CMLS, Cell. Mol. Life Sci. 55 (1999) 9–11
1420-682X/99/010009-03 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Introduction: p53 – the first twenty years

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Abstract. The p53 protein was discovered 20 years ago, as a cellular protein tightly bound to the large T oncoprotein of the SV40 DNA tumour virus. Since then, research on p53 has developed in many exciting and sometimes unexpected directions. p53 is now known to be the product of a major tumour suppressor gene that is the most common target for genetic alterations in human cancer. The nonmutated wild-type p53 protein (wtp53) is often found within cells in a latent state and is activated in response to various intracellular and extracellular signals. Activation involves an increase in overall p53 protein levels, as well as qualitative changes in the protein. Upon activation, wtp53 can induce a variety of cellular responses, most notable among which are cell cycle arrest and apoptosis. To a great extent, these effects are mediated by the ability of p53 to activate specific target genes. In addition, the p53 protein itself possesses biochemical functions which may facilitate DNA repair as well as apoptosis. The role of p53 in normal development and particularly in carcinogenesis has been elucidated in depth through the use of mouse model systems. The insights provided by p53 research over the years are now beginning to be utilized towards better diagnosis, prognosis and treatment of cancer.

Key words. p53; tumour suppressors; apoptosis; cell cycle; phosphorylation; DNA repair; cancer; differentiation.

Ever since its first description in 1979 [1–3] the p53 protein has been providing researchers with provocative questions and often surprising answers. p53 made its debut as a cellular protein found in tight association with the simian virus 40 large T protein (SV40LT). In those pioneering days, this oncogenic DNA virus was a popular tool for inducing experimental neoplastic transformation and for studying the biology of transformed cells. Early studies established that SV40LT, a nonstructural product of the viral genome, was largely responsible for the oncogenic capacity of SV40. However, these studies failed to provide a clear answer regarding the molecular processes underlying the transforming activity of SV40LT. It was therefore intriguing to discover that this potent viral oncoprotein chose a hitherto unknown cellular protein, p53, as a target for specific high-affinity interaction. This finding offered a promise that extensive characterization of p53 might provide new insights into the mechanism of transformation by DNA tumour viruses. In retrospect, this promise has been more than fulfilled. Indeed, subsequent work on p53 did result in much better understanding of how DNA tumour viruses subvert cellular regulatory pathways, eventually giving rise to neoplasia. However, it also led to the unexpected realization that p53 is a key player in practically all types of human cancer, far beyond its early suspected role in viral transformation. Twenty years later, p53 is now known to be a potent tumour suppressor, and the *p53* gene is very probably the most common target for genetic alterations in human cancer. Whereas normal cells express wild-type p53 (wtp53) protein, their cancerous derivatives often produce mutant forms of p53. Not surprisingly, the prominent position of p53 in tumour development has spurred extensive research into its basic and clinical aspects, resulting in a vast number of publications as well as many comprehensive reviews; for some recent reviews, see refs 4–11. The information

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accumulated as a result of this research has gradually given rise to a very complex picture. In this picture p53 emerges as a pivotal regulatory protein that listens to a variety of signals and recruits an array of biochemical activities in order to trigger diverse biological responses, at the level of a single cell as well as of the whole organism. In normal cells, wtp53 appears to be typically present in latent form. Moreover, the steady-state levels of this latent p53 are extremely low, owing to a very rapid rate of proteolytic degradation. In normal cells or tumour cells that still retain wtp53 expression, activation of the endogenous wtp53 in response to extracellular or intracellular stimuli results in accumulation of stabilized, biochemically altered protein.

Like everything else associated with p53, the molecular mechanisms responsible for the intracellular conversion of p53 from latent to active form are turning out to be very complex. Most probably, they involve a combination of posttranslational modifications in p53 itself and altered interactions of p53 with other proteins. In many cases, these events result in a conformational change within the p53 molecule, endowing it with new biochemical capabilities. Among activating posttranslational modifications, possibly the most important and by far the best characterized are changes in the phosphorylation status of p53, particularly within the N-terminal and C-terminal domains of the protein. The list of potential partners of p53 in its protein-protein interactions is continuously growing and already comprises an impressive and very provocative array of important regulatory proteins. The regulation of p53 function by covalent and noncovalent modifications, particularly in the context of activation mechanisms, is discussed in detail by L. Jayaraman and C. Prives and by T. Hupp.

In addition, wtp53 activity can be reconstituted experimentally in cells which have lost wtp53 expression, as is often the case with tumour-derived cells. The surge in wtp53 activity can lead to a number of cellular outcomes, most notable among which are the establishment of a cell cycle arrest, in G1 and often also in G2, and the induction of apoptotic cell death. It is believed that these dramatic biological effects of activated wtp53 may mediate much of its tumour suppressor function, particularly when they occur in cells which have accumulated defects in their DNA or chromosomes. In such situations, activation of p53 would prevent the perpetuation of the genomic damage, and ensure that these potentially dangerous cells will not multiply and take over the normal population [12]. In addition, p53 appears to have a direct role in maintaining genomic integrity through monitoring DNA damage, activation of genes whose products facilitate DNA repair, and also direct participation in DNA repair processes and perhaps also in DNA replication 'quality control'. Some of these latter activities may also be exerted by p53 in its unstimulated state, which is otherwise latent with regard to most known biochemical functions. The role of p53 in maintaining genomic integrity is discussed in detail by F. Janus et al., who also evaluate in depth the possible roles of the recently described 3′–5′ exonuclease activity of p53.

Much progress has been obtained with regard to the biochemical characterization of wtp53 protein. The cardinal feature of wtp53 is its ability to function as a sequence-specific transcriptional activator; in this capacity p53 can bind to specific DNA sequences, located within defined target genes, and strongly promote the transcription of such genes. Only a fraction of the full set of p53 target genes have so far been described, and the identification of additional genes is presently the focus of intensive efforts. Even so, several of the p53 target genes characterized to date already offer substantial clues to the mechanisms underlying the tumour suppressor function of p53. Perhaps the best understood of those is the p21/Waf1/Cip1 gene. This gene encodes an inhibitor of cyclin-dependent kinases [13, 14] which, when overexpressed as the result of transcriptional activation by p53, can effectively block cell cycle progression. Indeed, induction of p21 expression has been shown to underlie much, though not all, of the ability of p53 to enforce a G1 cell cycle arrest [15, 16]. Other target genes offer potential effector mechanisms for the apoptotic activity of p53, although the picture here is rather complex. In addition to several well-known death-promoting genes such as *Bax* and *CD95/Fas/Apo1*, this list comprises more recently discovered players in the death scene, including several genes whose products are involved in redox regulation and production of reactive oxygen species, as well as new death receptors. The complexity of p53-mediated apoptosis does not end here, though. In fact many lines of evidence suggest that, in addition to sequence-specific activation of multiple target genes, p53 also uses one or perhaps several other, less well characterized biochemical activities to promote apoptosis. The molecular biology of p53-mediated apoptosis, as well as the factors which influence the critical choice between death and viable growth arrest in response to p53 activation, are discussed in depth by S. Bates and K. Vousden.

Whereas the dramatic effects of p53 on cellular phenotype in culture and on cancer-related processes became evident early on, the assessment of its role in normal developmental processes turned out to be more demanding. In fact, contrary to many researchers' expectations, the first p53 knockout mice revealed a markedly nonperturbed pattern of development [17]. However, subsequent efforts in many laboratories have confirmed that p53 does play a role in a number of important developmental processes, as well as in in vitro differentiation models. These recent findings, as well as studies revealing a role for p53 in preventing embryonic malformations

(teratogenesis) are evaluated by J. Choi and L. Donehower.

Unlike the early difficulties in assessing the role of p53 in the normal development of whole organisms, the use of animal models for studying the role of p53 as a tumour suppressor was gratifying right from the beginning. In fact, the first knockout mice discussed above [17] already provided inarguable evidence in support of the potent tumour suppressor action of wtp53. Subsequent studies, based on detailed manipulation and analysis of p53-null mice and largely on crosses of such mice with other tumour-prone mouse strains, have provided a wealth of significant additional information. This information now allows us to evaluate critically the biological basis of p53-mediated tumour suppression, as well as to place it in the context of well-characterized in vivo signalling pathways. All these aspects are discussed in detail by L. Attardi and T. Jacks.

As discussed above, the intracellular activity of p53 can be regulated by a multitude of cellular proteins. One such protein which stands out in particular is MDM2. This protein, product of an oncogene involved in several types of human cancer, can form a very tight and specific association with p53. This association has two consequences: first, it prevents p53 from acting as a transcriptional activator; second, it actually targets p53 to rapid degradation through the ubiquitin-proteasome pathway. Thus, MDM2 acts as a potent physiological antagonist of p53. The critical role of MDM2 as a regulator of p53 activity in vivo is strongly supported by studies on *mdm2* gene knockout mice [18, 19]. Most significantly, this *mdm2* gene is a direct target for sequence-specific transcriptional activation by p53, thereby establishing a negative autoregulatory feedback loop in which p53 activates its own inhibitor. This loop probably serves to restrain p53 activity within cells, as well as to terminate p53-initiated responses once the inducing signal is turned off. The intricate interactions between p53 and MDM2, as well as additional features of MDM2 which are likely to contribute to its role in oncogenesis, are discussed by D. Freedman and A. J. Levine.

Finally, one has to keep in mind that much of the recent excitement about p53 stems from its conspicuous association with human cancer. Naturally, there is an extensive ongoing effort to translate the basic p53 knowledge into the clinic, be it as a tool for better diagnosis or prognosis, or eventually also as a basis for innovative anticancer therapies. While there are many hopes that this effort will eventually bear very valuable fruit, progress is significantly hindered by the vast complexity of the issues involved. The clinical implications of cancer-associated p53 mutations in particular, and p53 research in general, are critically evaluated by R. R. Wallace-Brodeur and S. W. Lowe.

Despite all that we already know about p53, much more remains to be uncovered. Hence, it can be taken for granted that the picture emerging from these *CMLS* reviews will keep evolving as more is learned and more is understood about this fascinating protein.

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