PRODUCTION OF RECOMBINANT GDNF IN MAMMALIAN CELLS FOR ITS ENCAPSULATION IN DRUG DELIVERY SYSTEMS

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Parkinson´s disease (PD) is a slowly progressive neurodegenerative disease that affects prominently the dopamine (DA) neurons of the substantia nigra. Current therapeutic strategies for PD remain symptomatic, and neither of them are neuroprotective. Data obtained from post mortem analysis of brains from PD patients suggest that a significant portion of the nigral DA neurons still remain alive, although in an atrophic and compromised stage (1,2). The progressive nature of the degenerative process provides a basis for the development of neuroprotective therapies aimed at blocking or slowing down the degenerative process, and stimulating recovery and regeneration of the remaining DA neurons.

Glial cell line-derived neurotrophic factor (GDNF) is one of the most potent neurotrophic factors for mesencephalic DA neurons identified so far and it has been shown to protect the DA neurons against toxic or mechanical insults and stimulate axonal sprouting and regrowth from lesioned nigral DA neurons. The therapeutic potential of GDNF for PD is likely to depend on sustained delivery of the appropriate amount to the target areas (3,4).

The aim of this work was to develop a procedure for the expression and purification of bioactive GDNF in view of its microencapsulation for the treatment of PD (see abstract by Garbayo *et al*).

The cDNA of the GDNF gene was cloned in the expression vector pDEST26 (Invitrogen) using the Gateway® Technology. Several eukaryotic mammalian cell lines (BHK, COS-7, and 293) were stably transfected with the construction. In those clones in which the presence of the mRNA of the GDNF was confirmed by PCR studies (Figure 1A), the expression of the recombinant protein in the serum free medium was analyzed by Western Blot studies (Figure 1B). The highest GDNF-producing clone, obtained from the BHK cell line, was cultured in roller bottles alternating the presence or the absence of FBS in the medium every 24 hours, being collected only the serum free medium for the production of the recombinant GDNF. Each cycle protein expression was analyzed by SDS-PAGE (Figure 2A) and by Western Blot (Figure 2B). The secreted protein was purified by several chromatography steps as follows:

1. A cationic ion exchange chromatography on XK 16/20 column packed with SP Sepharose High Performance (Amersham).
2. A gel filtration chromatography on Superdex 200 HR 10/30 (Amersham).
3. A second cationic ion exchange chromatography on Mono S HR 5/5 (Amersham).

After each step of the purification procedure the fractions obtained were analyzed by SDS-PAGE, Western Blot and silver staining analysis (Figure 3).

A neuronal differentiation PC-12 cell-based bioassay was also developed to confirm the biological activity of the purified recombinant protein (Figure 5). Previously, the presence of GFRα1 and RET, receptors required for GDNF activity were confirmed by PCR (Figure 4).

In conclusion, recombinant GDNF was produced in an eukaryotic mammalian cell line-based system. The protein purification procedure developed, allowed to obtain a highly purified recombinant GDNF. Furthermore, the recombinant protein was bioactive.

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References:

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[2] Schulzer, M., Lee, C.S., Mak, E.K., Vingerhoets, F.J. & Calne, D.B., Brain, **117** (1994) 509-516.
[3] Björklund, A., Rosenblad, C., Winkler, C. & Kirik, D, Neurobiol. Dis., **4** (1997) 186-200.
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Figures:

1- EXPRESSION AND PRODUCTION OF GDNF IN EUKARYOTIC MAMMALIAN CELLS

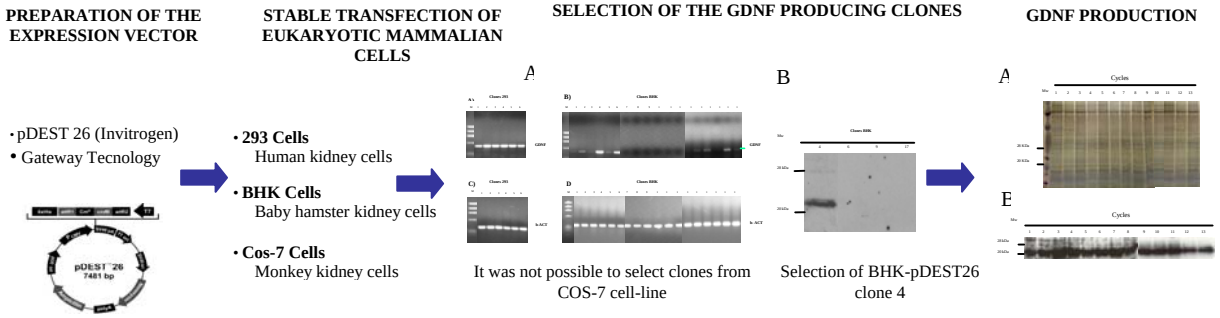

Figure 1. **A, PCR analysis of mRNA. B, Western Blot analysis of conditioned medium.**

Figure 2. **A, SDS-PAGE analysis. B, Western Blot analysis of conditioned medium.**

2- GDNF PURIFICATION

1 - A cationic ion exchange chromatography on XK 16/20 column packed with SP Sepharose High Performance (Amersham)
2 – A gel filtration chromatography on Superdex 200 HR 10/30 (Amersham)
3 - A second cationic ion exchange chromatography on Mono S HR 5/5 (Amersham)

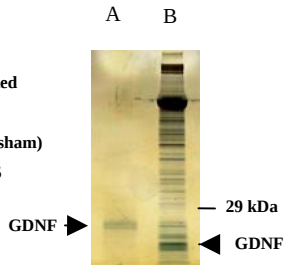

Figure 3. **A, purified GDNF. B, commercial GDNF. Silver staining reveals the presence of several impurities in the commercial GDNF (B) while the recombinant protein is highly pure (A)**

3- CELL-BASED BIOASSAY: DIFFERENTIATION OF PC-12 CELLS TO A NEURAL PHENOTYPE

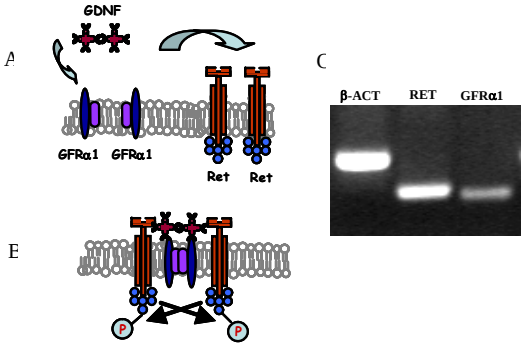

Figure 4. **GDNF-family ligand interaction with their receptors. A, GDNF dimer binds to two molecules of GFRα-1 and this complex binds to Ret that dimerizes. B, The dimerization of Ret causes the autophosphorylation of the tyrosine residues of the two subunits. C, PCR analysis of GFRα1 and RET, receptors necessary for the protein activity**

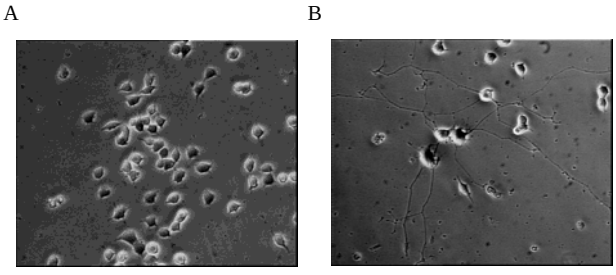

Figure 5. **Differentiation of PC-12 induced by GDNF. PC-12 cells cultured in 12 well plates; 20 cells/mm2. A, Cells without GDNF supplementation. B, Cells supplemented with 50 ng/ml of purified GDNF The differentiation of PC-12 cells to a neuronal phenotyphe confirm that recombinant purified protein is bioactive.**