Poster Session BP8: Stem Cell Biology

BP8-01

A novel method to enrich for neural stem cells

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Isolating a pure population of neural stem cells (NSCs) has been difficult since no exclusive surface markers have been identified *in vivo* or *in vitro* to use for panning or FACS purification. Current FACS protocols rely on negative sorting or transgenic mice that express GFP from either the nestin or the SOX2 promoter. While these methods produce highly enriched preparations of nestin+ or SOX2+ cells, only a fraction of the sorted cells are competent to form neurospheres, suggesting that a subset of the positive cells are stem cells. Here, we demonstrate that postnatal rat NSCs express low levels of pro-apoptotic molecules and resist PI3K inhibition, ERK1/2 inhibition, and glutamate receptor stimulation as compared with late oligodendrocyte progenitors. Therefore, we exploited the relative resistance of NSCs to these apoptotic stimuli to develop a novel method for stem cell enrichment. We find that maintaining SVZ cultures in the presence of LY294002 and PD98059, inhibitors of PI3K and ERK1/2 signaling, for 72 h eliminates lineage-restricted precursors, whereas stem cells survive. Not only do the surviving cells form neurospheres, but 89% of these neurospheres generate neurons, astrocytes, and oligodendrocytes. Without this enrichment step, only 42% of the neurospheres are multipotent. Additionally, the neurospheres enriched using this procedure produce 3-times more secondary neurospheres, further suggesting that this procedure increases the ratio of stem cells to lineage-restricted progenitors. This relatively simple and inexpensive means of enriching for NSCs will facilitate gene profiling experiments as well as other studies to reveal the fundamental properties of neural stem cells.

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BP8-02

Lysophosphatidic acid and its receptors as novel regulators of neural stem/progenitor cell growth and differentiation

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Neural stem/progenitor cells are able to self-renew as well as differentiate into neuronal cells and glia *in vitro*. These cells produce neurospheres in serum-free medium containing epidermal growth factor (EGF) and fibroblast growth factor (FGF2). We demonstrate that lysophosphatidic acid (LPA), a lysophospholipid growth factor, initiated the formation of neurospheres from dissociated mouse postnatal forebrain even in the absence of EGF and FGF2. The neurosphere growth induced by LPA was slower than by EGF and FGF2, sustained for up to 3 months, and associated with proliferation of the cells harboring Sca-1 antigen and AC133, markers of primitive stem cells of hematopoietic and neural origin. Sca-1 and AC133 positive cells also expressed LPA1, LPA2 and LPA3 subtypes of LPA receptor. The formation and growth of neurospheres by LPA was inhibited by diacylglycerol-pyrophosphate, an antagonist of LPA1/LPA3 receptors. When neurospheres were attached to the matrix and then induced to spread and differentiate in the presence of LPA, a significant delay of cell migration was observed as compared with EGF/FGF2. LPA receptors were expressed in the developing attached neurosphere, and co-localized with βIII-tubulin and nestin, and CNPase, but no with GFAP, a marker of astrocyte lineage. Sca-1 antigen and AC133 were still detected in the residual core of neurospheres grown attached in the presence of LPA, but not EGF/FGF, and co-localized with LPA receptors. Collectively, these data reveal novel properties of LPA toward initiation and regulation of neural stem/progenitor cell growth, maintaining undifferentiated phenotype as well as differentiation *in vitro*.

BP8-03

Dopamine specifically inhibits neural stem cell proliferation suggesting a novel mechanism of antipsychotic drug action

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Antipsychotic drugs increase brain tissue volume during treatment of schizophrenic patients. We examined the contribution of cell proliferation to this phenomenon. In adult rats chronic haloperidol (2 mg/kg/day via s.c. osmotic pump) significantly increases the numbers of neural stem cells and constitutively proliferating progenitors in the subependyma of the lateral ventricle, and results in more new neurons and glia in the olfactory bulbs and striatum, respectively. The increase in neural stem cells by haloperidol does not occur in dopamine D2 receptor null mice and peripheral D2 receptor antagonism does not alter neural stem cell number demonstrating that the effect of haloperidol on neural stem cells is dependent on central D2 receptors. D2 receptors are present in both adult lateral ventricle subependyma and in adult-derived neurospheres. Neurospheres are generated from cells FACS for D2 receptors demonstrating that neural stem cells express D2 receptors. Treatment of neurosphere cultures with dopamine or quinpirole significantly decreased the proliferation of neural stem cell from wildtype, but not D2 receptor null, mice and this effect is reversible by haloperidol. These data demonstrate that haloperidol increases cell proliferation in the adult mammalian brain by antagonizing dopamine at D2 receptors on neural stem cells. These findings implicate a role for cell genesis in the therapeutic effects of antipsychotic drugs.

BP8-04

Integration of transplanted neural progenitors in the brain of arylsulfatase a deficient mice

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Metachromatic leukodystrophy (MLD) is a dysmyelinating disease due to mutations in the arylsulfatase A (ARSA) gene. Central dysmyelination is consequent to oligodendrocyte cell death, likely due to the lysosomal accumulation of sulfatides. We tested the potential of oligodendrocyte progenitors (OPCs) and of multipotential neural progenitors (NSCs) to survive and integrate in the brain of mice affected of MLD. Two-day-old MLD pups received a single injection (105 cells/brain) of either LacZ + OPCs or GFP + NSCs in the lateral ventricle. One day after injection (1 dpi), LacZ + OPCs were detected within the delivery area in the MLD and control brains. However, LacZ+ cells were also detected in the contralateral MLD hemisphere, indicative of a robust migration in the mutant. At later time points, grafted cells migrated preferentially to the subventricular zone (SVZ), rostral migratory stream (RMS), olfactory bulb (OB), corpus callosum and cortex and to a lesser extent, to the thalamus, hypothalamus and cerebellum of the MLD brain. In wild type mice, LacZ + OPCs remained within the injection area in the ventricle but were not found in white matter areas or the SVZ-RMS-OB pathway. Most of LacZ + OPCs that migrated in the MLD SVZ-RMS-OB pathway remained undifferentiated while most of the LacZ+ cells that moved in the cortex and corpus callosum differentiated into PLP+/MBP+ myelin associated cells. GFP + NSCs showed similar patterns of migration but additionally, they robustly migrated to cortical areas where they adopted neuronal morphologies. Treatment with these cells led to a mild amelioration of central nerve conduction. These findings demonstrate that transplanted neural progenitors can survive, migrate and differentiate in the brain of MLD mice.