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Research Report

Differential modulation of nerve growth factor receptor (p75) and cholinergic gene expression in purified p75-expressing and non-expressing basal forebrain neurons by BMP9

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ABSTRACT

The synthesis of acetylcholine and its release from basal forebrain cholinergic neurons (BFCN) that innervate the cerebral cortex and hippocampus are considered essential processes for normal learning, memory and attention. We have developed a purification and cell culture method of BFCN in order to examine the regulation of their cholinergic phenotype. Cells isolated from the septal region of late embryonic mice were purified by fluorescence-activated cell sorting based on their expression of the nerve growth factor receptor (p75), a surface marker for mature BFCN. Consistent with previous reports, p75-postive (p75+) cells were enriched in choline acetyltransferase (ChAT) and the high-affinity choline transporter (ChT), as measured by reverse transcriptase PCR. In culture, these cells maintained their gene expression of p75, ChAT and ChT, while p75-negative (p75–) cells had a low expression of these genes. Incubation of the cells with BMP9 not only increased p75 and ChAT gene expression in p75– cells, but also augmented the expression of these genes in p75+ cells. Conversely, BMP9 decreased ChT gene expression in p75+ cells and had no such effect in p75– cells. Immunostaining confirmed that p75 protein expression was modulated by BMP9 in a similar way as p75 mRNA, and also revealed that only a subset of p75– cells respond to BMP9 in this manner. These data suggest that mature BFCN in culture may express their cholinergic phenotype in the absence of exogenous trophic input, but that BMP9 can further modulate this phenotype. Moreover, BMP9 induces the cholinergic phenotype in a set of basal forebrain non-cholinergic neurons or precursor cells.

1. Introduction

The long axon basal forebrain cholinergic neurons (BFCN) that project to the hippocampus and cerebral cortex regulate multiple processes including attention, learning, memory (Sarter and Parikh, 2005) and sleep (Jones, 2005). The cell bodies of these neurons reside in the medial septum, the diagonal band of Broca and the nucleus basalis magnocellularis (Dutar et al., 1995; Fibiger, 1982; Mesulam et al., 1983). The degeneration or malfunction of BFCN is thought to contribute to the pathophysiology of memory deficits associated with Alzheimer's Disease (Davies and Maloney, 1976). Study of the

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Abbreviations: BFCN, basal forebrain cholinergic neurons; ChAT, choline acetyltransferase; ChT, choline transporter; ACh, acetylcholine; BMP, bone morphogenetic proteins; FACS, fluorescence-activated cell sorting; NB, neurobasal medium; AraC, cytosine arabinoside; MFI, mean fluorescence intensity

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development of BFCN is fundamental to the understanding of learning and memory, and may help in the development of new treatments for diseases affecting these processes.

In the mouse, cholinergic neurons first arise in the basal forebrain at approximately embryonic day (E)11, and express cholinergic markers by E16 (Sweeney et al., 1989). These cells extend efferents toward hippocampal and cortical targets, with innervation occurring during the first postnatal weeks (Sweeney et al., 1989). In the septum and the nucleus of the diagonal band, 90% of cholinergic neurons express the low affinity nerve growth factor receptor (aka. neurotrophin receptor, p75), and 90% of p75-expressing (p75+) cells are cholinergic neurons (Dawbarn et al., 1988; Hartikka and Hefti, 1988; Heckers et al., 1994; Lin et al., 2007). Furthermore, p75 is one of the earliest markers expressed in these neurons (Koh and Loy, 1989). Acetylcholine (ACh) is synthesized from choline and acetyl-coenzyme A by the enzyme choline acetyltransferase (ChAT) (Tucek, 1985), a *sine qua non* marker for cholinergic neurons. The choline transporter (ChT, SLC5A7) is a transmembrane protein, co-localized with ChAT at the nerve terminals, that binds choline with high affinity and transports it into the cell (Okuda and Haga, 2003). Although choline from alternative sources, including low affinity uptake and phosphatidylcholine breakdown, may be used for ACh synthesis, uptake of choline by ChT is thought to be the rate-limiting step in the synthesis of ACh in most cases (Haga, 1971). Because very few non-cholinergic cells express ChT, it is also considered a marker of cholinergic neurons.

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor β superfamily of polypeptides that influence many processes such as developmental tissue patterning, apoptosis, cell proliferation, adhesion, positioning and fate decisions in a variety of organs (Massagué,, 2000; Massagué, et al., 2000; Massagué, 1998). Specialized functions for BMPs in CNS development include regulation of forebrain formation where a balance between BMP inductive actions and active repression of BMP signals is at play (Liu and Niswander, 2005). Our previous studies implicated BMP9 as a cholinergic differentiation factor in the developing septum (Lopez-Coviella et al., 2000, 2002, 2005). For example, *in vivo* administration of BMP9 into the cerebral ventricles of developing mouse embryos significantly increased the ACh levels in the forebrain. Furthermore, *in vitro* application of this factor to primary septal cells resulted in increased ChAT expression and ACh production; the latter potentiated by addition of bFGF (Lopez-Coviella et al., 2000). All previous *in vitro* studies of BFCN have examined the heterogeneous septal cell population, however BFCN comprise only a minority population in the septum (Greferath et al., 2000a; Naumann et al., 2002).

We have developed a method to isolate and culture BFCN utilizing fluorescence-activated cell sorting (FACS) in order to address fundamental questions concerning the development and nature of these neurons. Both purified BFCN and the non-

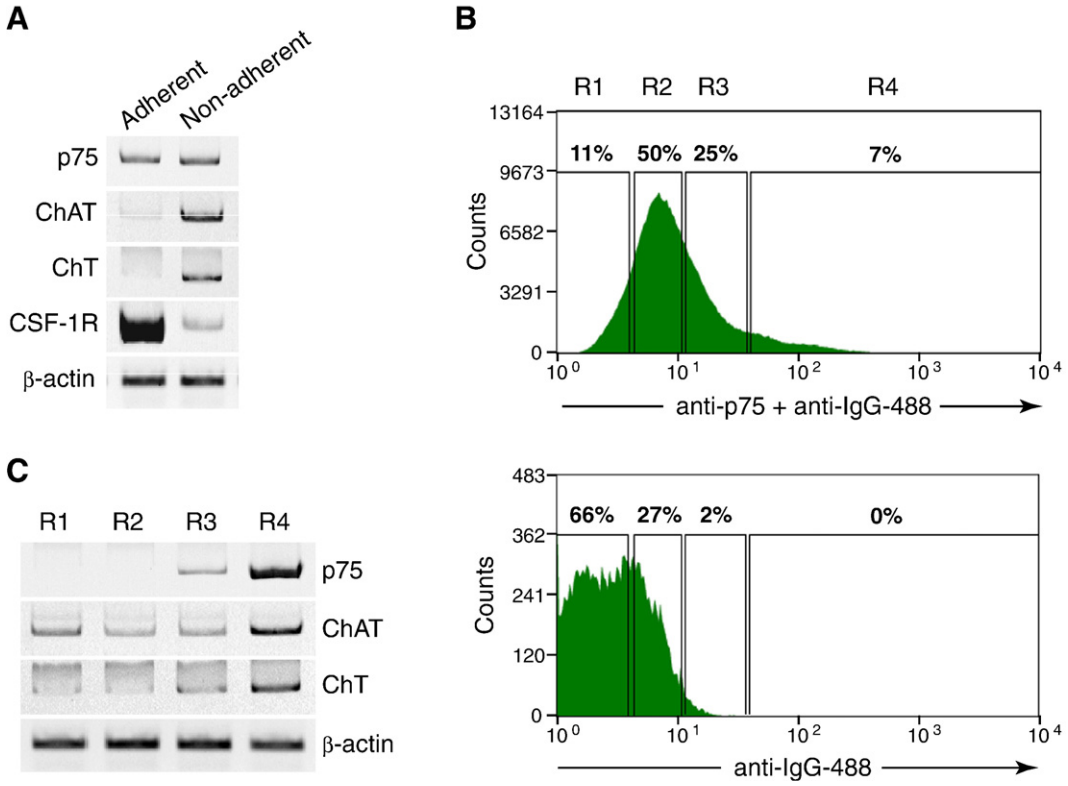

Fig. 1 – Isolation of p75+ BFCN via FACS. (A) Dissociated E18 septal cells were incubated in uncoated culture dishes for 20 min at room temperature, followed by analysis of the adherent and non-adherent fractions by RT-PCR. (B) Non-adherent, E18 septal cells were stained for p75 (anti-p75 primary antibody, anti-IgG-488 secondary antibody) and sorted based on p75 expression level into fractions R1–R4 (top panel). Cells stained with secondary antibody alone are shown in the bottom histogram. (C) RNA from fractions R1–R4 was analyzed by RT-PCR for expression levels of the indicated genes.