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# Embryonic Neural Stem Cell Differentiation to Aldynoglia Induced by Olfactory Bulb Ensheathing Cell-Conditioned Medium

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## 1. Introduction

Although the relevance of glial cells in regulating brain activity was predicted by Ramon y Cajal more than a century ago (García-Marín et al., 2007), it was not until almost fifty years ago that initial descriptions of a close functional relationship between neuroglia and neuronal perikarya (Hyden, 1962) or axonal processes (Blunt et al, 1965) began to reveal that neurons and glial cells operate as functional units in the central nervous system (CNS). However, this functional interaction has only been more carefully studied and analysed in the last few decades, generating a substantial increase in research on the roles of neuron–glia interactions in the control of brain function. Glial cells have subsequently been implicated in many functions, including: guiding the migration of neurons in early development, axonal guidance and being responsible for their integrity, forming the necessary scaffold for neuronal architecture and neural protection and proliferation by trophic effects, modulating neurodegenerative processes, and also being critical participants in synaptic transmission, and key regulators of neurotransmitter release.

Interactions between axons and glia are dynamic and reciprocal. Experiments carried out in several laboratories have shown that glial cells directly participate in synaptic signalling and potentially regulate synaptic plasticity and network excitability (e.g. Vijayaraghavan, 2007). There is also functional coupling between neurons and glia (Álvarez-Maubecín et al., 2000), and a variety of close anatomical relationship; on the one hand, white matter glia preserve axonal integrity (Edgar & Nave, 2009), and on the other hand neurons regulate migration, survival and proliferation of the glial cells that they need for guidance (Learte & Hidalgo, 2007). Hence, there are a plenty of examples regarding morphological and physiological

interactions between glial cells and neurons (Wigley, 2007). Such, communication between glial cells and neurons has led to the concept of the neuron–glial functional unit as the substrate of integration in the CNS.

Various glial cells types are involved in neuronal functioning. During the development of the CNS, the reciprocal communication between neurons and oligodendrocytes is essential for the generation of myelin (Simons & Trajkovic, 2006). These interconnections at multiple levels show how neurons and glia cooperate to build a complex network during development. In addition, although neurons are indispensable for brain function, an emerging alternative view holds that astrocytes, the dominant glial cell type, coordinate synaptic networks. Through the release of glutamate, astrocytes locally excite neurons, and via adenosine, which accumulates due to the hydrolysis of released ATP, astrocytes suppress distant synapses (Fellin et al, 2006).

Astrocytes respond to neuronal activity and neurotransmitters through the activation of metabotropic receptors, and can release gliotransmitters (ATP, d-serine, and glutamate), which act on neurons participating in a tripartite synapse (consisting of classic pre- and postsynaptic elements and an associated astrocytic process) (Halassa & Haydon, 2010). Moreover, astrocytes release several transmitters that affect neuronal activity and involve glial signalling in the modulation of mammalian behaviour (Halassa et al, 2009). By controlling neuronal A1-receptor signalling, astrocytes modulate mammalian sleep homeostasis and are essential for mediating the cognitive consequences of sleep deprivation. Slow-signalling glia modulate fast synaptic transmission and neuronal firing to impact behavioural output (Halassa et al, 2009). Cell type-specific molecular genetics has allowed a new level of examination of the role of astrocytes in brain function and has revealed an important role of these glial cells in gliotransmission, which is mainly mediated by adenosine accumulation, in the control of sleep and in cognitive impairments that follow sleep deprivation (Halassa & Haydon, 2010). Thus, a new challenge is to try to understand the functioning and rules of the neuron–glial networks (e.g. Araque & Navarrete, 2010).

These close functional interactions between glia and neurons are not completely unexpected given their intimate ontogenic relationship, despite the ancient and frequent postulation of classical neuroscience from the last millennium that neurons and glial cells were derived from different groups of precursor cells and that no new neurons were produced once development was completed. Henceforward, we used the term "precursor cell" to generally refer to a cell that precedes others in time and space and that gives rise to a variant, specialized or more mature form of cell in a cell lineage, instead of the term "progenitor cell" which vaguely indicates a biologically related ancestor.

Radial glia are now known to be the main source of neurons in several regions of the CNS, notably in the cerebral cortex (Malatesta et al, 2008), and they share several features with neuroepithelial cells, but also with astrocytes in the mature brain (Mori et al., 2005). However, they can be distinguished from neuroepithelial precursors by the expression of astroglial markers (Malatesta et al, 2008). During the last decade, the role of radial glia has been radically revaluated, and they are no longer considered to be a mere structural component serving to guide newborn neurons towards their final destinations.

Radial glial cells can be defined by morphological, cell biological and molecular criteria as true glial cells, like astroglia, they appear during neural development as a precursor cell intermediate between immature neuroepithelial cells and differentiating progeny, and they subsequently generate different cell lineages. In addition they persist into adulthood in various vertebrate classes ranging from fish to birds, while neurogenic glial cells become

restricted to a few small regions of the adult forebrain in mice and humans (Pinto & Gôtz, 2007). This leads us to consider the molecular mechanisms involved in the regulation of the heterogeneity of the radial glial cells lineage, including their diversity in distinct regions of the CNS, their identity, heterogeneity and their functions.

Surprisingly, when the primary precursor cells (stem cells) of new neurons in neurogenic regions were identified, they exhibited the structural and biological markers of astrocytes (Ihrie & Alvarez-Buylla, 2008), and subsequently became for the focus of research related to the mechanisms of control of the proliferation and differentiation of these precursor cells.

One of the key advances in the field of neurobiology was the discovery that astroglial cells can generate neurons not only during development, but also throughout adult life and potentially even after brain lesion. Interestingly, only some astrocytes maintain their neurogenic potential and continue to generate neurons throughout life (Mori et al., 2005). At late-embryonic and postnatal stages, radial glial cells give rise to the neural stem cells responsible for adult neurogenesis. Embryonic pluripotent radial glia and adult neural stem cells may be clonally linked, thus representing a lineage displaying stem cell features in both the developing and mature CNS (Malatesta et al, 2008).

Adult neural precursor cells appear to be strongly influenced by their local microenvironment, while also contributing significantly to the architecture of germinal zones. However, environment alone does not seem to be sufficient to induce non-germinal astrocytes to behave as neural precursor cells. Although emerging evidence suggests that there is significant heterogeneity within populations of germinal zone astrocytes, the way that these differences are encoded remains unclear (Ihrie & Alvarez-Buylla, 2008).

Glial function is fundamental to the CNS, however the *in vitro* differentiation of embryonic stem cells into glia has received relatively limited attention when compared with the interest in the generation of neurons. This was the case until the last decade, when the study of astrocytes, oligodendrocytes and other macroglial cells lineages became a very interesting and productive area of research. The further characterization of glial cells should eventually provide a body of knowledge central to the understanding of cell differentiation during brain development and also of brain response during disease.

## 2. Aldynoglia: not a particular cell lineage but a glial cell phenotype

The former view that glial cells can be classified based on the expression of various biochemical markers has been overturned by the recognition that different cell types in the brain have common precursors (Alvarez-Buylla et al., 2001) that can be phenotypically interconvertible.

All the neurons and glial cells of the CNS are generated from the neuroepithelial cells in the walls of the embryonic neural tube, the ”embryonic neural stem cells”. The stem cells seem to be equivalent to the radial glial cells, which for many years were regarded as a specialized type of glial cell (Kessaris et al, 2008).

Glial cells are derived from precursor cells that mature through specific stages of development to generate fully differentiated astrocytes and oligodendrocytes as well as other macroglial cell types. Several types of intermediate precursors have been described and in some cases lineage relationships have been identified, although these remain controversial.

Glial precursor cells (GPCs) comprise the most abundant population of precursor cells in the adult human brain. They are responsible for CNS remyelination, and may contribute to the astrogliotic response to brain injury and degeneration. Adult human GPCs are biased to

differentiate as oligodendrocytes and elaborate new myelin, and yet they retain multilineage plasticity, and can give rise to neurons as well as astrocytes and oligodendrocytes once removed from the adult parenchymal environment. GPCs retain the capacity for cell-autonomous self-renewal, and yet both their phenotype and fate may be dictated by their microenvironment (Sim et al, 2009).

Various other glial precursor cells have been partially described, probably all deriving from earlier appearing precursor cells but segregating at different stages in development. These include; motoneuron-oligodendrocyte precursors (MNOPs), white matter precursor cells (WMPCs), polydendrocytes, glial restricted precursors (GRPs), astrocyte precursor cells (APCs), and oligodendroblasts (e.g. Liu & Rao, 2004). Some of these precursors persist in the adult, and there are also intermediate glial precursors, rather than stem cells, that respond after injury and participate in the repairing process. Nevertheless, it remains unclear which specific glial precursor responds under different situations, and therefore new consensus sets of markers need to be identified, in order to improve our ability to define more clearly the different stages of glial maturation as well as the different glial phenotypes.

Besides the range of glia in the CNS, aldynoglia have recently emerged as a novel concept (Gudiño Cabrera & Nieto-Sampedro, 2000; Rojas-Mayorquín et al., 2008; 2010) and are beginning to be recognized as a specific glial phenotype (Panicker and Rao, 2001; Boyd et al., 2003; Liu and Rao, 2004; Rojas-Mayorquín et al., 2008; 2010) that includes and describes a group of macroglial cells that could constitute a separate functional group of CNS macroglia. These possess the ability to myelinate dorsal root ganglia neurites *in vitro* and provide the scaffold to guide neurons to their ultimate destinations (Gudiño Cabrera & Nieto-Sampedro, 1996; 2000). Functionally, aldynoglia differ from astrocytes and oligodendrocytes, but maintain the expression of some molecular markers that are common to both, and also to Schwann cells, which can be considerered as peripheral aldynoglia (Table 1). The prototype of this central glial cell is the olfactory bulb ensheathing cell (OBEC) (Gudiño-Cabrera & Nieto-Sampedro, 1999).

| Gene name | OBECs | OB | SCs | ACs | OLs |
|---|---|---|---|---|---|
| GFAP | + | + | + | + | - |
| S100a6 | + | + | + | + | - |
| N-myc | + | + | + | + | - |
| Adcy5 | + | + | + | - | - |
| p75 (NGFR) | + | + | + | - | - |
| Cdkn2b | + | + | - | + | + |
| Lyn | + | + | - | - | + |
| Mtmr2 | + | - | + | - | - |
| F2 | + | - | - | + | + |
| Abcc1 | + | - | - | + | - |
| Apbb1 | + | - | - | + | - |
| Eotaxin | + | - | - | - | + |

Table 1. Genes expressed in common among glial cells. OBECs - olfactory bulb ensheathing cells in culture, OB – olfactory bulb, SCs – Schwann cells, ACs – astrocytes, OLs – oligodendrocytes, (+) reported as expressed, (-) not yet reported or expression not yet verified. All (+) expression from OBECs was reported in Rojas-Mayorquín et al, 2008, and all other expression was from bona fide literature.

On the basis of an analysis of the expression profile by microarrays (Rojas-Mayorquín et al, 2008), we propose that OBECs are more closely related to Schwann cells, and that astrocytes are also more closely related to OBECs than to oligodendrocytes, although there are some similarities between OBECs and oligodendrocytes that are consistent with their respective phenotype as well as behavioural characteristics.

It should be emphasized that the concept of the aldynoglia refers to a glial cell phenotype and not a particular cell lineage (Rojas-Mayorquín et al., 2008; 2010).

The lineages of both astrocytes and oligodendrocytes have received more attention in the last decade, because the source of these cells in the mature CNS is relevant to the study of the cellular response to CNS injury. Some authors have argued that there is a common glial precursor cell from which both differentiate and that the microenvironment surrounding the injury determines the fate of the stimulated precursor cell (Rao & Mayer-Proschel, 1997; Rao et al., 1998). However, the precise origin of these glial cells is still not completely understood, though it appears that they derive from multiple regions of the CNS rather than from a single location (Kessaris et al., 2006; Richardson et al., 2006). A significant amount of evidence suggests that resident precursor cells proliferate and differentiate into mature glial cells that facilitate tissue repair and recovery. Additionally, the re-entry of mature astrocytes into the cell cycle can also contribute to the pool of new astrocytes that are observed following CNS injury. Better understanding of the origin of new glial cells in the injured CNS will facilitate the development of therapeutics targeted at altering the glial response in a beneficial way (e.g. Carmen et al, 2007).

Among the intermediate glial precursor cells, NG2 cells, first described more than two decades ago, are glial cells that specifically express the NG2 chondroitin sulphate proteoglycan (CSPG) and platelet-derived growth factor receptor alpha. Also known as synantocytes or polydendrocytes, to reflect their multi-processed morphology and lineal relationship to oligodendrocytes, they constitute a population of CNS cells distinct from neurons, mature oligodendrocytes, astrocytes, other macroglia and microglia.

Because they differentiate into oligodendrocytes *in vitro*, NG2-expressing glia were considered to be oligodendrocyte precursor cells (OPC; e.g. Nishiyama et al, 2009). Moreover, these cells persist in the adult CNS to generate oligodendrocytes throughout life (Hermann et al, 2010). However, they are widespread in the CNS, and in the adult human cerebral cortex and white matter they represent 10–15% of non-neuronal cells (Butt et al, 2005). Labelling techniques have shown them to have more than just precursor functions. A large proportion of NG2 cells do not appear to divide or generate oligodendrocytes, instead they form interactive domains with astrocytes and neurons (Wigley & Butt, 2009).

The morphology and distribution of NG2 glia are similar to, but distinct from, both microglia and astrocytes. The antigenic profile and morphology of NG2 glia in human tissues are consistent with an OPC function and this been well established in rodent models (Staugaitis & Trapp, 2009). NG2 proteoglycan expression could label newly generated cells or be inherited by resident cell populations that produce oligodendrocytes for remyelination, astrocytes that provide trophic support and other cells that contribute to CNS function (Sellers & Horner, 2005).

During embryonic development, multipotential neural precursor cells (MNP) possess the ability to self-renew and to generate the major CNS cell types: neurons, astrocytes and oligodendrocytes. However, the molecular mechanisms that control MNP fate specification are not yet fully understood. Recent studies have provided evidence that soluble protein

mediators such as cytokines and transcriptional factors play critical roles in cell fate determination.

Furthermore, it has become apparent that epigenetic gene regulation plays an important intracellular role as cell-intrinsic programs in the specification of cell lineages (Abematsu et al, 2006). This process appears to gradually reduce the neurogenic potential of the astrocytic progeny, through mechanisms involving the epigenetic silencing of neurogenic fate determinants (Hirabayashi et al., 2009). The epigenetic "programming" of precursor cells into oligodendrocytes, for example, moves through three sequential stages of lineage progression (Liu & Casaccia, 2010), first from pluripotent precursor cells to MNP, from this to the oligodendrocyte precursor, and finally to differentiation into myelin-forming cells.

Consequently, it is possible to depict a model that combines genetic and epigenetic clues to define the differentiation pathway in such a way that the intermediate stages transiently reveal certain phenotypes, allowing parallels to be drawn with a river crossing, where the process of differentiation itself is the river and the cells that undergo differentiation need to cross from one riverbank to the other. Hence, we can consider that during embryonic development differentiation of neural cells initially starts from pluripotent precursor cells (ectodermal stem cells) to MNP (both located at one side of the river).

As shown in Figure 1, the first step in neuroglial differentiation in the neuroepithelium is characterized by the repression of pluripotency genes and restriction of the lineage potential to the neural fate, which is equivalent to starting to cross the river. The next step involves the generation, sequentially or in parallel, of a large amount of intermediate precursor cells from MNP with a neural fate. This step is associated with the progressive loss of plasticity and the expression/repression of neuronal- or glial-specific genes, for astrocytes, oligodendrocytes, polydendrocytes, aldynoglial and other phenotypes accordingly.

These intermediate precursors are crossing the river of differentiation; they are at different stages, and most of them (if not all) are interconvertible depending on the environmental signals that they receive, which indicates location and timing. And finally, differentiation into a particular phenotype comes when the cells cross the river and arrive at the other side, defined by the expression/repression of a particular set of genes that confers the diverse phenotypes (Figure 1).

Therefore, it seem feasible that CNS macroglial cells such as astrocytes and olygodendrocytes, or even polydendrocytes (NG2+), aldynoglia (p75+,GFAP+) and others, could be identified at one time as functionally segregated cell populations, but that during development or under certain circumstances, such as response to injury or neurodegenerative diseases, their precursors could constitute interconvertible cell populations.

## 3. Microarrays and proteomics as tools for the study of the mechanisms of differentiation

The mechanisms of differentiation are very complex, and thus microarrays and subtractive cDNA libraries, as well as proteomics as experimental approaches for their initial analysis, provide powerful techniques for studying differential gene expression.

It is therefore obvious at this point that new fate-mapping tools must be developed to allow us to unambiguously distinguish between different multipotential, pluripotential or even more restricted intermediate neural precursor cell populations in the adult cerebral cortex and neurogenic zones. With this in mind, one needs first to identify genes that are

selectively expressed in one population, but not in others. To accomplish this it is quite important to use an experimental model in which the participation of different proteins can be reproducibly tested.

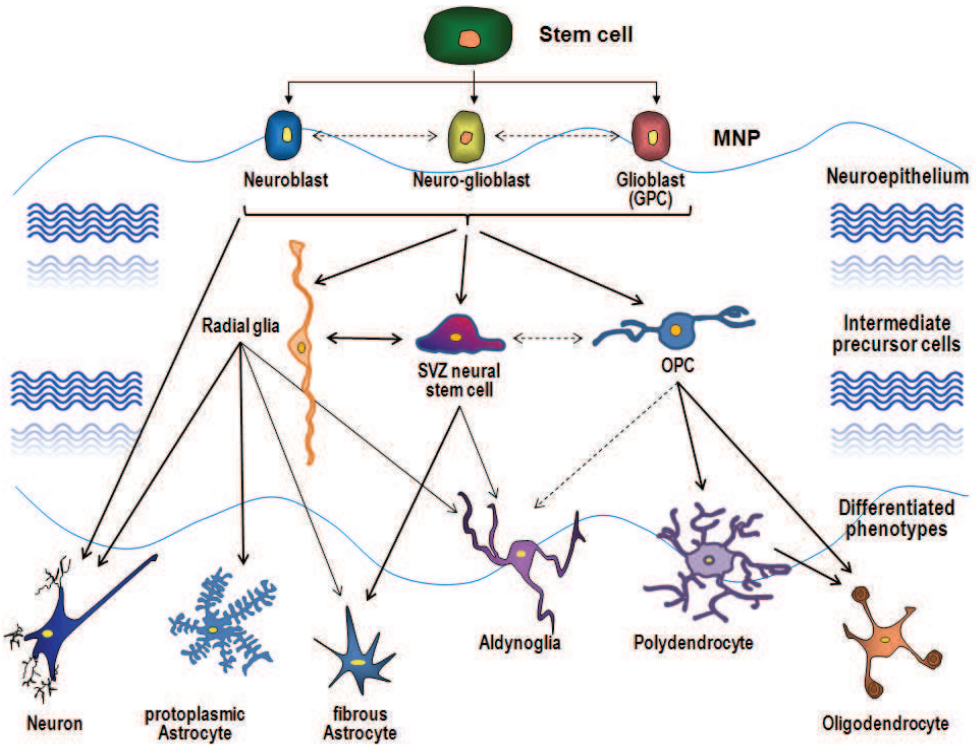


Fig. 1. Crossing the river of differentiation. The strength of a lineage relationship is indicated by bold (generally accepted), solid (recognized by some) or dashed arrows (hypothetical). MNP – multipotential neural precursor; GPC – glial precursor cell; OPC – oligodendroglial precursor cell; SVZ – subventricular zone.

As a first step, microarrays provide a powerful technique with which to study differential gene expression as they permit the expression of thousands of genes to be assessed in parallel in a single experiment (Figure 2). This approach has identified genes with potential relevance to cell differentiation in several cases (Roupioz et al., 2005), and in particular for MNP (Sousa et al., 2007).

Since cell differentiation is a complex process that can be regulated by intrinsic signals as well as by the extrinsic environment, a detailed analysis of microarray results (Rojas-Mayorquín et al., 2008) has enabled us to generate an expression profile for cells before and after they are subjected to different conditions or treatments. Along with other integral approaches, such as subtractive cDNA libraries (Rojas-Mayorquín et al., 2010) and proteomics, microarrays will help to establish a more comprehensive model of the molecular mechanisms involved in MNP differentiation that are directed by OBEC-conditioned media.

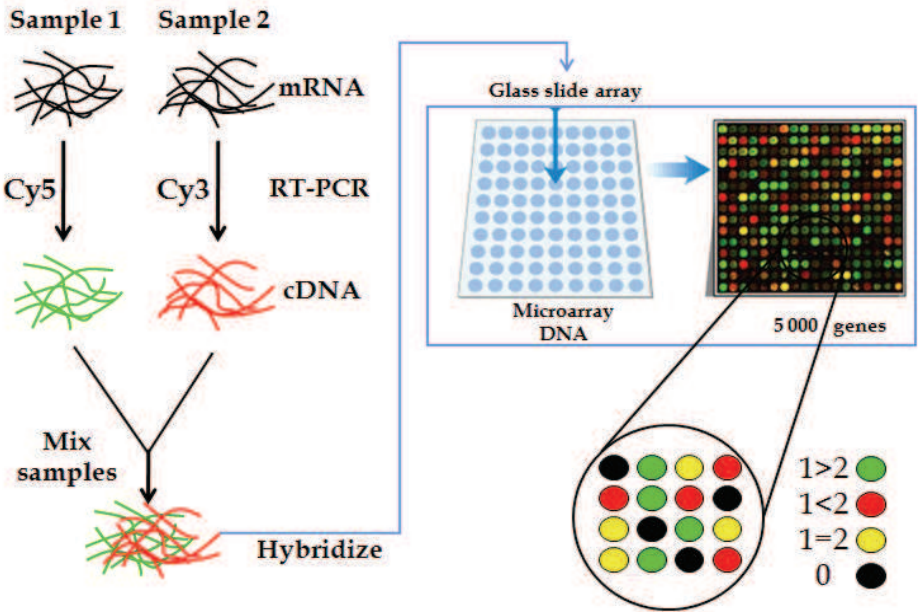


Fig. 2. Microarray procedure. This is used to compare the gene expression profile of thousand of genes from two different cell populations simultaneously.

## 4. OBEC-conditioned medium is a suitable model for inducing and directing stem cell differentiation *in vitro* towards an aldynoglia phenotype

Initially, OBEC-conditioned medium was used to elicit neuritogenesis *in vitro* via the co-cultures of neurons and EC (Kafitz & Greer, 1999; Sonigra et al., 1999; Wang et al., 2003), and more recently, we and other groups have been studying the capacity of the OBEC-conditioned medium to induce and direct *in vitro* differentiation from neural stem cells (Rojas-Mayorquín et al., 2008; 2010; Zhang et al., 2008; Doncel-Pérez et al., 2009) or even from other stem cells (Wang et al., 2007).

Given that OBECs are able to produce, secrete and also respond to a variety of growth factors (Table 2) and because the OB displays constant neuroglial replacement (Altman, 1969; Graziadei and Monti Graziadei, 1978), it is therefore reasonable to propose that the OBEC could induce its own differentiation from stem cell precursors located in the OB *in vivo*, or even that of other stem cells *in vitro* (Rojas-Mayorquín et al., 2008; 2010).

To analyse the differentiation process we employed a cell culture model that can be applied to study the possible molecular mechanisms that direct embryonic stem cell differentiation through aldynoglial cells. It also provides new insight into the molecular events and biological features that occur during the transition from MNP toward an aldynoglia phenotype, particularly when induced by EC-conditioned medium. In this model, we first obtained primary cultures of EC from OB, and then performed an immunopurification of ECs that express the p75 receptor, and finally obtained from these ECs the conditioned medium to be used to induce the differentiation of a heterogenic embryonic stem cell population from the striatum (figure 3).

| Growth factor | Receptor | *In vivo* expression | *In vitro* expression | *In vitro* response |
|---|---|---|---|---|
| **BDNF** | | | (4) (19) (21) (28) (30) | |
| **BMP** | | | | |
| **BMP-4** | | (20) | | |
| **CNTF** | | | (19) (29) | |
| | **CNTFR-a** | | (29) | |
| **Estrogen** | **ER-alpha** | | (14) | |
| **FGF1** | | (7) (17) | | |
| **FGF2** | | (7) | (22) | (2) (32) |
| | **FGFr1** | (16) | | |
| **GDNF** | | | (19) (22) (30) | |
| | **GFRalpha-1** | | (30) | |
| | **GFRalpha-2** | | (30) | |
| **HGF** | | | | (33) |
| **IGF-1** | | (27) | (15) | |
| | **IGF-1r** | | | (32) |
| **Neuregulin (EGF-like)** | | (5) (25) | (4) (26) (30) | (9) (24) (26) |
| | **ErbB-2** | (23) | (9) | |
| | **ErbB-3** | (5) | (9) | |
| | **ErbB-4** | | (9) | |
| **GGF2** | | | (8) | (8) |
| **Neurturin (NTN)** | | | (30) | |
| **NGF** | | | (4)(6)(19)(22)(28)(30) | |
| | **NGFR** | | (30) | |
| **NT-3** | | | (4) (28) | |
| **NT-4/5** | **Trk B** | | (30) | (3) |
| | **p75 (Ngfr)** | (11) (12) | (10) (13) | |
| **PDGF** | | (18) | | (32) |
| **VEGF** | | | (1) | |

Table 2. Growth factors and their receptors expressed by OBEC both *in vivo* and *in vitro* and their response *in vitro*.

(1) Au & Roskams, 2003; (2) Barraud et al., 2007; (3) Bianco et al., 2004; (4) Boruch et al., 2001; (5) Bovetti et al., 2006; (6) Cao 2007; (7) Chuah & Teague 1999; (8) Chuah et al., 2000; (9) De Mello et al., 2007; (10) Franceschini & Barnett, 1996; (11) Gómez-Pinilla et al., 1987; (12) Gong et al., 1994; (13) Gudiño-Cabrera & Nieto-Sampedro, 1996; (14) Gudiño-Cabrera & Nieto-Sampedro, 1999; (15) Gudiño-Cabrera & Nieto-Sampedro, 2000; (16) Hsu et al., 2001; (17) Key et al., 1996; (18) Kott et al., 1994; (19) Lipson et al., 2003; (20) Liu et al., 2010; (21) Pastrana et al., 2007; (22) Pellitteri et al., 2007; (23) Perroteau et al., 1998; (24) Pollock et al.,1999; (25) Salehi-Ashtiani & Farbman, 1996; (26) Thompson et al., 2000; (27) Vicario-Abejón et al., 2003; (28) Vincent et al., 2003; (29) Wewetzer et al., 2001; (30) Woodhall et al., 2001; (31) Woodhall et al., 2003; (32) Yan et al., 2001a; (33) Yan et al., 2001b.

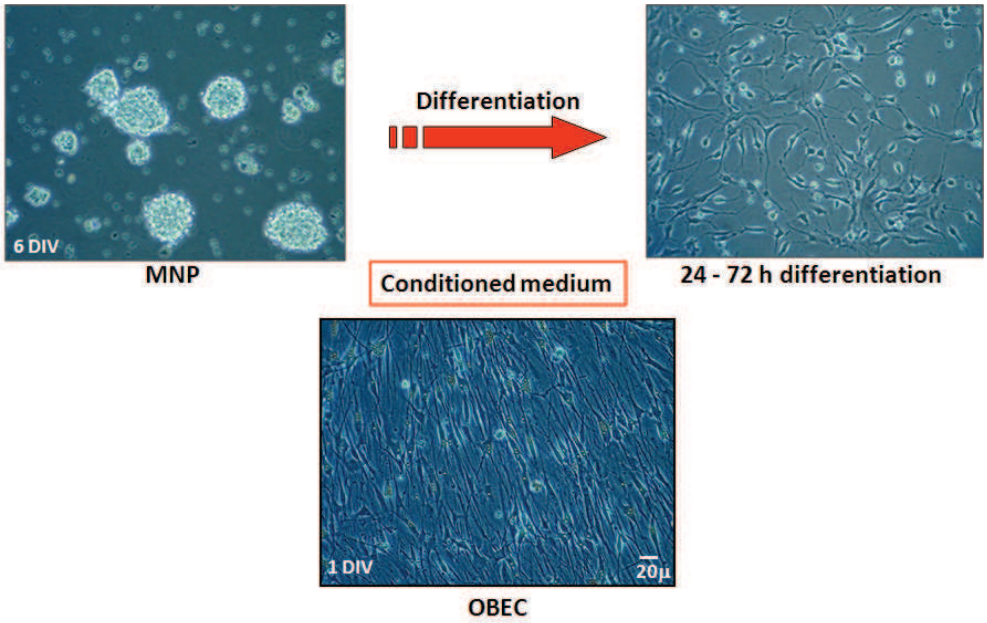


Fig. 3. Cell culture model of differentiation. First, a proliferating and undifferentiated embryonic neural stem cell culture of MNP was maintained. Then, neurospheres were cultured in conditioned medium from an immunopurified EC culture for 24 to 72 hours to achieve almost complete differentiation to an aldynoglia phenotype. MNP–multipotential neural precursors, OBECs–olfactory bulb ensheathing cells, DIV–days *in vitro*. Bar 20 µm.

## 5. Differentiation of the aldynoglia phenotype can be achieved by blocking Wnt signalling and activating the BMP pathway, with the involvement of IGF-1

Glial cells were long considered to be supportive cells with a different origin from neurons. New studies have shown that some glial cells function as primary precursors or MNP, deviating from the classical view that glia and neurons ontogenically develop from early separate lineages and demonstrating that during development and in the adult brain, many neurons and glial cells are not the direct progeny of neural stem cells (NSC), but instead originate from transit-amplifying, or intermediate precursor cells (IPC) (e.g. Kriegstein & Alvarez-Buylla, 2009). Thus, a clearer identification of NSC and IPC is critical to understand brain development and adult neurogenesis and to develop new strategies for brain repair.
Recent findings have revealed part of the underlying mechanistic basis for this preferential differentiation into astroglia. The more oxidized state of pathological brain tissue leads to upregulation of the protein deacetylase sirtuin 1 (Sirt1). Sirt1 appears to stabilize a co-repressor complex of Hairy/enhancer of split (Hes)1, thereby suppressing expression of the proneural transcription factor Mash1, and directs precursor cell differentiation towards the glial lineage. Sirt1 upregulated by CNS inflammation may also inhibit neuronal differentiation. Myelin-associated inhibitors such as Nogo, acting through the Nogo-66 receptor (NgR), also appear to promote neural stem/precursor cell differentiation into

astrocytes. Understanding the molecular basis of the glial lineage restriction of neural precursors in the injured or diseased CNS would provide clues to improving the success of stem cell-based transplantation therapy (Teng et al, 2009).

The specification of cells into the oligodendrocyte lineage is largely the result of interplay between the bone morphogenetic protein, sonic hedgehog and Notch signalling pathways, which regulate the expression of transcription factors dictating the spatial and temporal aspects of oligodendrogenesis. Many of these transcription factors and others then direct oligodendrocyte development through to a mature myelinating oligodendrocyte both in the spinal cord and brain (Nicolay et al, 2007).

Based on our results and those reported in the literature, we propose a gene expression profile that accompanies the induced differentiation of MNP into the aldynoglia phenotype. Under the conditions tested, OBEC-conditioned medium could induce MNP differentiation by inactivating Wnt signalling and promoting BMP signalling through the BMP1rA receptor with the possible involvement of IGF-1 (Rojas-Mayorquín et al., 2008; 2010).

IGF-1 is produced by the OBECs (Gudiño-Cabrera & Nieto-Sampedro, 2000) and it regulates the continuous generation of OBECs from local precursor cells within the OB (Vicario-Abejón et al., 2003). IGF-1 also induces Igfbp-5 expression, which is associated with the ECM (Jones et al., 1993), thereby promoting IGF activity (Cheng & Feldman, 1997; Duan et al., 1999) and resulting in positive regulation. In fact, Igfbp-5 was strongly expressed by immunopurified OBEC *in vitro*, and its expression was induced by conditioned medium following MNP differentiation (Rojas-Mayorquín et al., 2010). Hence, it seems probable that IGF-1 participates in the initial induction of MNP differentiation by OBEC-conditioned medium.

Moreover, it has been reported that IGF-1 also induces the expression of Tn-C (Kenney et al., 2003), which is also strongly expressed in the OBEC *in vitro*, and is induced in differentiated MNP in OBEC-conditioned medium (Rojas-Mayorquín et al., 2010). Tn-C could in turn be involved in the inactivation of the Wnt signalling pathway (Kakinuma et al., 2004) that we suggested could be inactivated during induced MNP differentiation (Rojas-Mayorquín et al., 2008). Additionally, the expression of BMPr1A is also increased (Rojas-Mayorquín et al., 2008), this being a BMP receptor that preferentially binds BMP-2 and BMP-4 (Chen et al., 2004). It has also been reported that BMP-2 increases IGF-1 binding to growth plate chondrocytes, suggesting that the BMPs may modulate the action of IGF-I via the type 1 IGF receptor and/or IGF binding proteins (Takahashi et al., 2007).

Given that cell differentiation is a complex process that can be regulated by intrinsic signals as well as by the extrinsic environment, the data obtained from integral approaches such as microarray analysis (Rojas-Mayorquín et al., 2008), subtractive cDNA libraries (Rojas-Mayorquín et al., 2010) and proteomics, will help to establish a more comprehensive model of the molecular mechanisms involved in MNP differentiation directed by OBEC-conditioned medium.

## 6. Perspectives and future use of aldynoglia cell transplants as a complementary therapy for neurodegenerative disorders

In the CNS of mammals, axonal regeneration is limited by two main factors: first, the low intrinsic regenerative potential of adult CNS neurons, and second the inhibitory influences of the glial and extracellular environment. Myelin-associated inhibitors of neurite growth as well as some properties of the so called "reactive astrocytes" contribute to the non-

permissiveness of CNS tissue for axonal growth. In contrast, the peripheral nervous system environment is supportive of regeneration because Schwann cells provide suitable substrates for regrowing axons (Hirsch & Bähr, 1999; Lavdas et al, 2008).

Knowledge of the mechanisms that direct stem cell differentiation to diverse cell phenotypes raises the possibility of generating *in vitro* a large population of cells of specific phenotypic characteristics that can be used to promote regeneration after a CNS injury by means of autologous transplantation from previously differentiated precursor cells (Franklin, 2003), in our case this particular phenotype is represented by the aldynoglia, which can be obtained by inducing MNP cells to differentiate using OBEC-conditioned medium. It would also be feasible to attempt to generate new cells endogenously by activating specific signalling pathways that elicit the production of regeneration-promoting glial cells (Kulbatski et al., 2008) of the aldynoglia phenotype.

There are two experimental strategies for developing cell-based therapies for neurodegenerative diseases: replacing degenerated cells (neurons and glia) via transplantation of cells that have been expanded *in vitro* and subsequently specified into the desired cell type, or recruiting endogenous cells for brain repair, either from endogenous adult neural stem cells that reside in neurogenic zones such as the adult subependymal zone lining the lateral ventricle (Saghatelyan et al., 2004), or from stem cell-like potential cells that are present to a varying degree throughout the brain (Nakatomi et al, 2002; Nunes et al, 2003).

The capacity to instruct endogenous precursor cells to generate neurons takes advantage of the important pool of endogenous and consequently autologous cells, thereby circumventing many of the problems associated with therapeutic strategies based on transplantation. Moreover, it has been shown that astroglia can be reprogrammed *in vitro* by forced expression of neurogenic transcription factors to transgress their lineage restriction and stably acquire a neuronal identity (e.g. Berninger, 2010).

In conclusion, it is clear that to treat complex pathologies in neurodegenerative diseases such as Alzheimer's, Parkinson's disease or multiple sclerosis, among others, it is not sufficient to use just one approach, and that it would be more helpful and convenient to combine therapies, making it possible to take care of patients with neurodegenerative diseases with a combination of neuronal replacement, either from endogenous or exogenous precursor cells, and the use of regenerative glia such as aldynoglia, in transplants that promote functional restoration.

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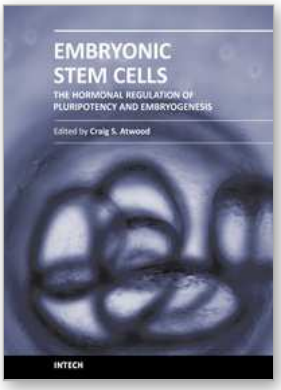


**Embryonic Stem Cells: The Hormonal Regulation of Pluripotency and Embryogenesis**

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