Undergraduate Research Spotlights

Molecular Biology of ASPM and Possible Insight into the Evolution of the Cerebral Cortex

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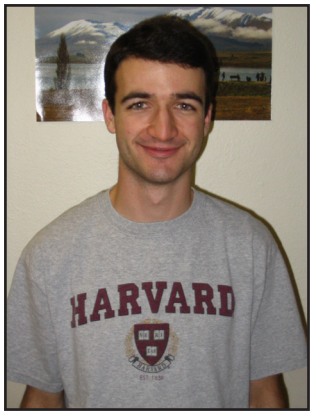
The development of the cerebral cortex is a highly defined and regulated spatial and temporal sequence of events. Dysfunctional neurogenesis during proliferation affects eventual brain size and results in a condition called microcephaly, meaning "small brain." The effect is a 70 percent reduction in brain size with the cerebral cortex being most severely affected. Research suggests that microcephaly may be due to a reduced production of neurons. The genes that are linked to microcephaly may offer potential insight into the development and evolution of the cerebral cortex. ASPM is a gene involved in the regulation of neurogenesis and is found to be mutated in human microcephaly with seizures. The ASPM protein is conserved across species between mammals, Drosophila and nematodes. It is hypothesized that ASPM may be one of the genetic components underlying the expansion of the human brain, and that the positive selection of parts of ASPM is related to the differences in cerebral cortical size found between different species. I am studying the effect of loss of ASPM in a strain of ASPM knockout mice. My hypothesis is that neuronal differentiation occurs earlier in ASPM knockout mice, so I expect that the upper layers of the cortex that form later will be thinner in the knockouts. In the cortex of mice at postnatal day 4, the thickness of upper layers 2-4 as indicated by the Cux-1 stain in the knockout mouse seems to be thinner than in the wild-type mouse by about 10 percent. This would suggest that neuronal-glial precursors in the knockout mouse are differentiating into neurons sooner than in the wild-type mouse. Further avenues of study include the continued characterization of the difference between ASPM knockout and wild-type mice, analyzing the mutant phenotype and studying the role of ASPM in effecting this phenotype. H

Conjoint analysis: A new method of capturing implicit stereotypes



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Area of study: Psychology
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Much research has been conducted on the topic of implicit attitudes – attitudes that people are not completely aware of and can sometimes be opposite to one's explicit beliefs. Traditional methods of measuring implicit attitudes have limitations, such as restricted predictive ability, which necessitate the invention of new measuring techniques. We hypothesized that conjoint analysis, an already well-developed marketing research method, has the capability of capturing implicit stereotypes. In a conjoint study, people are asked to make a series of choices between different products that have various attributes (for instance, varying price levels). We also adapted the traditional procedure to include a socially sensitive attribute. In one study, we asked 105 college students to choose a partner for a trivia game. Possible partners varied on 4 dimensions: education, intelligence, previous experience with the game, and their weight. We manipulated the last dimension using software that allowed us to manipulate people's appearance as either thin or overweight in different parts of the study.

The results showed a strong preference for thin people. On average, people were willing to give up 9 IQ points in their partner's intelligence in exchange for a thin appearance. However, when explicitly asked in the end of the procedure, people claimed that weight made little or no difference. The study gives support to the hypothesis that conjoint analysis can reveal implicit stereotypes. H

Undergraduate Research Spotlights

Transcription Factor AP-2 expression in mouse mammary epithelial cells

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The AP-2 transcription factor family contains five members, and mutant mice lacking them exhibit lethal phenotypes. Levels of AP-2α and γ in vivo are constantly high during pregnancy, decline and are no longer detectable during lactation (Zhang et al. 2003), but increase again during involution. Transgenic experiments show that AP-2 overexpression impairs secretory differentiation, which is necessary for lactation, and increases apoptosis, which is necessary for the completion of involution.

Using a dexamehtazone-insulin-prolactin(DIP) medium to stimulate in vitro cell differentiation, we observed that HC11 cells fail to simulate the in vivo pregnancy or lactation. Specifically, cells treated with DIP for 72h showed no reduction in AP-2 levels as compared to the undifferentiated state. Examining the presence of pregnancy markers beta-casein and wap by Northern Blot yielded interesting results. Beta-casein, a good marker for early pregnancy, was detected in DIP-treated HC11 cells; however expression levels of beta-casein in DIP-treated cells were much lower than those observed in day 13.5 of pregnancy or in the lactating gland. Wap, a good marker of late pregnancy, was undetectable in the DIP-treated cells, indicating that the cells had not undergone full differentiation. We conclude that DIP-induced in vitro differentiation does not faithfully simulate the secretory differentiation achieved in vivo during pregnancy and lactation. The cells were readily susceptible to transfection with plasmids containing the G418 resistance gene under control of the PGK promoter or RSV-LTR using lipofection, and easily apoptosed under serum and insulin starvation. In contrast, mouse fibroblasts do not undergo apoptosis in conditions lacking serum or insulin. (Evan et al. 1992)

Given the results of transgenic AP-2 expression experiments, it seems possible that AP-2 is an inhibitor of differentiation in the HC11 cells. To determine this, the AP-2 genes must be silenced by siRNA separately. Then HC11 cells have to be examined, to determine if they can undergo full differentiation. The greatest step, of course, in accelerating the study of the relationship between AP-2 levels and differentiation would be to generate a suitable 3-D cell culture system simulating the in vivo state. H

Cancer Stem Cells in Zebrafish Model of Human Melanoma

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Recent evidence suggests that a rare population of tumor cells--the cancer stem cells--is responsible for giving rise to the cells in the tumor mass. These cancer stem cells are thought to arise from the transformation of normal tissue stem cells. Using a zebrafish model of the human skin cancer melanoma, we asked whether embryonic development can give us insights into the earliest events in transforming normal stem cells into cancer stem cells. Discovery of novel signaling pathways required for the development of melanoma stem cells may contribute towards finding a therapeutic treatment of human melanoma. Previous work has demonstrated the critical role of the notch signaling pathway in the development of normal tissue stem cells, including those of the melanocyte lineage from which melanoma is ultimately derived. We therefore hypothesize that alterations in this pathway occur early in embryogenesis in zebrafish that highly dispose the organism toward development of adult melanomas. We are analyzing embryonic expression of several genes in the notch pathway to determine if their increased expression is related to the eventual development of tumors in adults. Through whole-mount embryo in situ hybridization, we can directly observe anatomic localization of cells expressing these genes, which can then be correlated to locations where tumors eventually develop months later. In examining gene expression in melanoma prone BRAF/p53 mutant zebrafish, we find a 3-fold greater expression of notch1b, by as early as the first 24 hours of life. Further downstream in the pathway, the expression of the notch dependent gene crestin is markedly disorganized, and appears to be strongly related to the tumors that develop in adults. Given our findings, this pathway may represent an attractive target for the treatment of human melanoma. H