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Expression of the wild type p53 tumor supressor gene in normal cells and its deregulation in cancer cells

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p53, the tumor suppressor gene that functions as the "guardian of the genome" plays a pivotal role in "sensing" damaged DNA and in making critical decisions of whether a cell should repair the damaged DNA or whether it should be induced to undergo apoptosis. Mutant p53 has lost these activities and thus permits the proliferation of cells which carry damaged DNA, eventually leading to their malignant transformation. More than 50% of human primary tumors have lost the wild type p53 suppressor gene and instead express accentuated levels of mutant p53 protein. Designing efficient ways to restore wild type p53 and concomitantly eliminate the expression of mutant p53 are therefore key issues in cancer research. Achievement of these long-term goals are greatly dependent upon the elucidation of the molecular mechanisms that control the activity of wild type p53 in normal cells and its loss in the process of tumor development. Moreover, understanding the pathways that lead to accumulation of mutant p53 and its possible role in progression and establishment of the malignant phenotype are critical.

Function of wild type p53 in normal cells

The goal of our research is to evaluate the molecular mechanism that underlies the decisions for selecting and inducing the specific p53-dependent pathways in the cells following genotoxic stress. Elucidating the role of the various functional domains of the molecule in these conjectures is expected to yield important clues to the understanding of how p53 protects the cell from undergoing malignant transformation.

Role of the C- terminus in the life of the p53 protein

In an effort to resolve the activity of the wild type p53 protein we are focussing our study on the elucidation of the activity of the C-terminal domain of the molecule. In our previous studies we found that the C-terminus is essential for the protein to induce apoptosis.

Recently, we found that although the interaction of mdm-2 and p53 occurs through the N terminus of the p53 protein, the C terminus plays an important role in the regulation of the p53/mdm-2 loop. Comparative analysis of the murine regularly spliced form of p53 (RSp53) and a physiological C-terminally modified p53 protein, which results from alternative splicing of the p53 mRNA (ASp53), indicated that the two isoforms behave differently in the p53/mdm-2 loop. We suggest, therefore, a new mechanism for the regulation of p53, and show that alteration of the p53 extreme C-terminus can significantly change the transcription activity and the resistance to degradation properties of the p53 protein.

p53 and DNA repair

The working hypothesis that we would like to examine is that p53 is not only associated with the initial steps of recognizing damaged DNA, but also takes part in the DNA repair process itself. To elucidate the nature of the cross talk between the p53 protein and the DNA repair machinery we have investigated the relationship between the two throughout the cell cycle. Base Excision Repair (BER) was analyzed in cell cycle phase enriched populations of lymphoid cells expressing wild type p53. Our study yielded the following novel findings. First, BER exhibited two distinct peaks of activity. One associated with the G0/G1 checkpoint and the second with the G2/M checkpoint. Secondly, although the overall BER activity was reduced following exposure of cells to 400R, there was an augmentation of the G0/G1 associated BER activity and a reduction in the G2/M associated BER activity. Thirdly, modulations in these patterns of BER following genotoxic stress were found to be p53 regulated. p53 protein levels induced following g-irradiation were evenly distributed in the various cell cycle populations (analyzed by the PAb-248 anti-p53 monoclonal antibody). However, both the dephosphorylation of serine 376 of p53 (contained in the PAb-421epitope) and the specific DNA binding activity, as well as apoptosis, were enhanced towards the G2/M populations. Furthermore, inactivation of wild type p53, mediated by mutant p53 expression, abolished the alterations in the BER pattern and showed no induction of a G2/M-associated apoptosis following γ-irradiation. These results suggest that following genotoxic stress, stabilized p53 enhances the G0/G1-associated BER activity, while it predominantly reduces BER activity at the G2/M enriched populations and instead induces apoptosis. Following genotoxic stress p53 functions as a modulator that determines the pattern of BER activity and apoptosis in a cell cycle specific manner.

Interestingly, our results indicate that this involvement is

independent of the transcriptional activity of the p53 molecule. We found that both under in vitro and in vivo conditions, a p53 transactivation-deficient molecule, p53 22-23 was more efficient in BER activity than was wild type p53. However, mutations in the core domain or C-terminal alterations strongly reduced p53-mediated BER activity. These results are consistent with the hypothesis that the involvement of p53 in BER activity, a housekeeping DNA repair pathway, is a prompt and immediate one that does not involve the activation of p53 transactivation-dependent mechanisms, but rather concerns with the p53 protein itself. In an endogenous DNA damage status p53 is active in BER pathways as a protein and not as a transcription factor.

Mutant p53 gain of function

There is ample evidence of participation of wild type p53 in the positive regulation of apoptosis. Much less is known about the effects of various p53 mutants on apoptosis. Conceivably, in cells which have endogenous wild type p53, the addition of mutant p53 could inhibit the apoptotic activity of this endogenous wild type p53 in a negative transdominant fashion. However, the more challenging issue is whether mutant p53, on its own, has any effects on the cellular response to p53-independent apoptotic signals. This question is highly important since most tumors express mutant p53 protein types, hence a negative dominant effect over endogenous wild type p53 is irrelevant there. We have found that introduction of the mutant p53 into p53-null cells protects them from p53-independent apoptosis that is induced by survival factor deprivation and a number of chemotherapeutic drugs. Wild type p53, however, seems to induce an apoptotic response that co-operated with the p53-independent one. This is direct evidence that at least certain types of mutant p53 can exert a direct oncogenic effect by interfering with general apoptotic pathways.

Recently, we found that the ability of mutant p53 to block apoptosis dependents on its transcriptional activity. A core domain mutant p53 (143 Val to Ala) in which two N-terminal residues (22,23) essential for transactivation were also mutated (Leu to Glu and Trp to Ser, respectively), was examined. While p53 containing the core mutation only, efficiently interfered with drug-induced apoptosis, further modification at the N-terminus abolished this blocking activity. Furthermore, expression of c-myc, a suggested target for core mutant p53 transactivation, was elevated in the core mutant p53 expressing cells but was abolished in the presence of the transcription deficient p53 core mutant. In addition, wild-type p53, mutated in the N-terminus (22,23), was unable to induce apoptosis by itself. Nevertheless, it synergized with drugs in the induction of apoptosis. This suggests that the integrity of the N-terminus is essential for both the activity of wild-type p53 in apoptosis and for mutant p53 mediated block of drug-induced apoptosis. Thus supporting the notion that core p53 mutants act via a mechanism of 'gain of function'.

Selected publication

Almog, N., Goldfinger, N., and Rotter, V. (2000). p53-dependent apoptosis is regulated by a C-terminally alternatively spliced form of murine p53. Oncogene 19, 3395-403.

Sigal, A., and Rotter, V. (2000). Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. Cancer Research 60 6788-6793.

Offer, H., Zurer, I., Banfalvi, G., Reha'k, M., Goldfinger, N., and Rotter, V. (2001). Modulations in the pattern of p53-dependent base excision repair activity following genotoxic stress are cell cycle specific. Cancer Research. 61, 88-96.

Seluanov, A., ., Gorbunova, V., Falcovitz, A., Sigal, A., Milyavsky, M., Zurer, I., Goldfinger, N, and Rotter, V. (2001). The inability to stabilize p53 in near-senescent human fibroblasts is responsible for the change of the death pathway in response to DNA damage. Molecular and Cell Biology 21, 1552-1564

Offer, H., Milyavsky, M., Erez, N., Matas, D., Zurer, I., Harris, C. C., and Rotter, V. (2001). Structural and functional involvement of p53 in BER in vitro and in vivo. Oncogene 20, 581-9.

Almog, N., Milyavsky, M., Stambolsky, P., Falcovitz, A., Goldfinger, N., and Rotter, V. (2001). The role of the C' terminus of murine p53 in the p53/mdm-2 regulatory loop. Carcinogenesis 22, 779-85.

Sigal, A., Matas, D., Almog, N., Goldfinger, N., and Rotter, V., Anti-apoptotic activity of mutant p53 is dependent on the C-terminus (2001). Oncogene 20, 4891-4898.

Matas, D., Sigal, A., Stambolsky, P., Milyavsky, M., Weisz, L., Schwartz, D., Goldfinger, N., and Rotter, V. (2001). Integrity of the N-terminal transcription domain of p53 is required for mutant p53 interference with drug-induced apoptosis. Embo J 20, 4163-72.

Milyavsky, M., Mimran A., Senderovich, S., Zure r I., Erez I., Shats I., Gomoldfinge r N., Cohen I., Rotter, V., (2001). Activation of p53 Protein by Telomeric (TTAGGG)n Repeat Nucleic Acid Research, In press.

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