SELECTION OF MUTATED EPITOPE VARIANTS OF Erbb2/HER2 EPITOPES BY PHAGE DISPLAY MEDIATED SCREENING APPROACH

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Introduction. Immunological approaches for cancer research aim to promote and to improve the immune response against tumor cells[1].ErbB2/HER2 over expression has been reported in about 20-30 % of breast cancers[2], and it represents a self tumor antigen weakly immunogenic and subjected to immune tolerance. A possibility to enhance the immunogenicity of this antigen could result in a better anti-tumor response[3]. Therefore there is needed to expand the spectrum of epitopes targeted by immune response and of course the corresponding identification methods have to be improved. In that sense our experimental work applies the phage display in order to study an alternative of enhancing the immunogenicity of an ErbB2/HER 2 peptide/epitope related to breast cancer.

Methods. Our strategy consists on selection and identification of peptide/epitopes by cell proliferation assays.
We work with a variable epitope library (VEL), which was constructed by mutating residues for T Cell Receptor (TCR) contact, on an immunodominant epitope (TYLPANASL) of the receptor "Erbb2" related to breast cancer. The VEL contains up to 8,000 different peptides of 9 aminoacids expressed on *M13* phage surface. The peptide is fused with the major phage coat protein (cpVIII) by cloning in *pG8SAET* phagemid vector, which is kept in *E.coli* TG1 cells. By this way it is possible to rescue, amplify and obtain recombinant phages using *M13K07* helper phage[4] (Fig 1).

Proliferation assays are performed with spleen cells from *BALB/c* mouse on day 18th after tumor development by subcutaneous implant of 4T1 cells. These spleen cells are labeled with carboxifluorescein (CellTrace™ CFSE Cell Proliferation Kit, Molecular Probes. Invitrogen), then they are cultured in a 96-well flat-bottom plate (2×10^5 cells/well) with 3×10^9 phage particles. Every well has only one type of recombinant phage. The culture is kept for 72 h, after that we determine the proliferation percentage by flow citometry (FACS) using a FACScan and "Cyflogic 1.2.1" software.

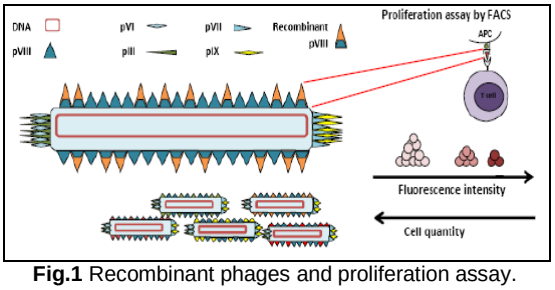

Fig.1 Recombinant phages and proliferation assay. Peptides fused with major phage coat protein (cpVIII) and principle of the proliferation assay.

Results. Figure 2 presents the result for a proliferation assay by FACS where we tested 89 individual peptides derived from wild type epitope (TYLPANASL) displayed on phage surface. Some peptides elicited a higher proliferation than wild type epitope (red bar).

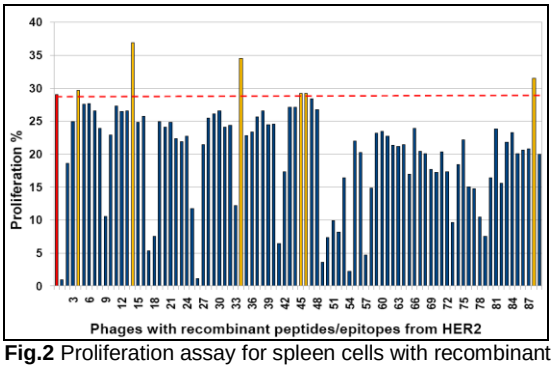

Fig.2 Proliferation assay for spleen cells with recombinant phages as antigens.

Conclusions. As we can see, these results suggest that subtle changes on the sequence of a peptide/epitope can improve its immunogenicity *in vitro*, which could be translated into a better immune response *in vivo*. On the other hand this method can be used as a massive searching tool to identify new immunogenic peptides for the development of better therapeutic alternatives.

References.

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