



# CONSTRUCTION AND CHARACTERIZATION OF A REPLICATION-DEFICIENT ADENOVIRUS EXPRESSING IP10-LYMPHOTACTIN FUSOKINE

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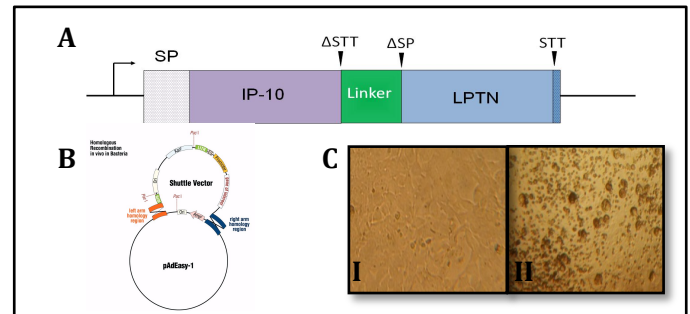
**Key words:** adenovirus, IP10, Lymphotactin

**Introduction.** Fusion proteins have emerged as an alternative to improve the expression of individual proteins and facilitate its administration in various therapies. Among some fusion proteins can be found fusokines that is a term recently coined to describe fusion proteins formed by two cytokines (1). The chemokine gamma interferon inducible protein 10 (IP10) has been described as a modulator of other antitumor cytokines. This may be due to its vascular regulating activity and acts as an antiangiostatic thereby inhibiting tumor growth. Lymphotactin (LPTN) is the only family member with chemokine receptor C. This chemokine is secreted by activated T lymphocytes and chemo-attracting CD4 + and CD8 + and NK cells. Recently it has proven a synergistic antitumor activity from these two chemokines that resulted in regression of established tumors in a mouse model (2). In this work we constructed a replication-deficient adenovirus that expresses a fusokine formed by IP10 and Lymphotactin.

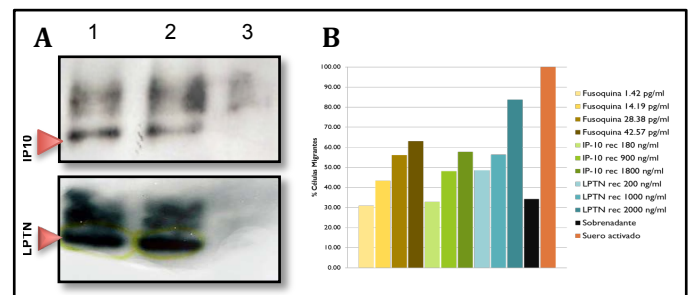
**Methods.** Fusokine gene construction included the open reading frame for both cytokines. IP10 signal peptide (SP) was removed as well as the stop codon ( $\Delta$ STT) and the signal peptide sequence of the second cytokine ( $\Delta$ SP). Furthermore a linker sequence was placed between the two cytokines. GeneArt Company performed gene synthesis and codon optimization. To construct the vector system we used Ad-Easy System (Quantum Biotech). This is based on the cloning of the gene of interest in a plasmid carrier for subsequent recombination with a larger plasmid that contains the adenoviral genome with deletions on genes necessary for virus replication. Once obtaining the complete vector it was transfected into HEK-293 cells that expresses genes needed for viral production. From the culture medium of infected cells we performed a Western blot using antibodies specific for H-IP-10 and H-LPTN to effectively demonstrate that cells infected with this adenovirus are able to secrete IP10/LPTN fusokine. Lastly we assayed the biological activity of this recombinant fusokine in a chemotaxis assay with human peripheral lymphocytes using commercial recombinant chemokines as positive controls.

**Results.** In Fig. 1A we observe the schematic design of our gene of interest with the elements described in Methods. In Fig. 1B is a representation of the two plasmids used for integration of our gene of interest in the adenoviral genome through homology recombination. In Fig 1C we observe the morphology changes in HEK293 cell line (cytopathic effect) after transfection with adenoviral genome; this is indicative of viral particle

production. In Fig 2A we demonstrate the secretion of fusokine by cells infected with the adenovirus resulting in a band of approx. 22 KDa detected with antibodies against IP10 and LPTN. In Fig 2B we observe that the recombinant fusokine is biologically active.



**Fig.1. Adenoviral construction.** A) Schematic design of IP10-LPTN fusokine expression gene. B) AdEasy system used for adenoviral construction. C) Viral particle production in HEK293 cells transfected (II) and non-transfected (I) with adenoviral genome.



**Fig. 2. Expression analysis of IP10-LPTN fusokine and its biological activity.** Supernatant from cell cultures infected with adenovirus were analyzed by Western blot using antibodies anti IP10 and LPTN, Lines 1,2: two different adenoviral clones, 3 AdLacZ as negative control. B) Chemotaxis assay.

**Conclusions.** We constructed a replication-deficient adenoviral vector that directs the expression of a fusokine formed by IP10 and Lymphotactin. The recombinant fusokine is biologically active.

This adenovirus may be used in a future as an alternative in cancer therapy.

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## References.

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