

FATE DECISIONS DURING NEURAL CREST ONTOGENY

Chaya Kalcheim

*Department of Medical Neurobiology, Hebrew University of Jerusalem and Hadassah
Medical School*

The dorsal neural tube first generates neural crest cells that exit the neural primordium following an epithelial-to-mesenchymal conversion to become sympathetic ganglia, Schwann cells, dorsal root sensory ganglia and melanocytes of the skin. Following the end of crest emigration, the dorsal midline of the neural tube becomes the roof plate, a signaling center for the organization of dorsal neuronal cell types. Recent lineage analysis performed before the onset of crest delamination revealed that the dorsal tube is a highly dynamic region sequentially traversed by fate-restricted crest progenitors. Furthermore, prospective roof plate cells were shown to originate ventral to presumptive crest and to progressively relocate dorsalward to occupy their definitive midline position following crest delamination. These data raise important questions regarding the mechanisms of cell emigration in relation to fate acquisition, and suggest the possibility that spatial and/or temporal information in the dorsal neural tube determines initial segregation of neural crest cells into their derivatives. In addition, they emphasize the need to address what controls the end of neural crest production and consequent roof plate formation, a fundamental issue for understanding the separation between central and peripheral lineages during development of the nervous system.

A GENETIC APPROACH FOR INTRA-CEREBRO-VENTRICULAR DELIVERY

Alon Chen

Department of Neurobiology Weizmann Institute of Science, Rehovot, Israel

Administration of synthetic or purified peptides directly into the brain ventricles is a method commonly used by neuroscientists for exploring physiological and behavioral functions of gene products. Intravenous administration is controlled by the blood-brain barrier, which limits its effectiveness, and current approaches for acute or chronic intra-cerebro-ventricular delivery have significant technical drawbacks resulting from both the chemical properties of the delivered substance, and the experimental procedures.

This lecture will describe a genetic approach for the delivery of secreted peptides or proteins into the cerebrospinal fluid (CSF) using a choroid plexus-specific and lentiviral-based genetic system. Using a choroid plexus-specific promoter, we established a lentiviral-based system, which offers inducible and reversible delivery of a gene product into the CSF. The system is composed of two complementary lentiviral vectors. The 'Effector' construct consists of a choroid plexus-specific promoter that drives the expression of reverse tetracycline trans activator (rtTA) protein and the reporter green fluorescent protein (GFP). The 'Target' construct includes the tetracycline-responsive element (TRE) DNA sequence, upstream to the nucleotide coding sequence of the requested gene of interest, followed by the reporter red fluorescent protein (RFP). Transcription initiation of the gene of interest and the RFP is mediated only in the presence of the inducer, doxycycline (Dox). A mixture of the two lentiviruses is injected ICV and the delivered genes are incorporated into the DNA of the choroid plexus cells. Initiation of transcription, limited to the choroid plexus cells by the choroid plexus-specific promoter, is induced by administering Dox-containing drinking water, and results in secretion of the final processed gene product into the CSF. The functionality of this system was demonstrated using the over-expression of the two established neuropeptides, corticotropin-releasing factor and gonadotropin-releasing hormone, modulating anxiety-like behavior and estrus cycle, respectively.

NEURODEGENERATION AND NEUROPROTECTION IN AN ANIMAL MODEL OF MULTIPLE SCLEROSIS

Stella Elkabes

Department of Neurological Surgery, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, NJ, USA

Multiple sclerosis (MS) is an inflammatory, neurodegenerative and demyelinating disease of the central nervous system. Despite consensus about the importance of axonal damage and neuronal death in MS, the underlying molecular mechanisms have not been well defined.

To elucidate the molecular events leading to neurodegeneration, we used experimental autoimmune encephalomyelitis (EAE), an animal model mimicking some features of MS. We investigated the contribution of plasma membrane calcium ATPase 2 (PMCA2) to neuronal pathology and death in the inflamed spinal cord. As PMCA2 is an essential calcium pump and PMCA2 expression is reduced in spinal cord neurons during EAE, we hypothesized that perturbations in PMCA2 levels could be a cause of neuronal damage. In agreement with this concept, silencing of PMCA2 expression induced death of spinal cord neurons. Knockdown of PMCA2 was followed by a decrease in collapsing response mediator protein 1 (CRMP1). CRMPs have been implicated in microtubule assembly and dendritic integrity and therefore, such changes could cause cytoskeletal and synaptic abnormalities leading to neuronal dysfunction and loss. Consistent with this notion, silencing of CRMP1 expression was followed by neuronal death, *in vitro*. CRMP1 expression was also decreased in EAE and administration of an AMPA/kainate receptor antagonist at onset or peak of the disease restored both PMCA2 and CRMP1 levels to control values and ameliorated clinical symptoms. Thus, perturbations in PMCA2 and CRMP1 expression could be additional mechanisms associated with AMPA/kainate receptor-mediated glutamate excitotoxicity in EAE.

Highlighting further the importance of PMCA2 in EAE, neurological deficits were more severe in PMCA2-heterozygous mice than in wild-type littermates. Accordingly, axonal loss was more pronounced in the spinal cord of PMCA2-heterozygous mice than wild type controls whereas the inflammatory reaction and glial activation did not show major differences. These findings support the notion that PMCA2 plays a critical role in neuronal injury during EAE.

INTERACTIONS BETWEEN CANCER GENES AND THE IGF-I SIGNALING PATHWAY

Haim Werner

Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University

The involvement of the insulin-like growth factors (IGF) in cancer biology has been the focus of extensive research. The IGF-I receptor (IGF-IR) is overexpressed in most malignant cells, where it displays potent antiapoptotic, pro-survival activities. Transcriptional regulation of IGF-IR gene expression constitutes a key control mechanism with important roles in normal growth control as well as in cancer development. Using a DNA affinity chromatography protocol linked to mass spectroscopic proteomic analyses we identified a number of nuclear proteins with oncogenic or antioncogenic properties that regulate IGF-IR transcription. Transcription factors with tumor suppressor activity, including p53, BRCA1, VHL, WT1, and others, were shown to negatively regulate IGF-IR expression. The etiology of neoplasias associated with *loss-of-function* mutation of tumor suppressors is, in many cases, linked to the inability of mutant forms to suppress IGF-IR gene transcription. In addition, we have recently identified a novel mechanism for IGF-IR autoregulation. Specifically, we showed that IGF-IR is localized in the nucleus of breast cancer cells. Furthermore, the IGF-IR (or fragments of the protein) binds to the IGF-IR gene promoter in an estrogen receptor-dependent fashion and controls IGF-IR gene expression. These data is consistent with the notion that the IGF-IR, in addition to its classical role at the cell membrane level, can also function as a transcriptional enhancer in breast cancer. Understanding the molecular basis of these complex interactions will be of significant value both in basic as well as translational terms.

MULTI-CELLULAR, MULTI-AGENT ANGIOGENIC CONTROL MECHANISMS: THE CORPUS LUTEUM AS A MODEL

Rina Meidan¹, Yulia Zalman¹, Klipper Eyal¹, Farberov Svetlana¹,
George W. Smith²

¹*Department of Animal Sciences, The Robert H Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, 76100 Israel*

²*Laboratory of Mammalian Reproductive Biology and Genomics, Michigan State University, East Lansing, MI 48824*

The corpus luteum (CL) lifespan is characterized by a rapid growth, differentiation and controlled regression, accompanied by an intense angiogenesis and angioregression, respectively. The short period of angiogenesis (until day 5 of cycle) is followed either by maintenance and stabilization of the vasculature during pregnancy, or controlled regression (induced by prostaglandin F2a ;PG) of the microvascular tree in a non-fertile cycle. Uncontrolled angiogenesis will cause reproductive defects and pathologies such as cancer, atherosclerosis and infectious diseases.

Using functional genomics we compared PG induced gene expression profiles in PG refractory (d 4 of estrous cycle) versus responsive (d 11) bovine CL. Interestingly, quite a few of the novel PG-regulated genes identified were related to angiogenesis (being either pro or anti-angiogenic): FGF2, pentraxin 3 (PTX3), thrombospondins (THBSs) and their cell-adhesion receptor (CD36). However, pro and anti-angiogenic factors were distinctly regulated by PG; there was a pronounced PG-induced upregulation of the FGF2 in d 4 CL while THBSs, CD36 and PTX3 were dramatically induced after PG in d 11 CL, associated with luteolysis. FGF2 is a well-known angiogenesis inducer while THBSs and PTX3 bind and inhibit FGF2 actions. We next examined if genes differentially expressed in d 4 vs d 11 CL were confined to a specific cell type. RNA was isolated from steroidogenic and endothelial cells (EC) enriched from the CL using magnetic beads. While some PG- regulated genes: NRG-1, *SELE* and *SELP* mRNAs showed cell-specific localization, *FGF2*, *PTX3*, *THBSs* were present in both steroidogenic and EC compartments of the CL. These results suggest a functional relationship between FGF2 activity and the luteolytic response. Moreover, administration of PTX3 and THBS-mimetic peptides into early stage CL may counteract FGF2 action and restore sensitivity to luteolytic actions of PG. The CL therefore provides a relevant model for studying the balance between angiogenic inducers and inhibitors.