The tumor supressor p53-our cell gurdian

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As it is 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 signatures that are associated with the individual steps of malignant transformation that we defined.

Two interesting gene signatures that we investigated in details seem to be associated with the phase were slow growing cells are turned into fast growing cells. At that point in time cells have lost the expression of several tumor suppressor genes such as p16 and myocardin. While the first gene signature is associated with this cell growth turning point represents a cluster of cell differentiation genes that are lost, the second gene signature identified at this point represent proliferation associated genes whose expression was amplified. In all we found at that phase of cells in culture a cell differentiation block that is partly regulated by p53.

A third cluster of genes that we have focused consistes of genes whose expression levels increased as a function of p53 and p16INK4A tumor suppressors' inactivation. This cluster predominantly consisted 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 regulatory motifs. 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.

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 down regulated by p53. This gene signature, which consists of a high number of chemokines, 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 unravelled a novel networks that connects the RAS and p53.

A fifth interesting cluster

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