Identification of transcriptional programs along defined stages of human carcinogenesis

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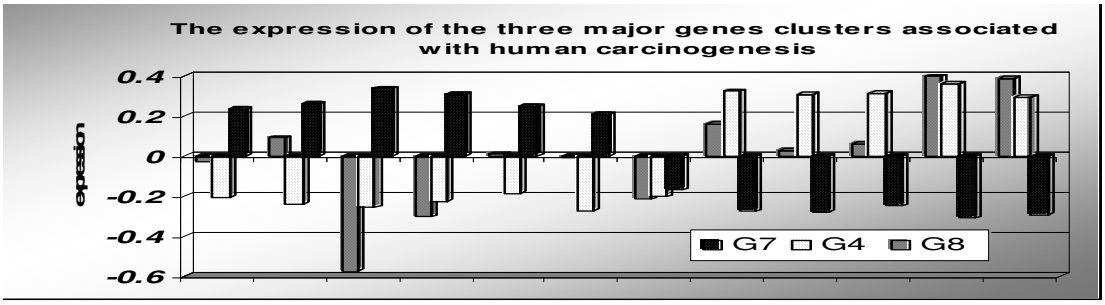
Global profiling of human transcriptome during distinct stages of carcinogenesis presents a challenge, which requires novel experimental models and advanced analytical tools. We developed such a model of the stepwise transformation in vitro, which is based on human primary fibroblasts[1]. Upon hTERT expression spontaneous variants with inactivated INK4A locus arose, allowing further transformation by H-Ras. In the present study we showed that cells with concomitant expression of H-Ras and dominant negative p53 were endowed with more transformed features, which were sufficient for tumorigenic potential in vivo. Distinct stages along the multistage carcinogenesis were selected for microarray profiling.

Unsupervised analysis of the stepwise transformation, using SuperParamagnetic Clustering (SPC) [2], identified specific genetic signatures that differentiate samples according to the INK4A locus status, presence of functional p53 and H-Ras overexpression. Several important conclusions were drawn from our analysis. Dramatic downregulation of growth inhibitory molecules, putative tumor suppressors and proapoptotic factors were evident at the earliest stages of the neoplastic process. In contrast, robust induction of protein translation machinery, antiapoptosis genes and multiple cancer specific antigens characterize progression from a slow to a fast growing stage. Inactivation of wild type p53 in the absence of INK4A resulted in upregulation of multiple genes required for cell cycle progression, mitosis regulation and rapid proliferation resembling "proliferation signature" found in many aggressive human tumors. Lastly, genes involved in chemotactic attraction of endothelial cells as well as in inflammatory response and metastasis were induced in a synergistic manner in cells expressing both H-Ras and dominant negative p53, adding novel facets to H-Ras transforming activities. The detailed understanding of the alterations in the transcriptional programs occurring during human carcinogenesis will ultimately lead to the identifications of the novel anticancer targets.



Days in culture	21	30	40	40	40	193	319	480	600	480	480	480
INK4A status	+	+	+	+	+	+	-	-	-	-	-	-
hTERT					Up	Up	Up	Up	Up	Up	Up	Up
p53		Down*	Up**	Down*		Down*					Down*	Down*
H-Ras V12										Up		Up

* GSE insertion: cause inactivation of p53

** p53 activity is increased in fibroblasts during senescence [3]

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Figure 1: The mean expression of the 3 clusters that are correlated with the malignance process. The clusters were identified by unsupervised analysis using the SPC procedure. The table represent the status of 3 manipulated genes: p53 that was repressed using GSE, hTERT, inserted to the cells in day 40, and Ras, inserted into two samples: with and without GSE. Cluster G7: 397 down regulated genes, that correlated with INK4A locus expression and anti-correlated with cell proliferation rate. Cluster G4: 250 up regulated genes, that anti-correlated with INK4A locus expression and with Cluster G7; correlated with cell proliferation rate. Cluster G8: A cluster of 168 genes, which showed associated with p53 activity, cell proliferation and tumor aggressiveness.

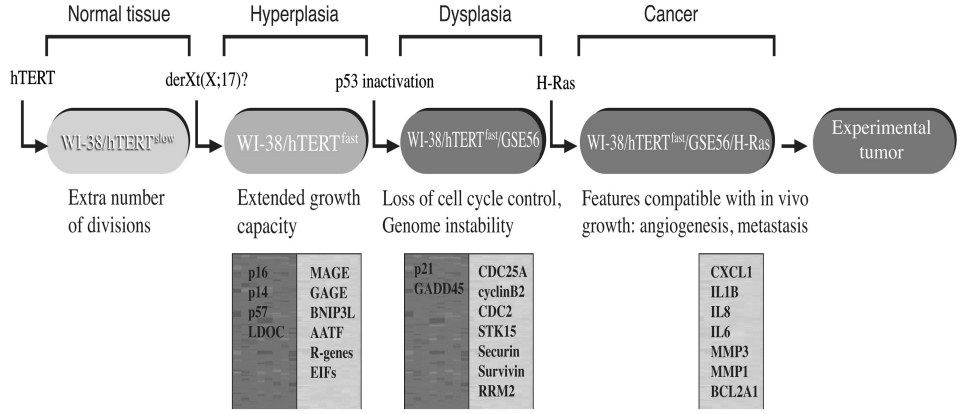

Figure 2: stepwise malignant transformation model of the human diploid fibroblast and the underlying transcriptional changes. Microarray profiling revealed specific genetic signatures, associated with the particular stages in the in vitro transformation model (selected genes in the boxes are colored according to their expression level: bright for high expression and dark for lower expression). These alteration reflect the biological features acquired by cells spontaneously (derX, t(X;17) or induced by engineered mutations (GSE56 and H-Ras) along the process. We hypothesize that genetic signatures identified by our study provide a conceptual framework of similar transcriptional alterations associated with transition from normal tissue to hyperplasia, dysplasia and then to cancer.

Data Analysis

Gene expression analysis was done on 12 data points with two replicates for each one of the data points. For probe-level data analysis, Affymetrix® Microarray Suite software 5.0 (MAS 5.0.) was used as follows. First, the abundance of each transcript represented on the array was estimated and was labeled as either present, absent, or marginal. Then absent /present and variance filters were applied; only those genes that were present in at least one data point in both repeats and had variance > 2 were kept. For each gene (row), the $\log_2$ of the expression was mean-centered (subtracting the average) and normalized to generate the final gene expression matrix. SPC [2], ANOVA, T-test and fold change were used to analyze the data.

References

1. Milyavsky, M., et al., Prolonged culture of telomerase-immortalized human fibroblasts leads to a premalignant phenotype. Cancer Res, 2003. 63(21): p. 7147-57.
2. Blatt, M., S. Wiseman, and E. Domany, Superparamagnetic clustering of data. Physical Review Letters, 1996. 76(18): p. 3251-3254.
3. Atadja, P., et al., Increased activity of p53 in senescing fibroblasts. Proc. Natl. Acad. Sci. USA, 1995. 92: p. 8348-8352.