Program/Abstract # 281
Notch activation is a more robust gliogenic inductor than leukemia inhibitory factor in rat brain cortex neural stem cells
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Neural stem cells (NSC) self-renew and generate specialized neural cell types. In vivo NSC generate neurons first and glial progeny later. Notch is involved in fate determination of NSC, either preventing differentiation, or promoting glial fates. We used retroviral vectors containing the active intracellular domain of Notch1 fused to green fluorescent protein (ICN1-GFP) to establish Notch effects in NSC, and compare them with those of leukemia inhibitory factor (LIF), a known inducer of astrocytic differentiation. Proliferating NSC from E14 rat cerebral cortex were treated with LIF (1000 U/ml), or transduced with control (GFP) or ICN1-GFP retroviruses, and then allowed them to differentiate. During proliferation, about 93% of ICN1-GFP transduced cells expressed nestin, a NSC marker, and 28% of this population co-expressed glial fibrillary acidic protein (GFAP; astrocytic marker). Morphologies resembling those of astrocytic cells were observed in NSC expressing ICN1-GFP. In contrast, 98% of cells transduced with the control virus were positive to nestin, and less than 1% of these cells co-expressed the differentiation markers GFAP or β-Tubulin III (neurons). Under differentiation conditions, we found a ratio of astrocytes/neurons of 0.83 with the control vector, while in cells expressing ICN1-GFP, this ratio was 149; for cells treated with LIF the ratio was 7.5. These data show a direct relationship between Notch1 activation and astrocytic differentiation in NSC, and that Notch activation is a stronger gliogenic stimulus than LIF.

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Program/Abstract # 282
Generation of transgenic mouse embryonic stem cells that express neurogenin 1
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Neuronal differentiation is essential for the generation of the central nervous system (CNS). Stem cell differentiation is influenced by transcription factors expression that regulates essential genes in neuronal fate specification. During CNS development, proneural and differentiation basic helix–loop–helix (bHLH) factors are expressed at low levels initially, and its expression increase during neurogenesis. Neurogenin1 (Ngn1) is a member of the bHLH family that is classified as a proneural gene. Ectopic expression of Ngn1 promotes neuronal differentiation in neural stem cells from the cerebral cortex. The aim of this work was to generate transgenic embryonic stem (ES) cell lines that over-express Ngn1. ES cells are blastocyst-derived pluripotent cells that do not express neuronal markers under normal conditions. We constructed expression vectors that contain Ngn1 labelled with a c-myc epitope. These vectors were linearized and introduced by electroporation in mouse ES cells. Electroporated cells were cultured for 7 days in the presence of neomycin, resulting in 9 resistant colonies. Cells were expanded, fixed and immunostained with Oct-4, a transcription factor associated with pluripotency, with c-myc to confirm Ngn1 transgenic expression, and with anti-Ngn1. We also analyzed the presence of Ngn1 by c-myc immunoblot. Six of the transgenic ES cell lines expressed Oct-4, c-myc and Ngn1. These Ngn1 over-expressing cells will be useful to establish if pluripotent ES cells can be committed to neuronal fates by proneural gene expression.

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Program/Abstract # 283
Mediators of Hoxb4 hematopoietic-promoting activity
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Hematopoietic stem cells (HSCs) are responsible for the generation of all cell types within the hematopoietic lineage. HSCs must maintain a balance between self renewal and differentiation mechanisms. Several genes were described to be involved in these processes and some of them were directly related to the hematopoietic niche. Class I Homeobox (Hox) genes encode a family of transcription factors implicated in specifying positional identity and tissue fate in the embryo. They are also expressed postnatally, and several of them in primitive hematopoietic cells and committed progenitors. It has been shown that induction of Hoxb4 expression in embryonic stem (ES) cell-derived embryoid bodies (EBs) increased HSCs proliferation. To explore downstream effectors of Hoxb4 in this process we performed an Affymetrix GeneChip screen comparing Hoxb4-induced and -uninduced EBs. Genes belonging to the cell signalling, cell cycle and apoptosis categories were shown to be regulated by ectopic expression of the Hox gene. Next, tetracycline-inducible transgenic ES cell lines were produced with some of the candidate target genes and their effects on the generation of hematopoietic progenitors studied in vitro using the methylcellulose colony-forming assay. Overall, we presented here new effectors of Hoxb4 hematopoietic activity that will assist to elucidate the precise mechanism of action of this gene during expansion of early hematopoietic progenitors derived from ES cells.

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Program/Abstract # 284
Defining the earliest sites of definitive hematopoiesis based on Runx1 expression