The role of cigarette smoke-induced gene expression modifications in the etiogenesis of lung cancer

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THE AIM

The aim of our study is to identify and characterize genes potentially involved in lung tumorigenesis, the expression of which is changed due to exposure to cigarette smoke condensate (**CSC**).

STRATEGIES

Three different approaches are adopted in this study:

- analysis of differential gene expression in normal human bronchial epithelial cells (BEAS-2B) chronically exposed to low doses of CSC;
- analysis of re-activated genes in 10 lung cancer cell lines and in chronically (CSC) treated BEAS-2B after demethylation (5' Azadeoxycytidine and trychostatin A);
- analysis of gene expression profiles of normal and tumor lung tissue

We are using DNA microarray U133 2.0 plus Affymetrix chips containing probes representing the entire human transcriptome.

RESULTS

Fig. 1. Chronically treated BEAS-2B cells with CSC form more and larger colonies in soft agar as compared to DMSO treated cells

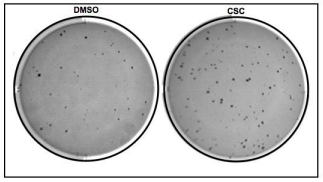


Fig. 2. Chronically treated BEAS-2B cells with CSC are clearly morphologically different as compared to control

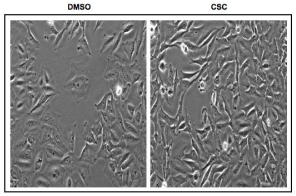


Table 1. Differentially expressed genes in BEAS-2B cells after 2 months of chronic treatment (CSC vs DMSO)

	Down in CSC treated	Up in CSC treated
Month 1	415	277
Month 2	104	162
Common down or up regulated genes after 1. and 2. months	56	74

Table 2. Downregulated genes in CSC treated cells

name	Fold change after 1 month	Fold change after 2 month	Symbol
Involved in cell adhesion and cell-cell interactions			
Occludin	8.8	4.6	OCLN
desmoplakin	3.9	2.9	DSP
cadherin 1, type 1, E-cadherin (epithelial)	17.0	30.5	CDH1
cadherin, EGF LAG seven-pass G-type receptor 1	4.4	2.5	CELSR1
plakophilin 3	2.5	2.6	PKP3
claudin 7	11.4	8.0	CLDN7
L1 cell adhesion molecule	3.4	7.3	L1CAM
kazrin	2.4	2.0	KIAA1026
lipocalin 2 (oncogene 24p3)	5.7	13.1	LCN2
G protein-coupled receptor 87	5.8	3.6	GPR87
Methylated in lung cancer			
TIMP metallopeptidase inhibitor 3	9.1	8.5	TIMP3
transcription factors			
GATA binding protein 3	5.9	6.3	GATA3
E74-like factor 3 (ets domain transcription factor, epithelial-specific)	12.1	9.8	ELF3
tripartite motif-containing 29	19.7	39.1	TRIM29
Other interesting candidate genes			
jagged 2	3.7	2.4	JAG2
BCL2-interacting killer (apoptosis-inducing)	9.0	4.5	BIK

Fig. 3. Unsupervised hierarchical clustering of BEAS-2B cells based on 25360 expressed genes

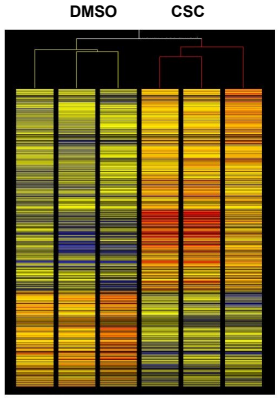


Fig. 4. Unsupervised hierarchical clustering of 10 lung cancer cell lines based on 23000 expressed genes (yellow branches -demethylated cell lines, red branches-control cell lines)

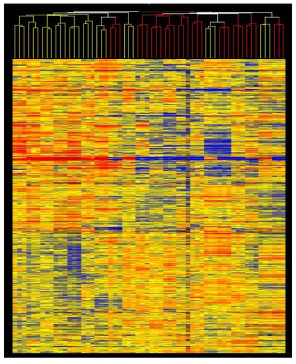


Fig. 5. Expression of BIK protein in lung tissue

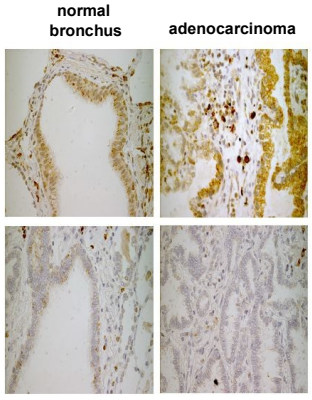
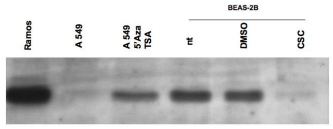


Fig. 6. BIK is re-activated after demethylation in lung cancer cell line A 549 and downregulated in CSC treated BEAS-2B cells



CONCLUSIONS

1. We have identified 650 differentially expressed genes after 1 month and 266 genes after two months of chronic treatment of BEAS-2B cells with CSC;
2. A group of down-regulated genes involved in cell adhesion and cell-cell contacts might be responsible for morphological transformation of CSC treated cells;
3. Proapoptotic gene BIK is downregulated upon CSC treatment of BEAS-2B cells and re-activated after demethylation in 3 lung cancer cell lines;
4. Protein expression of BIK is heterogeneous in lung tumors

OUTLOOK

1. Using LCM and DNA microarray methods on human lung tumor and normal epithelium from smokers, we expect to identify a group of genes that are common with those identified in the *in vitro* model;
2. Verification of selected candidates and development of functional assays