ANTITUMOR EFFECT OF A REPLICATION-DEFICIENT ADENOVIRUS EXPRESSING MODIFIED ANTIGENS E6 AND E7 FROM HPV-16 FUSED TO HUMAN CALRETICULIN

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Introduction. The causal association between human papilloma virus (HPV) infection and cervical cancer has been firmly established. Vaccines targeting the oncogenic proteins, E6 and E7 of HPV-16 are the focus of current vaccine development. Previous studies have shown that calreticulin (CRT), an endoplasmic reticulum (RE)-residing protein, enhances the MHC class I presentation of linked protein and may serve as an effective vaccination strategy for antigen-specific cancer treatment (1). Genetically modified adenoviruses (Ads) make attractive vectors for the delivery of exogenous DNA to mammalian cells for basic science and gene therapy applications. Ad vector production consists of cloning a transgene into an infectious plasmid by in vivo recombination in bacteria, rescuing and propagating the vector in complementing cells, and purifying the vector. Ads rarely integrate into the host genome and are relatively safe (2).
In this work we evaluated a novel fusión protein consisting in Human Calreticulin fused to modified versions of E6 and E7 antigens. This modifications consists in mutations that eliminates the oncogenic potential of these proteins and makes them more unstable to improve antigen presentation.

Methods. Gene construction consisted in the human Calreticulin fused to mutant E6 gene and mutant E7 gene. GeneArt Company performed gene synthesis and codon optimization. To construct the Adenovirus vector we used Ad-Easy System (Quantum Biotech). This is based on cloning the gene of interest in a shuttle plasmid for subsequent recombination with the adenoviral genome. This includes deletions on genes necessary for virus replication. Once obtaining the complete vector it is transfected into HEK-293 cell line that expresses genes needed for viral production. In order to confirm protein expression and cellular localization we analyzed cell cultures infected with the adenovirus by immunofluorescence using an anti-calnexin ER marker antibody. Antitumoral assays were performed in female C57BL/6 mice. Briefly, in prophylactic assay mice were immunized with adenoviral treatments and one week later they were injected with Tc1 cell line that expresess E6 and E7. In therapeutic assays mice were injected with Tc1 cells and then immunized with adenoviral treatments on days 2, 5 and 15.

Results. In Fig. 1A we observe the constructions used in this work, in last line is the novel fusion gene. In Fig. 1B we can observe that E7 antigen (red) is co-localized with an ER marker (green) in all constructs. In Fig 2A we observe the tumor growth is diminished in all the groups except the one that received negative control, Ad LacZ. More important is the therapeutic activity of this adenovirus since the group treated with Ad CRTH/E6/E7 showed the smallest tumors compared to other groups. We are going to increase adenoviral doses in order to improve antitumor effect.

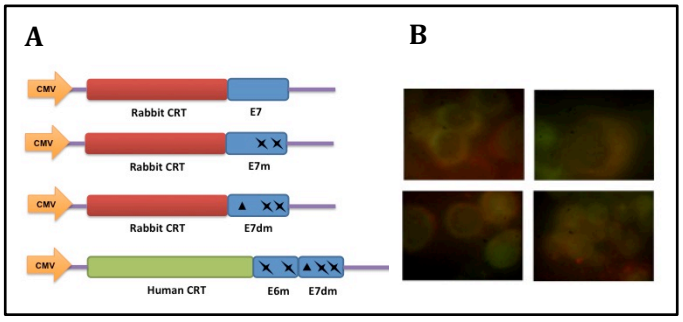

Fig.1. Adenoviral construction and protein localization. A) Schematic design of different adenovirus constructions used in this work. B) Protein localization in cells infected with different adenovirus (red signal= E7, green signal= endoplasmic reticulum).

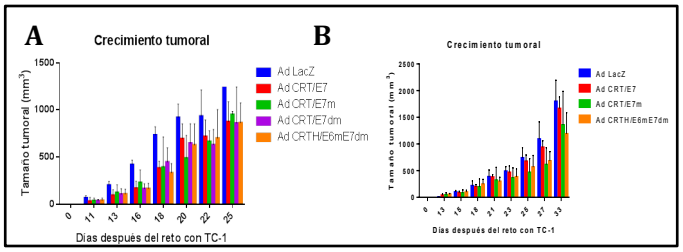

Fig. 2. Antitumoral effect. A) Tumor growth in prophylactic Assay. B) Tumor growth in therapeutic Assay.

Conclusions.
We constructed a replication-deficient adenovirus that expresses the Human Calreticulin fused to E6 and E7 antigens. This fusion protein is detected in endoplasmic reticulum. This adenovirus is able to diminish tumor growth in a murine tumor model. This adenovirus may be used in a future as an alternative in cancer therapy.

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