Wnt signaling and Shh may orchestrate floor plate neurogenesis, or lack thereof.

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Program/Abstract # 423
Functional analysis of CSPG in Danio rerio
Morris K. Jacqueline, Dan Hill, Brian Morningstar, Amanda Swenson, Sutherland Laura
Department of Biology, Baldwin-Wallace College, Berea, OH, USA

Oligodendrocyte progenitor cells (OPCs) have been identified in mammalian central nervous system by the expression of a chondroitin sulfate proteoglycan (designated CSPG4 in the human genome and NG2 in other vertebrates). Although several myelin genes have been identified in Danio rerio, NG-2 positive OPCs have yet to be identified in the adult teleost brain. In order to identify these cells BLAST analysis with a rat CSPG-4 protein sequence was conducted using the Ensemble Blast site of the Danio rerio genome sequencing project at Sanger. CSPG-4 was identified and syntenic to the human CSPG4. The entire sequence of the NG-2 cDNA has been confirmed through RACE analysis. Expression of CSPG4 was confirmed in Zebrafish adult brain using RT-PCR and in situ hybridization. Adult Zebrafish brains were positive for CSPG4 gene expression in a subset of cells suggesting that OPCs similar to mammalian OPCs are present in the Zebrafish brain. Additionally in situ hybridization demonstrated expression of CSPG-4 between 18 and 24hpf embryos in more distinct regions of somites. CSPG-4 gene expression has also been detected in mesenchymal condensations of developing chondrocytes within the jaw at 48hpf. By 4days post fertilization, CSPG-4 gene expression is downregulated in chondrocytes. In order to determine a function for CSPG-4 in developing chondrocytes and oligodendrocyte progenitors, morpholinos targeted to the CSPG-4 gene were injected into one-celled embryos. The morphology of the cranial skeleton was not altered by the morpholino knockdowns suggesting redundancy or nonessential function for CSPG-4 in Zebrafish chondrogenesis.

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Program/Abstract # 424
Knockdown of PAPSS1 expression in an inducible RNAi mouse model demonstrates the requirement for sulfation in the developing neocortex
Leslie A. King, Mauricio Cortes, Elviche Tsakem, Nancy B. Schwartz
Department of Pediatrics, The University of Chicago, Chicago, IL, USA

Sulfation of macromolecules is known to modulate a number of cellular processes from protein-protein interactions to growth factor signaling. The universal sulfate donor in the cell, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), is synthesized by two isoenzymes, PAPSS1 and PAPSS2, which are each expressed in a tissue- and temporal-specific manner. PAPSS1 is the predominant isoform expressed in the developing brain; therefore, to determine the importance of cellular sulfation during normal brain development, we developed a single-vector inducible RNAi construct containing the H1 promoter regulated by the tetracycline repressor flanked with loxp sites. Generation of transgenic mice expressing this construct and subsequent crossing with Nestin-Cre mice generated mice which express an RNAi hairpin directed to PAPSS1 in Nestin-expressing cells. Radiometric activity assays of whole brain lysates from these mice at E15.5 revealed an 85 reduction in PAPS synthetase activity over control mice, indicating the balanced production. LIM-only protein LMO4 controls this binary cell division and/or differentiation. Together these observations suggest distinct neuronal subtypes arise from progenitors with distinct cell division modes. Functional analysis of Ascl1 in these two distinct lineages is being examined using a conditional mutant allele of Ascl1.

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Program/Abstract # 425
Apical and basal Ascl1 (Mash1) progenitors in the dorsal neural tube contribute differentially to inhibitory and excitatory neuronal populations in the spinal cord
Euiseok J. Kim, Jane E. Johnson
Department of Neuroscience, U of Texas Southwestern Medical Center, Dallas, TX, USA

The dorsal spinal cord comprises a network of inhibitory and excitatory neurons that are required for relaying somatosensory information. The majority of these neurons (dILA, inhibitory and dILB, excitatory) arise at a relatively late phase of neurogenesis from a common progenitor domain defined by the neural BHLH transcription factor, Ascl1 (Mash1). Here we redefine this Ascl1+ progenitor domain into at least two subdomains (apical and basal) in the neural tube and show that these two spatially separable Ascl1+ lineages contribute differentially to inhibitory and excitatory neuronal populations. To reveal this novel lineage relationship, we generated two distinct inducible Ascl1-CreER transgenic mouse lines. In a line where CreERT2 was knocked into the Ascl1 locus, expression of CreERT2 recapitulates a complete Ascl1+ pattern. Whereas, a BAC transgenic line with CreERTM replacing Ascl1 in the BAC preferentially marks a basal subdomain at the lateral edge of the ventricular zone. Lineage analysis from these two Cre lines reveals that inhibitory neurons are generated from the apical subdomain of the Ascl1+ progenitor pool whereas excitatory neurons are generated from both the apical and basal subdomains. In addition, Insm1, a marker for symmetrically dividing neuronal progenitors, is restricted to the basal subdomain. Together these observations suggest distinct neuronal subtypes arise from progenitors with distinct cell division modes. Functional analysis of Ascl1 in these two distinct lineages is being examined using a conditional mutant allele of Ascl1.

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Program/Abstract # 426
LMO4 controls the balance between excitatory and inhibitory spinal V2 interneurons
Soo-Kyung Leea,c, Seunghee Leea,b,c, Bora Leeb,c, Jae W. Leed,c
aMolecular and Human Genetics, Baylor College of Medicine, USA
bMolecular Cellular Biology, Baylor College of Medicine, USA
cNeuroscience, Baylor College of Medicine, USA
dProgram in Developmental Biology, Baylor College of Medicine, USA

Multiple excitatory and inhibitory interneurons form the motor circuit with motor neurons in the ventral spinal cord. Notch signaling initiates the diversification of immature V2-interneurons into excitatory V2a-interneurons and inhibitory V2b-interneurons. Here we provide a transcriptional regulatory mechanism underlying their balanced production. LIM-only protein LMO4 controls this binary cell fate choice by regulating the activity of V2a- and V2b-specific LIM-complexes inversely. In the spinal cord, LMO4 induces GABAergic V2b-interneurons in collaboration with SCL and inhibits Lhx3 from...