An integrated approach of immunogenomics and bioinformatics to identify new Tumor Associates Antigens (TAA) for mammary cancer immuno-prevention

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Motivation

Neoplastic transformation is a multistep process in which gene products of specific regulatory pathways are involved at each stage. Identification of these over-expressed or mutated gene products provides an unprecedented opportunity to address the immune system against defined antigens and eliminate transformed cells. Mice transgenic for these oncogenes (e.g. HER-2/neu, a prototype of deregulated oncogenic protein kinase membrane receptors) are ideal experimental models for assessing the potential of active immunization. The demonstration that vaccines can cure HER-2/neu transplantable tumors, prevent their onset and delay the progression of preneoplastic lesions in mice at risk suggests that efficient immunological inhibition of HER-2/neu carcinogenesis can be achieved by specific vaccination. Since an important issue of mammary cancer immunoprevention is the definition of a set target genes other that HER-2/neu in order to make broader the coverage of the efficacy of the vaccination we decided to use part of the data previously described by us (Quaglino et al. J. Clical. Invest. 2004; Astolfi et al. Cancer Immunol., Immunot.2005) to identify a set of new putative targets to be used as TAA.

Methods

Microarray data: CEL files were derived from two previously published works (Quaglino et al. 2004, Astolfi et al. 2005). Data analysis: microarray data analysis was mainly performed using Bioconductor libraries. Probe sets intensities were calculated using the rma algorithm, normalized by the quantile method and.filtered to have IQR>0.5 within experiments. A statistical linear model was used to measure the effects of mice age on gene expression and to identify probe sets linearly correlated to the increment of the tumoral mass. Gene ontology class enrichment was evaluated using the Bioconductor GOstats package and the annotation library mgu74av2 1.6.5. Expression level of the selected probe sets within mouse tissues was evaluated using the GNF mouse atlas. Probe sets expression in tumoral samples was evaluated using transcription profiling studies available at GEO database (www.ncbi.nih.gov/GEO).

Results

Seven prototypic situations were evaluated: (a) wk6nt (6 weeks old mice) glands displaying atypical hyperplasia (b) wk10nt glands displaying multifocal preneoplastic lesions (c) wk15nt glands with alveolar groups of neoplastic cells (d) wk19nt glands with alveolar groups of neoplastic cells (e) wk22nt glands with invasive palpable carcinomas (f) wk26nt glands with large palpable lobular carcinomas in all 10 mammary glands, and (g) glands from wk2prg mice displaying marked pregnancy-related hyperplasia. As previously shown (Astolfi et al. 2005) we observed that the 6-26 week neuT mammary glands transcriptional profilings can be analyzed

together since they are well correlated to each other. Furthermore, we have shown (Astolfi et al. 2005) that the animals can be grouped as function of the animal age (i.e. in function of the increment of tumoral mass in the mammary gland). We used this clear correlation between the increment of the tumoral mass and the transcription profiles to identify genes which show expressions positively linearly correlated the increment of the tumoral mass. Using a simple linear model we selected 205 probe sets characterized by a r2 greater then 0.7 and a positive slope (205). The selected probe sets were linked to 204 gene IDs and one to an unmapped EST. Since an important issue for the identification of new vaccination target is its limited expression in normal tissue we selected from the putative TAA, having an expression linearly linked to the neuT mice tumoral mass increase, present in the GNF mouse expression atlas (Su et al. Proc. Natl. Acad. Sci USA 2004), those characterized by an overall low expression in normal tissues (log2(intensity signal)<7, IQR within GNF tissues <0.5). This selection produced 12 genes. These genes are actually under experimental validation for their ability to inhibit the tumoral progression i BALB-neuT mice.

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