

## Isolation and characterization of human cholinergic neurons from fetal nucleus basalis of Meynert

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The nucleus basalis of Meynert (NBM), the major source of cholinergic innervation to the neocortex, is selectively vulnerable to degeneration in Alzheimer's disease (AD; 1). To date no resolutive therapies exist. Among the therapeutic strategies currently in development, cell transplantation for the replacement of lost neurons is under consideration. The preliminary clinical experiences reported in the literature are limited to a very restricted number of patients and extensive studies are needed to identify the optimal cell source and the most effective methods for cell transplantation in AD. In this study we established a primary culture of human cholinergic neurons (hCN) isolated from the NBM of 12-week old fetuses. The primary culture was immunophenotyped with flow cytometry and resulted almost totally positive for the neuronal marker MAP2 and for the choline acetyltransferase (ChAT; 97±2 %). A small percentage of cells was positive for O4 (4±2.5%) and GFAP (1.6±0.05%), suggesting a marginal glial contamination. ChAT expression in hCN was confirmed by immunocytochemistry and western blot analyses. Moreover, hCN express the nerve growth factor (NGF) receptor TrkA, which is biologically active, as demonstrated by both increased phosphoTrkA detection and cell growth stimulation upon NGF treatment. Interestingly, hCN also express receptors for hormones of the hypothalamic-pituitary-gonadal (HPG) axis (ERs, LHR, GnRHR), indicating that our cellular model may be a useful tool to study in vitro the mechanisms underlying the known association between HPG hormones and neurodegeneration (2). To test whether hCN could be used in cell therapy, these cells were intravenously administered in NBM quisqualic acid-lesioned adult male rats. Histological examination of paraffin embedded brain slices from injected rats showed that hCN (labeled with PKH26 fluorescent kit before injection) migrated to the injected NBM and along the needle tract. Behavioral tests indicated a significant improvement in cognitive and spatial memory functions in hCN injected as compared to un-injected NBM-lesioned rats.

### References

- [1] Gratwicke et al. (2013). The nucleus basalis of Meynert: a new target for deep brain stimulation in dementia? *Neurosci Biobehav Rev.* 37, 2676.
- [2] Webber et al. (2007) Gonadotropins: A cohesive gender-based etiology of Alzheimer disease. *Mol Cell Endocrinol* 260–262, 271; doi:10.1016/j.mce.2006.01.018

### Keywords

Alzheimer's disease; fetal neurons; cell transplantation.