8. P53 AND OTHER CELL CYCLE REGULATORS

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INTRODUCTION

Most tumor suppressor genes (whose function in cancer biology was surmised by their inactivation or deletion) turn out to be important in normal cell growth and proliferation. Inactivation of these genes opens the gates to malignant transformation driven by aberrant growth signals. In general, tumor suppressor dysfunction does not initiate genomic instability or aneuploidy, hallmarks of the origin of cancer, but they are at least as susceptible as other genes to the consequences of these destabilizing phenomena. On the other hand, these genes have a predilection to inactivation by epigenetic mechanisms, such as aberrant methylation with the progression of cell transformation.

This chapter focuses on the pathways anchored by the two "big" regulators of the cell cycle: p53 and the retinoblastoma (Rb) genes. Until recently the connections between these two networks was obscure (1). It is now apparent that they regulate and counter-regulate each other in a Ying-Yang fashion. The network centred on p53 is denser and more intricate (2,3), receiving signals from upstream modulators and downstream effectors. In addition to their respective roles at crucial check-points in the cell cycle and DNA repair, the products of both these genes appear to have roles in embryogenesis, cell differentiation and cell fate including senesce(1,4,5).

THE P53 NETWORK

P53 is a transcription factor that transactivates a large number of genes. The abundance of the p53 protein is predominantly regulated through its degradation. Compared to

the other features of its biology, our knowledge of the transcriptional regulation is less than complete. The gene is induced by single DNA breaks, radiation, UV light, some chemotherapeutic agents (3) and as demonstrated beautifully recently by interferons α/β (6). P53 tansactivation of its target genes is regulated by posttranslational mechanisms including phosphorylation, acetylation and prolyl isomeration (3,7) or by protein-protein interaction (8). P53 thus modified may select subsets of target promoters by changing its shape and affinity to bind to regulatory DNA sequences that vary among the downstream genes. Little is, however, known about the mechanisms underpinning the selection of the target genes and the divergent cell fates triggered down a given pathway (3,7). In certain situations apparently non-modified P53 can select and activate genes (6).

MDM2 and networks cross-over

P53 is ubiquinated by MDM2, directing it to the proteosomes for degradation. P53/MDM2 makes a finely balanced tandem. Thus, while p53 up-regulates MDM2 gene transcription, the MDM2 protein marks p53 for proteolysis by attaching ubiquitin to its carboxy-terminus (2,3). Furthermore phosphorylation of p53 NH2-terminus (and its activation) influences its binding affinity to MDM2, and thus its degradation (2,3). A deubiquinating enzyme can counteract the degree to which P53 is ubiquinated (9). Moreover, the transcriptional response of P53 may be regulated by the SUMO-1 modification of its carboxy-terminus (10).

And the regulation of MDM2 gets even more complex, in that it is capable of self-ubiquination and is SUMO-1 modified. The latter modification prevents MDM2, s self-ubiquination and therefore, in turn, its ability to ubiquinate P53 (11) The phosphorylation of MDM2 through the phosphatidylinositol 3-kinase (PI3K) pathway (12,13) may also enhances its ability to ubiquinate and thus regulate the cellular level of p53.

Other cues for the p53—anchored pathway also feed through MDM2. Thus growth signals from a number of oncogenes e.g. RAS, induce the ARF gene or stabilize its protein. ARF promotes the accumulation of SUMO-1 modified MDM2 and block the shuffle of MDM2 from the nucleolus to the cytoplasm, thereby stabilizing P53 (14–16).

MDM2 is also the conduit for the regulation of growth factors and allied receptors relevant to proliferation and signaling (17,18).

The influence of ARF on MDM2 function is an important link between the P53 signaling network to that centered on Rb, in that ARF gene transcription is, in turn, regulated by E2F (1). And this influence is far from being unidirectional because MDM2 (which you will recall is transcriptionally regulated by P53) binds to Rb and E2F with resulting increase in E2F gene transcription (19 for review).

Cycle arrest & apoptosis

When the cell is stressed or its function impaired, p53 abundance is increased and the protein activated to arrest cell division until such time as repairs are affected (2,3). P53 mediates the arrest of the cell cycle at the G1/S restriction point and the G2/M phase through the increasing the transcription of $p21^{WAF}$, an inhibitor of cyclin-dependent