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Phil. Trans. R. Soc. Lond. B 1994 **345**, 277-280
doi: 10.1098/rstb.1994.0106

The role of the p53 protein in the apoptotic response

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SUMMARY

When mammalian cells or tissues are exposed to DNA damaging agents a programmed cell death pathway is induced as well as a cell cycle arrest. In mice in which the p53 gene has been inactivated by homologous recombination this response is profoundly diminished. These mice develop normally so that developmentally induced apoptotic events do not require p53. The p53 gene product is a 393 amino acid nuclear protein that binds specifically to DNA and can act as a positive transcription factor. High levels of p53 can induce the transcription of gene products involved in the cell cycle arrest and apoptotic pathway. The p53 proteins activity is very tightly controlled both by allosteric regulation of its DNA binding function and by regulation of the protein's stability. These results are discussed in the context of the mutations in p53 found in human tumours and their implications for the treatment of the disease by the use of radiation and chemotherapeutic agents that target DNA.

1. INTRODUCTION

The normal development of multicellular eukaryotic organisms requires an ordered process of cell divisions and, as has been appreciated more recently, programmed cell deaths. These cell death processes are tightly controlled both spatially and temporally. Elegant work in the nematode model has emphasized the precision of this process and identified critical gene products required for its execution. It has become evident from careful observations on cells in culture and from the regulation of cell number in adult tissues that programmed cell death is a normal mechanism of tissue homeostasis. Programmed cell death is often seen to occur at very high levels in tumours and is rapidly induced in response to hostile environmental or internal signals, such as the removal of polypeptide growth factors or the exposure of cells to DNA damaging agents. As the gene products required for the induction of apoptosis and for its regulation have been identified in mammalian cells it has been striking how many have been previously identified either as tumour suppressor genes or as oncogenes. This suggests that breakdown of the control of apoptosis may be a key step in the development of cancer. In support of this one of the genes most often found to be mutant in human tumours, the p53 tumour suppressor gene, has been found to be essential for the apoptotic response to DNA damage.

2. RESULTS AND DISCUSSION

(*a*) *p53 and apoptosis*

In many human tumours the p53 gene has been inactivated. Typically both alleles are affected, one allele is normally lost by a gross chromosomal deletion while the other allele typically has sustained a point mutation (Hollstein *et al.* 1991). These point mutant proteins continue to be expressed by the tumour cell and indeed may accumulate to high levels (Iggo *et al.* 1990). The point mutations in p53 are commonly found in the central domain of the protein that has recently been shown to constitute the sequence specific DNA binding domain of the molecule (Pavletich *et al.* 1993). In 1990 Moshe Oren and his colleagues discovered that one of these point mutant p53 proteins was in fact a temperature-sensitive mutant (Michalovitz *et al.* 1990). This mutation allowed rapid progress in understanding the function of the protein. When this gene is introduced with an activated ras gene to primary rat fibroblasts at 37°C the cells that express both the ts mutant p53 and the ras gene become immortal and transformed. These cells can be cultured continuously at 37°C, however when they are shifted down to 32°C the cells cease growing and instead arrest, principally at the G1-S phase border of the cell cycle. This arrest coincides with the activity of the ts mutant p53 protein. It is inactive as a transcription factor at 37°C but instead acts as a transforming gene probably by a dominant negative mechanism, whereby it complexes to, and inactivates, the transformed cells own p53 protein (Shaulian *et al.* 1992). As a result of a conformational shift the ts mutant protein regains wild-type activity at 32°C and stimulates the transcription of genes whose products ultimately result in growth arrest. Although an arrest response is seen in ras-gene-transformed fibroblasts this is not the case in other cell types. In some of these reactivation of the mutant p53 by temperature shift results in a dramatic apoptotic response. This

Phil. Trans. R. Soc. Lond. B (1994) **345,** 277–280
Printed in Great Britain

programmed cell death response to the ts mutant p53 has been seen in a wide variety of different cell types. It was first shown by Oren and his colleagues working with the M1 mouse myeloid leukaemia cell line (Yonish-Rouach *et al.* 1991) and subsequently seen by other groups working with adenovirus-E1A-transformed cells (Debbas & White 1993; White 1993). The response shows all the characteristic features of apoptosis at both the morphological and the biochemical level. Importantly the apoptotic response of the M1 cells could be blocked by the IL-6 polypeptide growth survival factor and the response in the E1A-transformed cells can be opposed by the action of the known anti-apoptotic genes bcl-2 and adenovirus E1b 19K.

These studies clearly demonstrated that the p53 gene product could induce apoptosis but they did not reveal how this signal might normally be triggered, nor establish the physiological role of the process. The recent production of mice in which both copies of the p53 gene have been inactivated by homologous recombination has allowed a critical test of the role of this gene product in apoptosis. The results have been dramatic as the p53 null mice develop normally to adulthood but are extraordinarily susceptible to the development of cancer (Donehower *et al.* 1992; Harvey *et al.* 1993). When thymocytes (Clarke *et al.* 1993; Lowe *et al.* 1993) or intestinal epithelial cells (Merritt *et al.* 1994) from these animals are exposed *in vivo* to ionising radiation they are found to be extremely resistant to DNA damage induced apoptosis in contrast to the cells of control littermates who possess one or two copies of an intact p53 gene. This phenotype indicates that the p53 protein is a key determinant of the apoptotic response to DNA damage. It is reasonable to assume that it is the loss of this response to damage, induced spontaneously by external environmental agents or internal processes, that accounts for the tumour susceptible phenotype of these animals. This model would also go some way towards explaining the high frequency of p53 mutations in human tumours because inactivation of p53 function would permit the growth of cells with abberant DNA. It further suggests that tumours in which p53 function is ablated may be more resistant to some types of cancer therapy (Lane 1992, 1993).

(*b*) *The induction of p53 by DNA damage*

The p53 protein normally has a very short half life and although p53 mRNA is present in all adult tissues examined the level of the protein product is so low as to be virtually undetectable. In 1984 it was found that p53 protein levels rose dramatically in cultured fibroblasts exposed to UV light (Maltzman & Czyzyk 1984). The mechanism responsible for this increase in p53 protein appeared to post-translational stabilization since no increase in mRNA was apparent but the p53 protein produced by the damaged cells had a much longer half life. The significance of these observations was not fully appreciated at the time but they now have been confirmed and extended by many groups (Kastan *et al.* 1991; Kuerbitz *et al.* 1992; Lu *et al.* 1992; Fritsche *et al.* 1993; Lu & Lane 1993). The response is clearly physiological as when human skin is exposed to quite low doses of UV light (recreational mild sunburn) a dramatic increase in p53 protein is seen in the cells of the epidermis and dermis (Hall *et al.* 1993). The increase in p53 protein detected by immunohistochemistry is confined to the cell nucleus and is associated with increased staining for PCNA, the proliferating cell nuclear antigen that is required both for replicative DNA synthesis and DNA repair. In this tissue no proliferative response was seen to radiation; rather it seems as if there may be a cell cycle arrest and repair response. Careful examination of the kinetics of the p53 response to different DNA damaging agents and the study of the effects of drugs that influence repair rates suggests a very close coupling between the actual lesions in the DNA and the increase in p53 protein stability (Lu & Lane 1993). In support of this we recently showed that the accumulation of p53 would occur in cells which had been exposed to a restriction enzyme in the presence of the porating agent streptolysin O (Lu & Lane 1993). Therefore pure double strand breaks in DNA are able to lead to the accumulation of p53. The mechanism underlying the regulation of p53 degradation by exposure of cells to DNA damage is still unresolved. This is a key point for further study as the accumulation of mutant p53 in tumour cells may have the same fundamental basis as the accumulation of the wild-type protein in normal cells exposed to DNA damaging agents.

(*c*) *The p53 that accumulates in cells exposed to DNA damage is transcriptionally active*

The p53 protein binds sequence specifically to DNA. The protein appears to bind as a tetramer and a consensus recognition sequence has been defined. The DNA binding domain in the central region of the p53 protein is flanked on the N-terminal side by a acidic domain that can act as a transcriptional transactivator element while the C terminus of the protein contains the regions required for oligomerization and nuclear transport. If a p53 consensus binding sequence is placed upstream of a minimal promoter and a reporter gene then transcription of the reporter gene will be p53 dependent. This was found to be the case using a line of prostate-derived cells into which a p53 responsive CAT gene had been introduced (Lu & Lane 1993). These cells contain very low levels of wild-type p53 protein transcribed from their endogenous normal p53 gene. However, when such cells are irradiated by UV light a dramatic increase in p53-dependent transcription of the reporter gene is seen. A number of cellular genes have recently been identified whose transcription is p53 dependent. These include the gene WAF-1 (El-Deiry *et al.* 1993) whose protein product (Harper *et al.* 1993) is a potent inhibitor of the Cyclin/cdk-2 protein kinase. This suggests a very attractive route by which at least some of the biological effects of p53 could be exerted. Inhibition of the cyclin/cdk2 complex would arrest the cell cycle and in some cells this arrest may provoke an apoptotic

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response. One mechanism by which this pathway could function is that inactivation of cyclin/cdk2 complexes by WAF-1 would result in accumulation of the dephosphorylated form of the retinoblastoma protein, p105 Rb. This form of Rb blocks the cell cycle in G-1 because it complexes to and inactivates the transcription factor E2F (Chellappan *et al.* 1991). E2F transcriptional activity is required for the syntheses of many S-phase proteins such as the replicative DNA polymerase. This model is attractive as we recently found that the cell cycle block imposed by the temperature sensitive mutant p53 can be by-passed by introduction of the adenoviral E1A and Papilloma virus E7 genes (Vousden *et al.* 1993). The products of these genes constitutivly activate E2F because they bind to and inactivate the dephosphorylated form of p105 Rb.

(*d*) *p53 is regulated by an allosteric control mechanism*

Because the activation of wild-type p53 as a transcription factor can result in the induction of cell death it is expected that p53 function will be tightly controlled. One mechanism of importance is clearly the regulation of the proteins stability discussed above. Work on the biochemical activity of p53 as a sequence specific DNA binding protein has revealed another important form of regulation (Hupp *et al.* 1992, 1993). When p53 is produced in bacterial or insect cell expression systems most of the protein is biochemically inactive in DNA binding assays. This latent form of the protein can be activated by an allosteric shift that is induced by phosphorylation of the penultimate amino acid of the protein by casein kinase 2. The latent form is maintained by the activity of the last 30 amino acids of the protein, the so called negative regulatory domain. Simply removing this region of the protein will result in constitutive activation of p53 as a DNA binding protein. The allosteric activation of p53 can also be brought about by non physiological events such as the binding of the monoclonal antibody PAb421 to the C terminus or the action of the bacterial chaperone protein dnaK (Hupp *et al.* 1992, 1993). Of great interest has been the discovery that certain point mutant p53 proteins can be activated to DNA binding forms only by dnaK and PAb421 but not by phosphorylation. Such mutant proteins may represent a powerful target for the development of novel therapeutics that would rescue mutant p53 proteins to induce apoptosis in tumour cells (Hupp *et al.* 1993).

(*e*) *Viruses and apoptosis*

The p53 protein was first discovered because it formed a tight protein complex with the viral oncogene product of the SV40 virus, the SV40 large T antigen (Lane & Crawford 1979). Many other DNA viruses of diverse evolutionary origin also produce oncogene products that interact with p53 suggesting that this is very important for this group of viruses. When the viral proteins bind to p53 they inactivate its function as a transcription factor (Mietz *et al.* 1992). This suggests that the viruses have evolved to inactivate p53 function. This can be understood if the viral life cycle would normally induce a p53-dependant apoptotic response. By blocking p53 function the infected cell will survive longer allowing the virus a longer period for propagation and possibly reducing the vulnerability of the infected cell to external inducing agents. The signal that the virus creates that would normally trigger the p53 pathway is not yet clear though an attractive possibility is that the signal is generated by the replication of the viral genome within the infected cell nucleus.

3. CONCLUSIONS

The p53 protein function in mediating the apoptotic response to DNA damage acts to protect the organism from the development of cancer. This response is highly regulated and its modulation may improve the therapeutic affect of many anti cancer agents. Tumours in which p53 function has been lost by point mutations in the p53 gene may still be susceptible to therapy if a practical route to rescuing some wild-type function from these mutant proteins can be found.

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