IDENTIFICATION OF GENETIC FACTORS AFFECTING RESISTANCE AND SUSCEPTIBILITY TO CHEMICAL CARCINOGENESIS AND THE DEGREE OF ACUTE INFLAMMATORY RESPONSE, BY USING A MODEL OF GENETICALLY SELECTED MOUSE LINES

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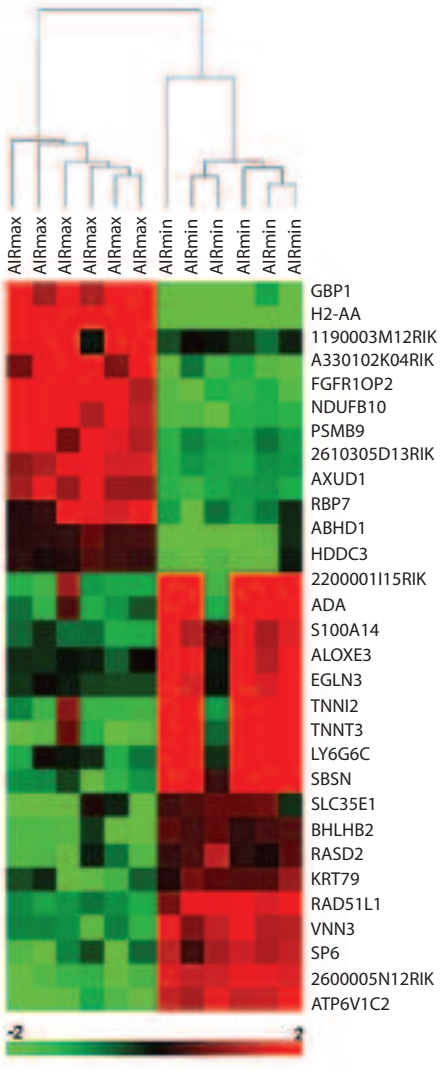

Genes differentially expressed in the normal lung tissue in AIRmax and AIRmin mice

Two non isogenic mouse lines phenotypically selected according to the maximal (AIRmax) or minimum (AIRmin) acute inflammatory response (AIR) show differential susceptibility to chemical carcinogenesis in the lungs and skin, AIRmax being resistant and AIRmin susceptible. As a consequence of the bi-directional selective process, the alleles of the genes relevant to the "maximal" and "minimum" inflammatory response phenotypes, specifically segregated in AIRmax and AIRmin lines, respectively, leading to homozygosity in AIR modifier *loci* but maintaining the background genetic heterogeneity in each line. The analysis of the heritability of the character during the bi-directional selective process, revealed the involvement of about 11 Quantitative Trait Loci (QTL) with additive effect, accounting for the phenotypic divergence between AIRmax and AIRmin mice.

Our project aims to evaluate whether functional polymorphisms in genes, which could explain the variations in the inflammatory response in the two selected mouse lines, harbor an altered risk to the development of neoplasic diseases. In order to identify these genes, complementary strategies will be applied:

1) the search of polymorphism in candidate genes indicated from precise phenotypes relevant to inflammatory response regulation and to chemical carcinogenesis predisposition which differentiate the two lines.

2) genome-wide screening using polymorphic genetic markers between the two lines for the identification of the fine chromosomal location of candidate genes.

3) comparative transcriptome and proteome analysis of the inflammatory exudates, bone marrow and target tissues preparations from AIRmax and AIRmin mice, before and after the stimulus with inflammatory or carcinogenic agents.

The expression profiling data will be then combined to the marker-based QTL mapping. This analysis might unravel gene-gene interactions that affect the inflammatory phenotypes and cancer resistance or susceptibility of the two lines. The identification of functional polymorphisms in genes relevant to the variations in inflammatory response could be useful for the assessment of genetic cancer risk factors and eventually to indicate their human counterparts.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

The biological significance of this model was evidenced by the huge differences found between the two mouse lines in bone marrow granulopoietic activity and in the natural resistance to various infections and autoimmune diseases. Furthermore, AIRmax mice are considerably more resistant than AIRmin to skin and lung tumorigenesis, independent of the chemical nature or the route of administration of the carcinogen, indicating a broad effect of germ line genes segregated in these mice in innate resistance to tumorigenesis. In fact, "high" AIR co-segregated with resistance and "low" AIR with susceptibility to skin and lung tumorigenesis in two independent co-segregation assays carried out in F2 (AIRmax x AIRmin) intercross populations, demonstrating that at least part of the genes which determine the degree of acute inflammation are, or are closely, linked to cancer modifier genes.

In this context we could demonstrate the segregation of resistance or susceptibility related alleles in a 452-kb region, containing 5 genes in linkage located at the distal region of chromosome 6 which regulate lung tumorigenesis (*Pas1* locus). We identified also polymorphism at aryl hydrocarbon receptor coding gene in chromosome 12 which is related to variations in the receptor affinity to polycyclic aromatic hydrocarbon carcinogens resulting in resistance (AIRmax) and in susceptibility (AIRmin) to skin and lung tumorigenesis by these compounds. Linkage analysis showed also, originally, the functional involvement of these gene loci in inflammatory response regulation.

Due to this grant we established collaboration with Dr. Tommaso Dragani at the Polygenic Inheritance Unit at Istituto dei Tumori in Milan, Italy, for the introduction in our laboratory at Institute Butantan of techniques for large scale genetic analysis. Micro arrays are being used for gene expression analysis in target organs and the results are validated by real-time PCR and confronted to the proteome profiles obtained in the same organs. Results in the bone marrow, in the lungs and skin of normal or carcinogen treated AIRmax and AIRmin mice revealed line-specific patterns of gene expression associated to inflammatory response.

Micro arrays containing thousands of oligonucleotides for the analysis of single nucleotide polymorphisms (SNP) are also being used for whole genomic screening of the two mouse lines in pedigree studies using parental (AIRmax and AIRmin), F1 hybrids and F2 intercross populations.

MAIN PUBLICATIONS

Ribeiro OG, Maria DA, Adriouch S, Pechberty S, Cabrera WH, Morisset J, Ibanez OM and Seman M. 2003. Convergent alteration of granulopoiesis, chemotactic activity, and neutrophil apoptosis during mouse selection for high acute inflammatory response. *Journal of Leukocyte Biology*. **74(4)**:497-506, 2003.

Maria DA, Manenti G, Galbiati F, et al. 2003. Pulmonary adenoma susceptibility (*Pas1*) locus affects inflammatory response. *Oncogene.* **22**:426-432.

de Souza CM, Morel L, Cabrera WH, et al. 2004. Quantitative trait loci in Chromosomes 3, 8, and 9 regulate antibody production against *Salmonella flagellar* antigens in the mouse. *Mammalian Genome.* **8**:630-636.

Zaffaroni D, Spinola M, Galvan A, Falvella FS, et al. 2005. *Met* proto-oncogene juxtamembrane rare variations in mouse and humans: differential effects of Arg and Cys alleles on mouse lung tumorigenesis. *Oncogene.* **24**:1084-1090.

Ribeiro OG, Cabrera WH, Maria DA, et al. 2005. Genetic selection for high acute inflammatory response confers resistance to lung carcinogenesis in the mouse. *Experimental Lung Research*. **31(1)**: 105-116.

Santos Junior RR, Sartori A, Ribeiro OG, et al. 2005. Immunomodulation and protection induced by DNA-hsp65 vaccination in an animal model of arthritis. *Human Gene Therapy*. USA. **16**: 1338-1345.

LaPrete AC, Maria DA, Rodrigues DG, et al. 2006. Evaluation in melanoma bearing mice of an etoposide derivative associated to a cholesterol-rich nanoemulsion. *Journal of Pharmacy and Pharmacology*. **58(6)**: 801-808.

Di Pace RF, Massa S, Ribeiro OG, Cabrera WHK, et al. 2006. Inverse genetic predisposition to colon vs. lung carcinogenesis in mouse lines selected based on acute inflammatory responsiveness. *Carcinogenesis*. **27 (8)**:1517-1525.

Peters LC, Jensen JR, Borrego A, Cabrera WHK, et al. 2007. *Slc*11A1 (formerly *Nramp*1) gene modulates both acute inflammatory reactions and pristane-induced arthritis in mice. *Genes and Immunity.* **8**: 51-56.

10- De Franco M, Carneiro OS, Peters LC, et al. 2007. *Slc*11A1 (*Nramp*1) alleles interact with acute inflammation loci to modulate wound-healing traits in mice. *Mammalian Genome*. **18(4)**:263-9.

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