J Segura[1,3], C Fillat[2], D Andreu[3], J Llop[4], O Millan[4], B G de la Torre[3], Z Nikolovski[1], V Gomez[4], N Andreu[2], A Pinyot[1], R Castelo[3], JD Gispert[4], JA Pascual[1,3]

Non invasive molecular imaging of gene expression potentially useful for doping control: Pilot study in cells and animals

Institut Municipal d'Investigació Mèdica IMIM-Hospital del Mar[1], Barcelona, Spain
Centre de Regulació Genòmica[2], Barcelona, Spain
Universitat Pompeu Fabra[3], Barcelona, Spain
Institut d'Alta Tecnologia[4], Barcelona, Spain

Abstract

Gene therapy is anticipated as an important medical development. Essential to its effectiveness is the appropriate activity (protein expression) in the expected target cells. A non invasive diagnostic procedure of successful gene expression will be of paramount importance to validate its use or its misuse (e.g. sports gene doping). Externally detectable labelled oligonucleotides hybridizing with the messenger RNA generated by the gene transferred has been proposed as a possibility to monitor successful gene therapy. Erythropoietin gene (*Epo*) has been selected for a pilot study on Erythropoietin protein (EPO) expression in mice muscle.

Oligonucleotides of peptide nucleic acid (PNA) type covalently linked to cell penetrating peptides (Tat from HIV virus) with antisense coupling properties towards mice genome unique *Epo*-mRNA sequences were synthesized by solid phase chemistry. They were labelled with fluorescence and radioactive tags to verify penetration and longer half-life properties in *Epo* gene transfected C2C12 muscle mice cells as compared with corresponding wild type cells. Down regulation of newly expressed EPO in such cells additionally confirmed the penetration and hybridizing properties of the selected labelled oligonucleotide.

^{123}I labelled Tat-PNAs were intravenously injected into the mice right lower extremity having been transferred with the *Epo* gene. Preferential accumulation of radioactivity in the transferred limb as compared to the contralateral limb was ascertained, especially for ^{123}I-Tat-

CTA CGT AGA CCA CT (labelled Tat-PNA 1). The overall study affords experimental data to support the potential use of external non-invasive image detection to monitor gene therapy. The extension of the approach to more sensitive methods for whole body external detection such as positron emission tomography (PET) appears feasible.

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