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DNA microarray analysis of gene expression regulated by p53

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Analysis of global gene expression becomes a major tool for characterizing cell phenotype in normal and diseased state. A large effort is devoted worldwide to categorize malignancies through the identification of sets of genes that can explain the origin of cancer as well as changes associated with therapy. We focus on experimental cellular systems that respond to stress or to tumor suppressors by expressing genes, which could be correlated with those modulated in cancer. We are interested in "synexpression" groups of genes which are coordinately regulated, through their promoter activation and in the signaling pathways that they control. Recently we analyzed the response of cell lines to p53, the most well studied tumor suppressor, in order to clarify mechanisms related to growth arrest and apoptosis.

A human lung cancer cell line that expresses the temperature sensitive murine p53 was utilized to quantitate mRNA levels of various genes at different time points after shifting the temperature to 32°C. Inhibition of protein synthesis by cycloheximide (CHX) was used to distinguish between primary and secondary target genes regulated by p53. We used DNA microarrays (Affymetrix) containing probes for 12,000 genes to analyze mRNA expression profile at different time points. In the absence of CHX, 259 and 125 genes were up or down-regulated respectively; only 38 and 24 of these genes were up and down-regulated by p53 also in the presence of CHX and are considered primary targets in this cell line. Cluster analysis of these data using the super paramagnetic clustering (SPC) algorithm demonstrate that the primary target genes can be distinguished as a single cluster among a large pool of p53 regulated genes (Fig. 1). In collaboration with Prof. Eytan Domany and his group.

To study genes activated in the p53 induced apoptotic process, we used a mouse myeloid leukemic cell line (LTR6) expressing the temperature-sensitive p53 (val135) that undergoes apoptosis upon shifting the temperature to 32°C. We analysed the gene expression profile at different time points after p53 activation using oligonucleotide microarray (DNA Chips, Affymetrix) capable of detecting ~11 000 mRNA species. Cluster analysis of the p53-regulated genes indicate a pattern of early and

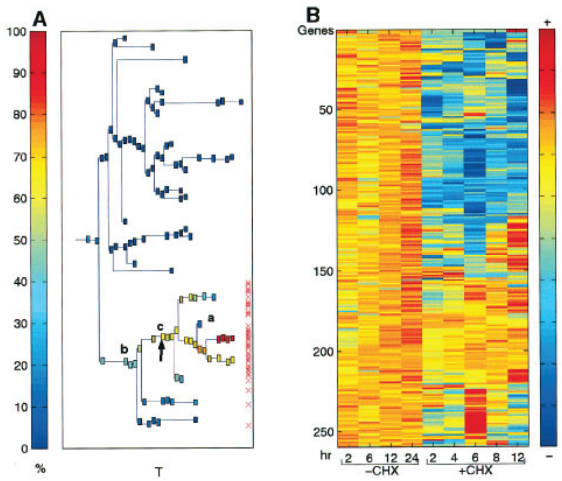

Fig. 1 *Clustering results using super-paramagnetic clustering (SPC) for the 259 genes that were activated by p53 without cycloheximide (CHX). (A) The dendrogram of the genes. Each cluster is represented by a box colored according to the per cent of primary target genes. (B) The genes are ordered according to the dendrogram on the left. The color represents induction (red) or repression (blue).*

late induced sets of genes. We show that 91 and 44 genes were substantially up and down regulated, respectively, by p53. Functional classification of these genes reveals that they are involved in many aspects of cell function, in addition to growth arrest and apoptosis. Comparison of p53 regulated gene expression profile in LTR6 cells to that of a human lung cancer cell line (H1299) that undergoes growth arrest but not apoptosis demonstrates that only 15% of the genes are common to both systems. This observation supports the presence of two distinct transcriptional programs in response to p53 signaling, one leading to growth arrest and the other to apoptosis.

At least two new p53-target genes were identified as proapoptotic (Fig. 2) . One is the gene called PUMA which was detected in our analysis and was characterized by others as a member of the BH-3 only family that antagonises bcl-2. The other is Apaf-1 which was induced by p53 as was also verified by Northern blot. Proapoptotic activity of p53 was shown to involve

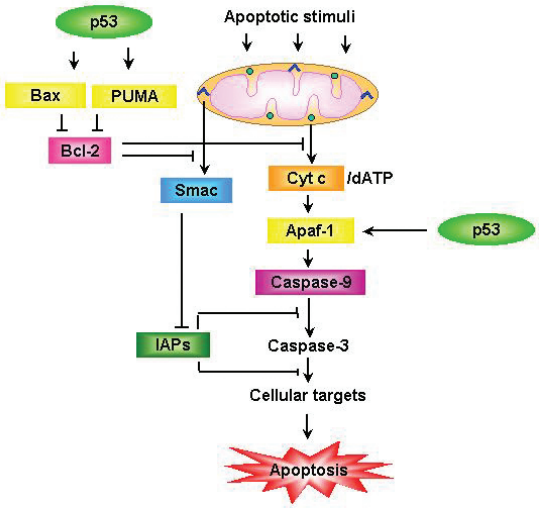

Fig. 2 *Schematic representation of p53 induced genes in the mitochondrial apoptotic pathway.*

several genes like Bax and Bak, which may function in the release of cytochorme C from the mitochondria. Cytochrome C associates with Apaf-1 and caspase-9 to form the apoptosome. Genetic and cellular data indicate that Apaf-1 deficiency abrogates the apoptotic effect of p53 and lack of Apaf-1 expression can substitute for p53 loss in promoting tumor formation. Our results of microarray analysis show that Apaf-1, the mammalian homologue of *C. elegans* CED4, is a direct target of p53. This was demonstrated by gel shift by p53 of the target site sequence located at –607 bp upstream to the RNA start site in Apaf-1 promoter. In addition p53 activates Apaf-1 promoter-luciferase construct using transient transfection. We also show that the p53 activation of the Apaf-1 luciferase construct can be enhanced by the putative tumor suppressor gene product, Zac-1, a transcription factor that has previously been shown to inhibit cell proliferation. An attractive possibility that emerges from defining Apaf-1 as a p53 target gene, is using the Apaf-1 promoter as a target for reagents that can activate this gene in presence of inactive p53.

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