycin is a small molecule immunosuppressant that selectively inhibits FRAP function. Using Western blot analysis for p53 and p21 after UVB treatment of normal human fibroblasts, we tested the effect of blocking FRAP function with rapamycin. Rapamycin had no effect on p53 or p21 induction by UVB. Control experiments done in parallel showed that rapamycin did indeed completely block FRAP function in these cells, as assessed by p70 S6 kinase activity. We therefore concluded FRAP was not required for this pathway, and efforts were focused on ATR.

ATR is difficult to study because no small molecule inhibitor exists, the transgenic ATR-deficient mouse has an early embryonic lethal phenotype,[10] and ATR is too large to be expressed in a retrovirus. Therefore transfection of wild-type and kinase inactive forms of ATR was used to disrupt its function. Cotransfection of green fluorescent protein was used to selectively sort or analyze the cells that received the ATR construct. ATR is part of a large multi-subunit complex, and its expression level is closely regulated. Overexpression of ATR likely disrupts its function by altering the normal stoichiometry of this complex and has been used to study the role of ATR in ionizing radiation.[11] By selecting a cell line that has an intact p53 response and can be transfected with high efficiency, it was possible to directly assess the effect of disrupting ATR function on the UV response. Cells transfected with a control plasmid showed a normal cell cycle arrest after UVB damage (200 J/m^2). If, however, an ATR expression plasmid was transfected (either wild-type or a kinase-inactive mutant form of ATR), the cell cycle arrest no longer occurred after UVB treatment. Studies are ongoing to more completely characterize

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the role of ATR in the UV response using newly created p53 wild-type stable cell lines in which either ATR–kinase inactive or ATR–wild type can be induced using doxycycline.

The major goal of this project is to elucidate the molecular detail of the UV response. Our hypothesis is that a PIK-related kinase, possibly ATR, mediates this checkpoint, much as *ATM* signals to p53 after ionizing radiation. If ATR is required for the UV response, it is possible that mutations in ATR itself could lead to a defective UV response. ATR could be mutated in cases of squamous cell carcinoma, leading to a defect in this pathway even though p53 is normal.[3] An improved understanding of this pathway will elucidate the molecular basis of UV carcinogenesis and may suggest novel targets for cancer treatment and prevention.

REFERENCES

1. Poon RYC, Jiang W, Toyoshima H, Hunter T. Cyclin-dependent kinases are inactivated by a combination of p21 and Thr-14/Tyr-15 phosphorylation after UV-induced DNA damage. J Biol Chem 1996;271:13283-91.
2. Smith ML, Chen IT, Zhan Q, O'Connor PM, Fornace AJ Jr. Involvement of the p53 tumor suppressor in repair of UV-type DNA damage. Oncogene 1995;10:1053-9.
3. Ziegler A, Jonason AS, Leffell DJ, Simon JA, Sharma HW, Kimmelman J, et al. Sunburn and p53 in the onset of skin cancer. Nature 1994;372:773-6.
4. Gailani MR, Leffell DJ, Ziegler A, Gross EG, Brash DE, Bale AE. Relationship between sunlight exposure and a key genetic alteration in basal cell carcinoma. J Natl Cancer Inst 1996;88: 349-54.
5. Nghiem P, Kupper T. Basal- and squamous-cell carcinomas. In: Jameson JL, editor. Principles of molecular medicine. Totowa (NJ): Humana Press; 1998. p. 785-8.
6. Lu X, Lane DP. Differential induction of transcriptionally active p53 following UV or ionizing radiation: defects in chromosome instability syndromes? Cell 1993;75:765-78.
7. Jimenez GS, Bryntesson F, Torres-Arzayus MI, Priestley A, Beeche M, Saito S, et al. DNA-dependent protein kinase is not required for the p53-dependent response to DNA damage. Nature 1999;400:81-3.
8. Brown EJ, Schreiber SL. A signaling pathway to translational control. Cell 1996;86:517-20.
9. Cimprich KA, Shin TB, Keith CT, Schreiber SL. cDNA cloning and gene mapping of a candidate human cell cycle checkpoint protein. Proc Natl Acad Sci U S A 1996;93:2850-5.
10. Brown EJ, Baltimore D. ATR disruption leads to chromosomal fragmentation and early embryonic lethality. Genes Dev 2000;14:397-402.
11. Smith L, Liu SJ, Goodrich L, Jacobson D, Degnin C, Bentley N, et al. Duplication of ATR inhibits MyoD, induces aneuploidy and eliminates radiation-induced G1 arrest. Nat Genet 1998;19:39-46.

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