ORAL-02-01

TRANSCRIPTIONAL CONTROL OF GLIOGENESIS IN THE DEVELOPING MOUSE BRAIN AND IN GLIOBLASTOMA

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The transcription factor *Nuclear Factor One b (Nfib)* regulates gliogenesis within the developing mouse forebrain, but the mechanism underlying this process remains unknown. Here we show that *Nfib* promotes gliogenesis via repression of *Ezh2* expression. *Ezh2*, which encodes a chromatin modifying protein of the Polycomb complex, contributes to the repression of differentiation-specific genes epigenetically, via the tri-methylation of lysine residues on Histone H3. *Nfib-/-* mice fail to form mature glia within the forebrain, and levels of *Ezh2* are significantly up-regulated in these mice. NFIB binds to the promoter of the *Ezh2* gene *in vivo*, and is able to repress *Ezh2* promoter-driven transcriptional activity *in vitro*. Furthermore, the increased *Ezh2* expression in *Nfib-/-* mice culminates in downstream epigenetic chromatin modifications, including increased repressive histone modifications on tumour suppressor genes such as *CDKN2A*. Finally, alterations in *Nfib* expression may also be relevant to glioblastoma multiforme (GBM), the most aggressive form of brain cancer. Significantly, elevated levels of *Ezh2* are a known hallmark of GBM. *Nfib* is markedly down-regulated in human GBM and over-expression of *Nfib* in GBM cell-lines attenuates their proliferative capacity. Importantly, *Nfib* over-expression also induces a transient reduction in *Ezh2* expression and results in increased expression of glial differentiation-specific genes. Thus, these studies provide a mechanistic insight into how *Nfib* drives gliogenesis via repression of *Ezh2*, a finding with relevance both to the developing mouse forebrain and to human brain cancer.

ORAL-02-02

OLFACTORY ENSHEATHING CELLS PROLIFERATE FROM STEM CELLS AFTER INJURY

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Olfactory ensheathing cells (OECs) support the regeneration of olfactory sensory neurons throughout life. However, it remains unclear how OECs respond to a major injury and whether stem cells give rise to new OECs in those conditions. **Purpose**: To identify where OECs proliferate in the olfactory pathway and the regions to whic they migrate. **Methods**: We examined the proliferation and migration of OECs by surgically removing an olfactory bulb from neonatal mice. The outer layer of the olfactory bulb, the nerve fibre layer, is rich with OECs and thus bulbectomy removed the OECs of the olfactory bulb. The peripheral region of the olfactory nerve within the nasal cavity and the olfactory epithelium where the stem cells are located remained untouched. Proliferating cells were labelled by the thymidine analogue, ethynyl deoxyuridine (EdU) at different days after surgery (days 0-14) and animals (n=3 at each time point) were harvested either 4 hr later or up to 14 days later. **Results**: In the unilateral bulbectomy model, there was a large stimulation of OEC proliferation throughout the olfactory nerve up to 14 days after bulbectomy. Tracking cells that had proliferated revealed that stem cells lining the basal layer of the olfactory epithelium also gave rise to OECs that subsequently migrated along the length of the olfactory nerve. **Conclusion**: These results demonstrate that OECs actively respond to widespread degeneration of olfactory axons and that both local proliferation of OECs as well as stem cells give rise to new OECs that migrate along the olfactory nerve to the regions of need.

ORAL-02-03

THE ACTIVATION OF NEURAL PRECURSOR CELLS DURING PHYSICAL EXERCISE IS DEPENDENT UPON THE INVOLVEMENT OF THE GH ACTIVATION PATHWAY

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Purpose: There is growing evidence that growth hormone (GH) plays multiple roles within the central nervous system. Utilising a mutant mouse model we recently demonstrated a possible function of GH in the activation of neural precursor cells during voluntary exercise in the subventricular zone (SVZ) of aging animals. Here we examine wild-type mice to further define the pathway by which GH activates neural precursor cells in vivo and in vitro. **Methods**: Cohorts of 12 month-old female C57Bl/6 mice were placed into cages with or without access to running wheels. In order to examine changes in precursor numbers along the ventral neuraxis we employed a technique whereby serial coronal sections were collected by vibratome and prepared for the neurosphere assay. **Results**: Addition of GH to dissociated SVZ cells increased neurosphere number (696±14 vs. 958±27 per animal, $p<0.01$) whereas addition of a competitive GH antagonist, G120R, abolished this effect (667±47). To directly examine the effect of blocking the GH activation pathway during exercise, 12 month-old mice were implanted with osmotic pumps containing the GH antagonist. Following 21 days exercise, neurosphere numbers from vehicle only controls increased from 834±7 to 1320±43 ($p<0.01$) whereas GH antagonist infusion prevented this increase (675±67). **Conclusion**: These findings demonstrate that GH is essential for the exercise-dependent activation of SVZ precursor cells within the adult brain. This may present a novel method where neural precursors can be activated within aging and diseased brains to prevent precursor cell loss and demonstrates a mechanism by which activation is possible.

ORAL-02-04

TROPOMYOSINS INDUCE DIFFERENTIATION OF B35 NEUROBLASTOMA CELLS AND CONTROL NEURITE BRANCHING AND GROWTH CONE MORPHOLOGY

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The regulation of the actin cytoskeleton is critical in early mechanisms of neuronal differentiation and neurite formation. The dynamics and structural properties of the actin cytoskeleton are regulated by a number of actin associated proteins, including tropomyosin (Tm). In neurons, products from three different Tm genes (α-, γ- and δ-Tm gene) are expressed. **Purpose**: The current study aims to understand the role of α-, and δ-Tm gene products in early neuronal development. **Methods**: We used B35 neuroblastoma cells to investigate the impact of the actin cytoskeleton on neuronal morphology and early stages of neurite formation using stable clones of B35 cells overexpressing the Tm isoforms TmBr1, TmBr2, TmBr3 (α-Tm gene products) or Tm4 (δ-Tm gene product). **Results**: Our data show that overexpression of Tms is sufficient to induce neurite formation associated with an upregulation of the neuronal differentiation marker MAP2c. Tm gene products differentially control elongation and branching of processes in cAMP stimulated B35 cells. TmBr2 is the only Tm isoform that increases neurite length. TmBr1 attenuates while TmBr3 and Tm4 increase the degree of branching as compared to control B35 cells (n=450). The differential impact of Tm isoforms on neurite branching is associated with an isoform dependent change of growth cone size: TmBr3 and Tm4 induce an increase while TmBr1 induces a decrease in size. **Conclusion**: This association implicates Tms in the regulation of neurite branching by impacting on the actin cytoskeleton in the growth cone compartment. Our work provides strong evidence for a central role for Tms in neuronal differentiation and the establishment of complex neuronal networks.