MOLECULAR CONSTRUCTIONS FOR NOD 30-3 EXPRESSION: A POTENTIAL GENE FOR THE INDUCTION OF PROGRAMMED CELL DEATH

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Introduction. Currently, treatments for cancer disease are based on nonspecific methods, so the search for more efficient therapies is always on the table[1,2]. Gene therapy has been considered as an emerging technology for correcting defective genes that are responsible of the disease progress[2]. One of the gene therapy strategies for cancer treatment is the induction of programmed cell death (PCD) by proteins that carry out such a function[3]. Nodulin 30-3 (Nod30-3) is a scarcely studied plant protein that has demonstrated a potential use as PCD induction in preliminary studies. Therefore, this work focuses on developing expression vectors for animal cells: a plasmid and an adenovirus first generation that express Nod30-3 transgene as a potential transgene for gene therapy against cancer.

Methods. Nod30-3 gene was amplified by the polymerase chain reaction technique; thereby the amplified fragment was cloned into the pVAX1 expression plasmid and into the pAd/CMV/V5DEST adenoviral plasmid. Competent *E. coli* DH5α strain was transformed with each plasmid and the selection of positive clones was made by PCR, digestion pattern and sequencing. The pAd/CMV/V5DEST/Nod30-3 plasmid was transfected into 293A cells to generate first generation recombinant adenovirus which was amplified by subsequent subcultures. Nod30-3 was measured by qPCR. Then, different cell lines: HEK293, A549, MCF7 and C33A were transfected or infected with the resulting plasmid and recombinant first generation Ad5, respectively. Such experiments were performed into 24-well dish plates using different concentrations of Lipofectamine 2000 (Invitrogen) in the case of the pVAX1/Nod30-3 plasmid and, a MOI of 5 for the Ad5. PCD was measured by flow cytometry, epifluorescence, DNA specific degradation and caspase-3 activation as an apoptotic marker in the cultures.

Results. Two molecular constructions were generated to test the expression efficacy of Nod30-3. pVAX1/Nod30-3 plasmid (3657 bp) was generated by cloning into *E. coli* DH5α cells. Figure 1A, shows the PCR amplification. On the other hand, an adenoviral plasmid carrying the Nod30-3 gene was engineered and constructed as a pAd/CMV/V5DEST/Nod30-3 of 36657 bp with the aim to obtain adenoviral particles (Figure 1B). After amplification, Nod30-3 was measured by qPCR and by the induction of PCD into different cell lines. Results showed that the cell viability percentage in A549 and C33A transfected with pVAX1/Nod30-3 was significantly lower than in the case of HEK293 cells. In addition, a higher expression in the case of Ad5 vector was obtained compared to the plasmid transfection.

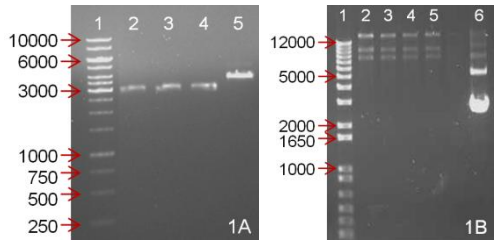

Fig.1 Molecular constructions. A) Molecular structure of pVAX1/Nod30-3 plasmid, lanes 2-4 pVAX1 plasmid digestion in 3000 bp and lane 5, pVAX1/Nod30-3 plasmid digestion in 3657 bp. B) Adenoviral plasmid digestion pAd/CMV/V5DEST/Nod30-3 with NdeI, enzyme that makes three cuts (17800, 9600 and 6900 bp) in lanes 2-5; lane 6, digestion negative control.

Conclusions. In general we observed that the Ad5 construction expresses substantially more protein than a conventional expression plasmid. In both cases protein expression reduces the viability percentage, probably indicating that Nod30-3 plays a role in the induction of PCD.

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