CONSTRUCTION AND EVALUATION OF MOLECULAR VECTORS THAT EXPRESS IFN-γ IN HUMAN TUMOROGENIC CELLS

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Introduction. Interferon-gamma (IFN-γ) is a 166 amino-acid protein (501bp) that has demonstrated an inmunomodulatory activity[1] and is mostly secreted by the activated $CD4^+$, $CD8^+$ T cells and NK cells[2]. Its importance as a biopharmaceutical compound is related with different functions on the body. The antitumoral effects can be mediated directly through inhibition of tumor cell growth by recruitment and activation of cells which are involved in innate as well as adaptive antitumor immune response[3].
In such a way, the purpose of this work was to develop different types of plasmids that contain the human IFN-γ and to evaluate its expression on some tumoral human cell lines to test its use as a genetic therapy tool.

Methods. Human IFN-γ gene was purchased from GeneScript. IFN-γ cDNA was amplified by polymerase chain reaction (PCR) and the amplified fragment was cloned on the vectors pDream2.1 and pShuttle2 (to obtain an Ad5 1st generation).
DH5α *E.coli* chemical-competent strain was transformed with such vectors using thermal shock. Such molecular constructions were transfected into different cell lines (A549, C33A, HEK293) and the expression of IFN-γ was evaluated by Western Blot.

Results. IFN-γ cDNA was amplified by PCR and was cloned into pDream2.1 and pShuttle2 vectors. The sequence data revealed that the sequenced fragment was the same as human IFN-γ cDNA (BC070256) in the GenBank. pDreamIFN-γ (7.6kb) DNA was isolated and its quality and quantity was evaluated in order to establish the transfection conditions into different cell lines. Transfection efficiency was measured by flow cytometry; the percentage of transfection in HEK293 line was around 30%, and a decrease on such a value was observed in A549 and C33A tumor cell lines.
On the other hand, pShuttleIFN-γ was made using the kit Adeno-X expression system 1, by digestion and ligation *in vitro* of the PCR fragment IFN-γ (527bp) and mammalian expression vector pShuttle2 (4000bp).

The pShuttleIFN-γ isolated DNA was digested by the restriction endonuclease NheI to verify the size of 4527bp (Figure 1). Such a vector was used in the construction of the recombinant adenoviral vector pAdenoIFN-γ, to produce adenoviral particles that were able to infect different mammalian cell lines.

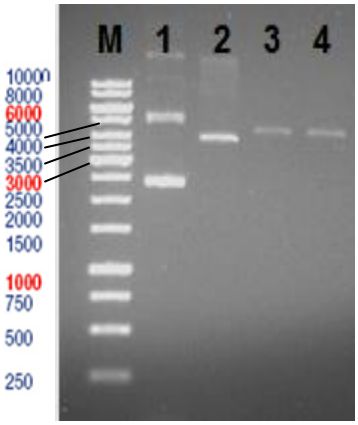

Fig.1 Restricted endonuclease enzymes analysis of pShuttleIFN-γ. Lane M: 1kb DNA ladder; lane 1: pShuttle2 undigested; lane 2: pShuttle2 digested by NheI; lane 3 and 4: pShuttleIFN-γ digested by NheI.

Conclusions. Different molecular constructions of vectors that express IFN-γ were performed and their efficacy on the expression of such a molecule was measured on different tumor cells lines. Results showed that oncogenic cells are less susceptible for transfection. Such results are helpful to establish new studies for measuring the immunomodulatory response against oncogenic cell lines.

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