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p53 tumor suppressor gene: function in normal cells and deregulation in cancer cells

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The role of p53 in normal cells and deregulation in cancer

The p53 tumor suppressor gene was shown to play a pivotal role in the regulation of the cell cycle. Inactivation of p53 in cells was found to induce malignant transformation. In our laboratory, we focus our research on further understanding of the role of p53 in normal cells and its deregulation upon malignant transformation. On the one hand, we focus on studying the involvement of p53 in the normal cell, and on the other, on the understanding of the way inactivation of p53 wild type and accumulation of mutant p53 acquire cells with a malignant phenotype.

p53 gain of function

Tumor-associated mutants of the p53 tumor suppressor protein exert biological activities compatible with an oncogenic gain of function. To explore the underlying molecular mechanism we performed microarray analysis, comparing p53-null cells to mutant p53-expressing cells. One of the genes upregulated in the presence of mutant p53 was EGR1, a transcription factor implicated in growth control, apoptosis and cancer. EGR1 induction by various types of stress is markedly augmented in cells expressing mutant p53, apparently through a physical association of mutant p53 with the EGR1 promoter. Functional assays indicate that induction of EGR1 by mutant p53 contributes to enhanced transformed properties and resistance to apoptosis. We propose that EGR1 is a significant contributor to mutant p53 gain of function.

p53 and telomerase activity

Inactivation of p53 and activation of telomerase occur in the majority of human cancers, raising the possibility of a link between the two pathways. We found that endogenous wild type p53 was able to downregulate telomerase activity, hTERT mRNA level and promoter activity, however the ability to repress hTERT expression was found to be cell type specific. The integrity of DNA binding core domain, the N-terminal transactivation domain and the C-terminal oligomerization domains of p53 were all essential for hTERT promoter repression, whereas the proline rich domain and the extreme C-terminus were dispensable. We found that p53 binds to this promoter, suggesting an indirect mechanism of repression. We propose a model in which p53 mediates the repression of hTERT expression via p21 induction, activation of the pRb family, and recruitment of a histone deacetylase-containing repressive complex to the hTERT promoter through an atypical E2F site.

p53 and senescence

Replicative senescence is an irreversible cell cycle arrest that limits the proliferation of damaged cells and may be an important tumor suppression mechanism *in vivo*. This process is regulated at critical steps by the tumor suppressor p53. To identify genes that may regulate the senescence process, we performed cDNA microarray analysis of gene expression in senescent, young proliferating, and hTERT-immortalized primary human fibroblasts. Activated p53 suppressed EZH2 gene expression through repression of the EZH2 gene promoter. This activity of p53 requires intact p53 transactivation and DNA binding domains. Furthermore, the repression of EZH2 promoter by p53 is dependent on p53 transcriptional target p21Waf1 inactivating RB/E2F pathways. In addition, the knockdown of EZH2 expression retards cell proliferation and induces G2/M arrest. We suggest that the p53 dependent suppression of EZH2 expression is a novel pathway that contributes to p53 mediated G2/M arrest. Activated p53 suppresses EZH2

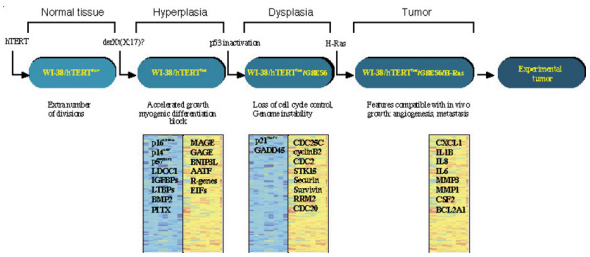
expression, suggesting a further role for p53 in epigenetic regulation and in the maintenance of genetic stability.

Development of an *in vitro* model to study malignant progression.

Prevailing theories of carcinogenesis suggest a stepwise transformation process. Nevertheless, the specific transcriptional programs initiated by oncogenic mutations and their contributions to cancer progression are largely unknown. To gain insights into the transcriptional programs involved, genome wide expression profiling coupled with Superparamagnetic Unsupervised Clustering (SPC) analysis was applied to human diploid fibroblasts gradually transformed by hTERT mediated immortalization, spontaneous silencing of the INK4A locus, mutant Ras expression, and p53 inactivation. Distinct genetic signatures revealed that a defect in myogenic differentiation associated with INK4A locus inactivation was the initiation event of the transformation process. During the next step, induction of the protein biosynthesis machinery allowed accelerated growth of a pre-malignant clone. p53 inactivation in these cells gave rise to the "proliferation signature" found in many aggressive human tumors. Finally, genes involved in angiogenesis and metastasis were induced in a synergistic manner by H-Ras and mutant p53, establishing a "tumor forming" genetic signature. The suggested model is described below (see Figure). We believe that these transcriptional programs faithfully reflect the *in vivo* stages of human carcinogenesis and will open new avenues for cancer diagnosis and treatment.

Outline of a stepwise malignant transformation process based on the transcriptional programs identified in this study:

Microarray profiling revealed specific genetic signatures, associated with the particular stages in our *in vitro* transformation model (Selected genes are shown in the boxes colored according to their expression level: yellow-red for high, and blue for low expression). These alterations in gene expression reflect the biological features spontaneously acquired by cells (derX, t(X;17)) or induced by engineered mutations (GSE56 and H-Ras) along the transformation process. We suggest that the genetic signatures identified in our study provide a conceptual framework for similar transcriptional alterations associated with the transition from normal tissue to hyperplasia, dysplasia, and then to cancer.



Selected Publications

Milyavsky, M., Shats, I., Erez, N., Tang, X., Senderovich, S., Meerson, A., Goldfinger, N., Ginsberg, D., and Rotter V. (2003). Evidence that prolonged culturing of hTERT immortalized human fibroblasts leads to a premalignant phenotype. Cancer Research 63, 7147-7157

Meerson, A., Milyavsky M., and Rotter V. (2004). p53 mediates density-dependent growth arrest. FEBS Letters 28053 1-7.

Matas, D., Milyavsky, M., Shats, I., Nissim, L., Goldfinger, N., and Rotter, V. (2004). p53 is a regulator of macrophage differentiation. Cell Death and differentiation in press

Tang, X., Milyavsky, M., Igor Shats, I. Erez, N., Goldfinger, N., and Rotter, V. (2004). Activated p53 Suppresses the Histone Methyltransferase EZH2 Gene. Oncogene in press

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Role of mutant p53 in the development of lung carcinoma

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