IDENTIFICATION OF ANTIGENS INVOLVED IN IMMUNE RESPONSE AGAINST BREAST CANCER IN MICE USING PHAGE DISPLAY cDNA EXPRESSION LIBRARY

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Introduction. Breast cancer is the most common cancer in women worldwide. Similar to other middle-income countries, cancer mortality has increased in Mexico, accounting for 14% of cancer-related deaths for women.(1)
Efforts to find an effective treatment for cancer are held back by the lack of more extensive knowledge on the interactions between the immune system and cancer cells. Thus we pretend to identify new peptide/epitopes which could be recognized by lymphocytes during the development of breast cancer in a murine model. This way the new epitopes could be considered as possible components of future vaccines or as candidates with immunodiagnostic potential.

Methods. We used a murine breast cancer cDNA library (NCI CGAP Lu29, Invitrogen, Life Technologies), which was expressed on the surface of M13 phage fused to the main coat protein PVIII. The resulting composition of the library is about $3x10^6$ variant peptides. Our selection strategy is based on cell proliferation assay using spleen cells from mice, which have developed tumors after an implant of 4T1 cells. Fourteen days after implant the spleen cells are obtained and stained by intracellular incorporation of CFSE (CellTrace™ CFSE Cell Proliferation Kit, Molecular Probes. Invitrogen). Then they are cultured with phage suspension for 72 hours after that we analyze the proliferation rate by flow cytometry and using "Cyflogic 1.2.1" sofware.
This way we do a massive selection in a format of 96 well flat bottom plate, placing a collection of recombinant phages per well. We perform rounds of selection choosing the clones with higher proliferation rate, and at the end we obtain individual clones.

Results. After 3 rounds of selection we obtained individual clones. The results for proliferation assay with individual clones are shown in Figure 1. We chose the three clones with the highest proliferation rate. Then they were purified, amplified and sequenced.
The sequence information for these clones is summarized on table 1 .

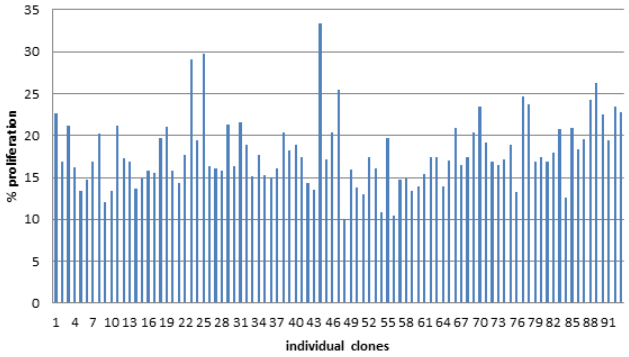

Fig.1 The graph shows the percentages of proliferation of individual clones

Clone 1	Mus musculus inositol monophosphatase domain containing 1 (Impad1), short fragment	not reported for cancer
Clone 2	Mus musculus inositol monophosphatase domain containing 1 (Impad1) (long fragment)	not reported for cancer
Clone 3	**Impad1+ Mus musculus lectin, mannose-binding 2 (Lman2),**	**Reported for cancer (2)**

Table 1: information reported in literature and databases

Clones 1 and 2 correspond to different fragments from chromosome 3 and also correspond to an exon fragment for Mus musculus inositol monophosphatase Domain Containing 1 (Impad1).
Clone 3 corresponds to a region from chromosome 13 with a fragment of the conserved domain of lectins, linked to fragment of clone 1

Conclusions.
As we have the phage display technique used in this work is efficient and inexpensive to select clones in vitro antigenic, we will test *in vivo* the immunogenicity of the selected clones in a mouse model of breast cancer.

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2. Klein-Scory et al. BMC Cancer 2010, 10:70