HOX GENES AND CELL ADHESION MOLECULES IN CANCERS

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Genomic changes are the most common feature of cancer cells. Usually multiple, they may involve all types of alterations, from single base mutations to whole chromosome losses or gains. Epigenetic modifications the HOX gene expression may also contribute to change the expression of a number of genes. In several cases, the role of oncogenes, tumor supressor and growth factor genes can be demonstrated. However, in the majority of solid tumors, there is no specific change, but recurrent patterns associated alterations, making it difficult to reconstruct their temporal or structural organization and assign them a precise role in the tumorigenesis process.

Edelman (1) proposes a model that links the HOX gene expression to morpho-regulatory molecules during development. All cells of the body assume shape, movement and proliferation by integrating three signals: homeodomain proteins, growth factors and adhesion molecules to follow cells (cell adhesion molecules, CAMs) and substrates (substrate adhesion molecules, SAMs). Cells presenting specific properties at given place and time will express a growth profile, an HOX gene pattern of expression and an adhesion molecule profile. According to this hypothesis, cells during cancer evolution undergo variations and generate changes at the level of each component of this model.

Homeobox genes are a family of genes containing a common 183-nucleotide sequence, the homeobox (2). The homeobox encodes for a 61 amino-acid domain, the homeodomain, which includes a helix-turn-helix motif responsible for the DNA-binding ability of homeobox-containing proteins. The homeobox was discovered in genes controlling *Drosophila* development and has been isolated in other evolutionary distant species, such as nematodes and vertebrates. The 38 mammalian class I homeobox genes (HOX genes) are clustered in restricted regions (100-120 kb) of the genome (HOX loci: HOXA, HOXB, HOXC and HOXD) on four chromosomes (7, 17, 12 and 2) respectively. Within each cluster, the position of the genes is highly conserved through species evolution suggesting that the physical order is essential for their expression and function.

HOX genes are expressed during embriogenesis in a tissue-specific fashion. After embryiogenesis, HOX genes continue their transcription according to a tissue specific pattern of expression. In normal tissues and cancer of several organs (kidney, colon, melanomas and lung) expression of the whole panel of 38 HOX genes has been studied (3,4,5). It was shown that the HOX gene expression pattern is specific for each organ. However, few data are presently available on HOX gene regulation in adult tissues or on the mechanisms responsibles for the changes observed in cancer cells. In tumoral tissue, the pattern of expression is modified and some alterations are characteristic of metastatic tumors. A link has been stablished between adhesion molecules and homeoproteins: the differential expression of a block of genes located at the 5' end of the HOXC locus allows melanoma clones to be classified into 2 major groups; the 2 patterns of HOX gene expression are inversely associated with 2 distinct surface phenotypes for integrins (VLA-2, VLA-5 and VLA-6) and the adhesion molecule ICAM-I (6). Furthermore, 2 adjacent HOX genes (HOX B8 and B9) can modulate N-CAM promoter activity (7). These results suggest that the expression of surface molecules involved in cell-cell and cell-matrix interactions may be related to the patterns of HOX gene expression.

In this work we have studied a possible correlation between HOX gene expression and cell-adhesion molecules (L-CAM and N-CAM) in SCLC-61 cell line obtained from a human *small cell lung carcinoma* previously characterized.

METHODS

The tumor samples used in our study (SCLC-61) were obtained from biopsy of human lymph node metastasis and xenografted into nude mice. Viable tumor cells were purified from specimen and maintained in RPMI supplemented with 10% of fetal calf serum. We stablished three goups of cultures: control (non-treated cells), 10^{-6} and 10^{-7} M Retinoic Acid-treated cells. We used this molecule because it has been shown that Retinoic Acid induce the expression of silent HOX-genes.

For the analysis of gene expression, total RNA was extracted from cells by two sucessive treatments with Trizol, treatment with chloroform and isopropyl alcohol. cDNA from the total extracted RNA was obtained by reverse transcription and gene

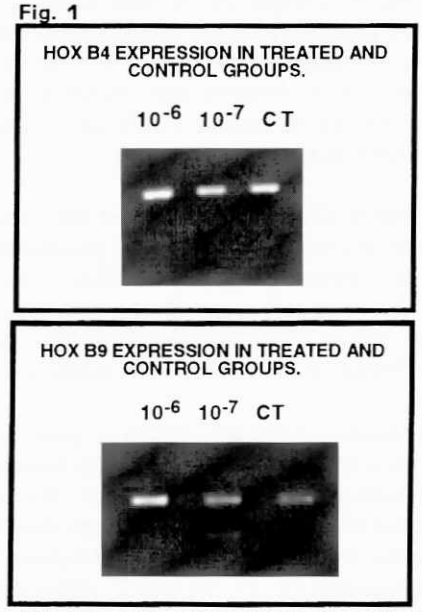
expression detected by specific polymerase chain reaction (denaturation at 94°C for 1 min, specific oligo annealing for 1 min and amplification at 72°C for 1 min during 40 PCR cycles).

For the analysis of expression of cell-adhesion molecules, monoclonal antibodies to L-CAM and N-CAM were used. Cells obtained from cultures were suspended at a concentration of 10^6 cells/ml in RPMI, incubated in 100 µl of 3 µg/ml of mouse anti-human L-CAM solution and 50 µl of 0.02mg/ml of mouse anti-human N-CAM antibody solution according to manufacturers instructions. After 30 minutes, cells were washed twice and incubated with anti mouse IgG-FITC antibody solution for 30 minutes in ice in the dark. Then, cells were washed twice and resuspended in 500 µl of culture medium. The analysis was achieved by flow cytometry, expressing the results as percentage of positive cells in each group of treatments. The levels of significance in the differences between mean values were computed with Student's *t*-test ($p<0.05$).

RESULTS

Expression of the HOXB gene cluster was analyzed by RT-PCR in SCLC-61 tumor cell line. Only HOXB4 and HOXB9 genes were actively expressed. Although HOXB4 was expressed at the same intensity in non-treated and Retinoic Acid treated cells, slight differences in the expression pattern was observed in HOXB9: SCLC-61 Retinoic Acid-treated cells expressed this gene more actively as compared with non-treated cells (Fig. 1).

Fig. 1



To investigate whether the patterns of HOX gene expression are associated with cell adhesion molecules in SCLC-61 tumor cells, we have analyzed the degree of expression of L-CAM and N-CAM molecules in the same groups of cells (control and treated cells) using immunodetection techniques and flow cytometric analysis. We have found a phenotypic heterogeneity regarding the expression of CAMs as shown in Table I: a) only 50% of cell population express N-CAM and there is no significant differences in N-CAM expression in Retinoic Acid treated cells as compared with control group; b) there is significant differences in L-CAM expression between control group (7%) and treated groups (35-40%).

Tabla I. Data express percentage of SCLC-61 positive labelling of L-CAM /N-CAM in control (CT) and Retinoic Acid treated groups (10^{-6} M and 10^{-7} M).

	L-CAM	N-CAM
	$\overline{X} \pm SD$	$\overline{X} \pm SD$
CT	7.33 ± 4.317	50.56 ± 11.96
10^{-6} M	36.48 ± 14.70 *	58.14 ± 3.107
10^{-7} M	39.15 ± 15.24 *	50.69 ± 9.306

* Statistically significance difference compared with the control group ($p< 0.05$).

CONCLUSIONS

Our preliminary results show an heterogeneity in the HOXB gene expression in SCLC-61 tumor cells. There is a correlation between HOXB9 and L-CAM expressions in SCLC-61 tumor cell line when treated with Retinoic Acid.

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