Title Role of p53 in Cancer Associated Gene Networks

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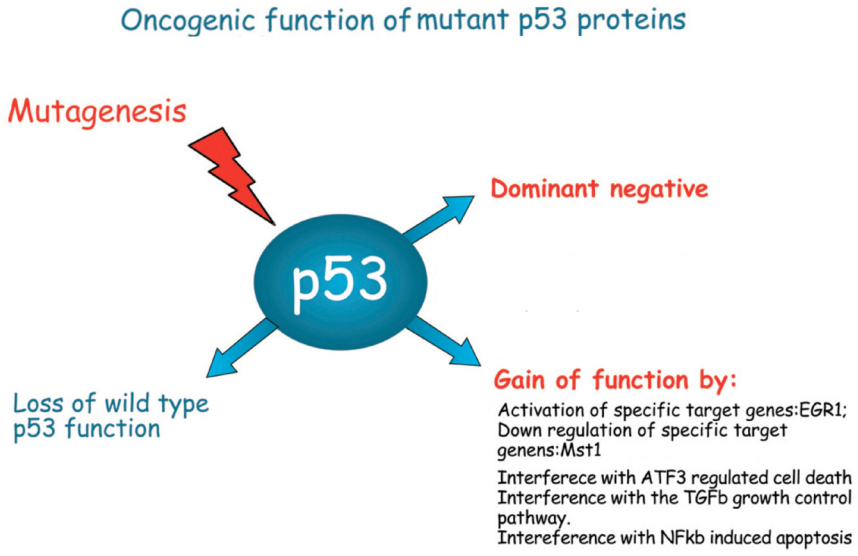
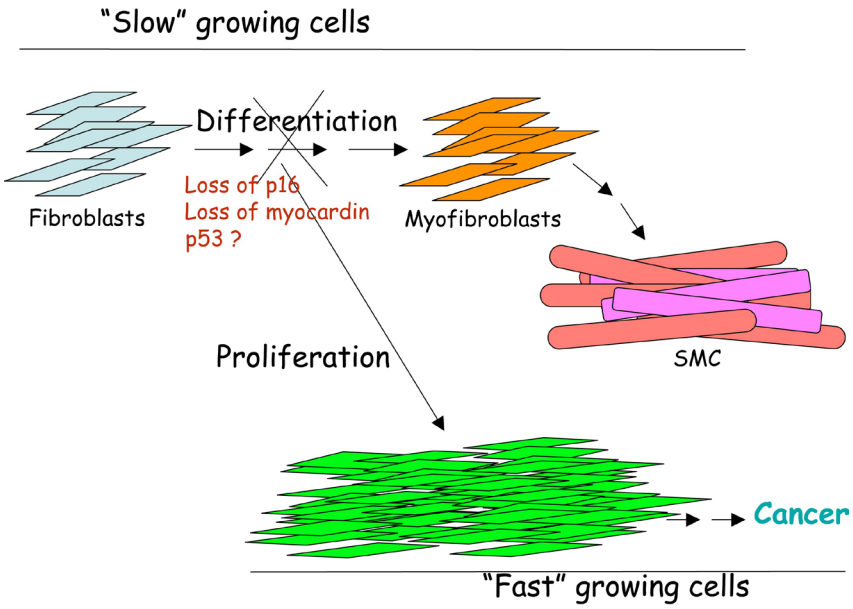
It is well accepted that the p53 tumor suppressor gene plays a pivotal role in protecting cells from cancer development. Inactivation of p53, the guardian of the human genome, causes genetic instability leading to the accumulation of genetic alterations, which induce malignant transformation of cells. In addition, it was found that mutant p53 protein that frequently accumulates in human tumors (Rotter 1983), facilitates tumor initiation and progression by a "gain of function" mechanism. Research in our laboratory is focused on revealing the mechanism by which wild type p53 acts in normal cells and the way by which mutant p53 contributes to cancer development. Our working hypothesis is that mutant p53 activate specific target genes which turn on specific gene networks which eventually lead cells to cancer (Segal and Rotter 2000). (Figure 1). In our experiments we found that expression of mutant p53 acquires cells with a higher chemotherapy resistance (Li et al., 1989). Cells expression mutant p53 which still express an intact N-terminal transcription domain are more resistance to drug induced apoptosis (Matas et al., 2001). In an effort to identify the mutant specific target genes we have adopted a genome wide analysis approach where we used cDNA micro-array technology and compared gene expression pattern of p53-null cells with their well-defined p53 mutant expressing counterpart cell lines that we have generated. This approach yielded lists of potential mutant p53 target genes. Interestingly, such targets are the EGR1 (Weisz et al., 2005), Mst1 (Zalcenstein et al., 2005) and others. In our study we could show that mutant p53 indeed interacts with their promoter regions and that knocking down these specific targets significantly modify the resistance of cells to chemotherapeutic drugs. In an independent studies, we observed that mutant p53 seems to affect TPA-induced cell death by attenuating ATF3 expression (Buganim et al.,2006) and was found to modify the activity of TGFb induced cell growth arrest (Kalo et al., 2007). We also found that NFkb induced networks (Weisz et al., 2007a). In all, our results suggest that mutant p53 actively modifies central cell growth control networks (Weisz et al., 2007b).

As it well accepted that malignant transformation is a step-wise process it is challenging to us to discover which of these events involve the p53 protein. To that end we have established several *in vitro* transformation models in which normal cells are transformed into cancer cells by well-controlled genetic alterations. In our experiments, we have immortalized various human primary cells of lung and prostate origin and engineered into them several defined cancer associated genetic alterations. These included inactivation of p53 tumor suppressors by several methods, over-expression of mutant p53, over-expression of the ras oncogene and various combinations of these modifications. As a result we have obtained transformed cells that are capable of developing tumors in mice, suggesting that the *in vitro* developed system represents an authentic model of cancer development. To evaluate the gene networks that are associated with the malignant defined steps, we have used a genome-wide approach, which permits the identification of gene clusters that are associated with the individual steps of malignant transformation that we defined. Deciphering the molecular regulatory events that drive malignant transformation represents a major challenge for systems biology. Taking this approach we identified specific gene signatures that are associated with the specific genetic alterations along the process of cancer development



In particular, we have focused on four important clusters that represent significant molecular networks altered in this process.

As we established our *in vitro* system, we noticed that although cells immortalized by hTERT grow well in vitro, at a certain time point, varying between the various cells types studied, cells seem to undergo a certain cell crisis which is then followed by the emergence of cells with a faster cell growth rate. We refer to this transition point as a junction where "slow" cells are turned into "fast" growing ones. Analysis of that checkpoint revealed a loss of the p16 tumor suppressor gene. This is in agreement with other reports



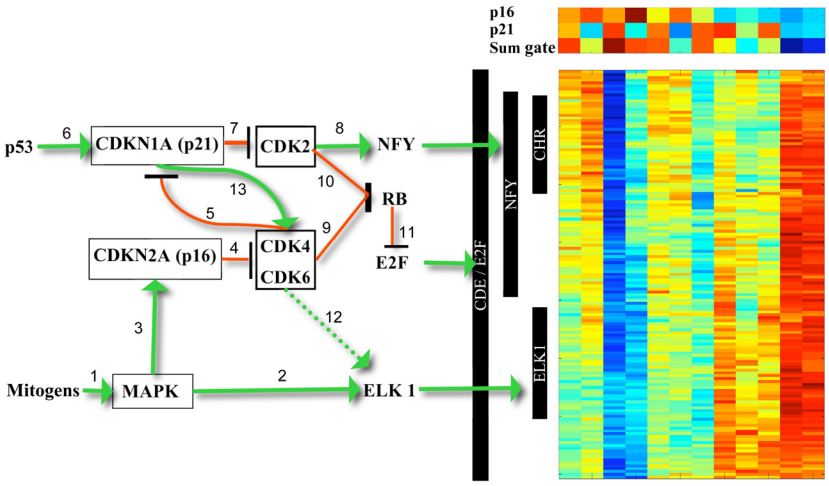
where it was shown that the p16 is undergoing methylation and thus is turned off.

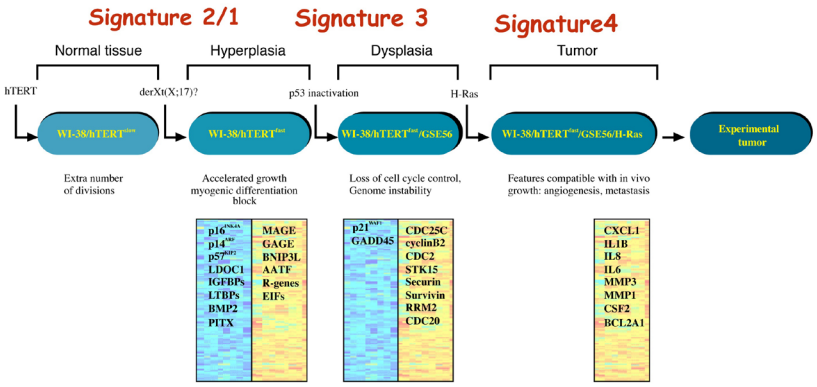
Gene Cluster analysis indicated that at that time point there is a specific loss of gene cluster, consisting of typical cell differentiation (cluster 1) and a concomitant again of a gene cluster that contains genes associated with cell proliferation (Cluster 2). We found that that at that turn point the immortalized WI-38 human fibroblasts lost their ability to undergo cell differentiation following a TGFβ trigger. Interestingly, restoration of p16 recovered the capacity to undergo TGF induced differentiation and express the typical differentiation markers.

In addition to the loss of p16, we noticed a reduction in the myocardin gene. This gene was shown to be a specific transcription factor that plays a central role in smooth muscle differentiation. Using several assays, we could show that the myocardin is lost at the point slow growing cells are converted into fast growers and that myocardin expression is essential for their differentiation (Milyavsky et al., 2007). Furthermore, our studies have shown that myocardin over-expression caused cell growth arrest. Analysis of human cell lines and primary tumors indicated a trend of losing myocardin gene expression. We therefore concluded that at this early step of cell transformation cell already underwent genetic changes that bring about a defect in their capacity to undergo cell differentiation. This defect is associated with a loss in the expression of at least two tumor suppressor genes, p16 and myocardin (Milyavsky et al., 2007) (Shatz et al., 2007) (Figure 2). The role p53 in the process leading to such a cell differentiation blockage is presently being investigated. We expect that such an approach will indicate as to a defined role of p53 in cell differentiation at large.

A third cluster of genes that we focus on consists on genes whose expression levels increased as a function of p53 and p16INK4A tumor suppressors' inactivation. This cluster predominantly consists of cell cycle-related genes and constitutes a signature of a diverse group of cancers. Promoters of the genes in this cluster are enriched with NFY, E2F, Elk1, CHR, and CDE regulatory motifs. The promoter architecture of many of the genes constitutes a "sum gate" – output mRNA levels correlate with the summated activity of the two suppressive channels of p53 and p16INK4A. Taking components of the mitotic spindle as an example, we experimentally verified our predictions that p53-mediated transcriptional repression of several of these novel targets is dependent on the activities of p21, NFY and E2F. Our study demonstrates how a well-controlled transformation process allows linking three levels in a complex regulatory network, namely gene expression, promoter architecture and activity of upstream tumor suppressors (Tabach et al., 2005).

A fourth-interesting cluster observed in this study is a group of genes that are up-regulated by over-expression of the RAS oncogene and concomitantly downregulated by p53. This gene signature, which consists of a high number of chimokines, shows a significant increase and in some cases, a synergism in their expression as a result of overproducing RAS and are inactivated or knock-down of p53 expression. Based on the information obtained by this cluster that agrees with an advanced phase of transformation we





unrevealed a novel networks that connect the RAS and p53.

In general it seems that the clusters that we have discovered using this in vitro model seem to agree to specific steps in transformation and thus may serve as specific hallmark signature of this stepwise malignant process.

More recently we also became interested in studying prostate cancer, which is the most commonly diagnosed type of cancer in men but yet there is no available cure for patients with advanced disease. Our working hypothesis is that developing an in vitro model will provide new tools to address this issue to that end immortalization without viral oncogenes of prostate epithelial cells and, prostate stromal cells. These cells exhibit a significant pattern of authentic prostate-specific features. In particular, the epithelial cell culture is able to differentiate into glandular buds that closely resemble the structures formed by primary prostate epithelial cells. The stromal cells have typical characteristics of prostate smooth muscle cells (Kogan et al., 2006). These immortalized cultures may serve as a unique experimental platform to permit several research directions, including the study of cell-cell interactions in an authentic prostate micro-environment, prostate cell differentiation, and most significantly, the complex multi-step process leading to prostate cell transformation.

In summary, our in vitro systems offer an interesting models that are expected to resolve and identify the various gene networks that are associated with defined steps of malignant transformation and are associated with defined genetic alteration with a particular focus on the p53 tumor suppressor gene. We except that deciphering universal transcriptional programs, which are affected by the most common oncogenic mutations, will provide considerable insight into regulatory circuits controlling malignant transformation and will, hopefully, open new avenues for rational therapeutic decisions including p53-based therapy.

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