YALE JOURNAL OF BIOLOGY AND MEDICINE 86 (2013), pp.397-402.

Yale School of Medicine Thesis Award Recipients — 2013

The Role of Fibroblast Growth Factors in Cortical Regeneration after Perinatal Hypoxia: A Model for Neurological Recovery in Premature Children. Devon Fagel. Louis G. Welt Prize.

Chronic perinatal hypoxia causes a significant loss of total brain volume, brain weight, and cortical neuron number. These measures are completely reversed following recovery in normoxic conditions. Yet, the cellular and molecular mechanisms underlying this plasticity are not well understood. Here, we show that hypoxia from postnatal days 3 (P3) to 10-11 causes a 30 percent decrease in cortical neurons and a 24 percent decrease in cortical volume. Excitatory neuron numbers were completely recovered 1 month after the insult, but the mice showed a residual deficit in GABAergic interneurons. In contrast, hypoxic mice carrying a disrupted fibroblast growth factor receptor-1 (*Fgfr1*) gene in GFAP+ cells [*Fgfr1* conditional knock-out (cKO)], showed a persistent loss of excitatory cortical neurons and an increased interneuron defect. Labeling proliferating progenitors at P17-18 revealed increased generation of cortical NeuN+ and Tbr1+ neurons in wild-type mice subjected to hypoxic insult, whereas *Fgfr1* cKO failed to mount a cortical neurogenetic response. Hypoxic wild-type mice also demonstrated a two-fold increase in cell proliferation in the subventricular zone (SVZ) at P17-18 and a three-fold increase in neurogenesis in the olfactory bulb (OB) at P48-49, compared with normoxic mice. In contrast, *Fgfr1* cKO mice had decreased SVZ cell proliferation and curtailed reactive neurogenesis in the OB. Thus, the activation of FGFR1 in GFAP+ cells is required for neuronal recovery after perinatal hypoxic injury. In contrast, there is incomplete recovery of inhibitory neurons following injury, which may account for persistent behavioral deficits in adult mice following early perinatal injury.

siRNA Therapy in Glioblastoma Stem Cells: Identification of Target Genes and Potential Therapeutic Implications. Benjamin Himes. Dr. Harold H. Lamport Biomedical Research Prize.

Glioblastoma multiforme (GBM), the most common primary brain malignancy, carries a grim prognosis: Survival statistics have scarcely improved in decades. Even with the development of temozolomide, the current front-line chemotherapeutic agent for GBM, improvement in long-term survival has been minimal, with recurrence virtually assured. One explanation for the persistence of this disease is the presence of a stem-like cell population within GBM (glioblastoma stem cells, or GSCs). These cells are capable of self-renewal and tumor initiation and are resistant to chemotherapy. We hypothesized that derangement in the expression of genes critical for the maintenance of GSCs could eliminate these cells outright or induce sufficient cell differentiation to sensitize them to existing chemotherapeutic agents. To this end, we performed a genome-wide small interfering RNA (siRNA) screen in search of genes that, when reduced in expression, cause GSC cell death or induce differentiation as measured by changes in nestin expression or cell morphology. Our screening yielded a number of candidate siRNAs. Their efficacy in reducing cell viability was demonstrated across a number of genetically distinct GSC cell lines. We further identified two siRNAs, targeting ubiquitin C (UBC) and disheveled 2 (DVL2), respectively, that significantly sensitize GSCs to the effects of temozolomide ($p < 0.05$). A similar but not significant effect was also observed in combination treatment with siRNA and either paclitaxel or doxorubicin. We conclude from these observations that siRNA-mediated gene knock-

down presents a promising avenue in the development of novel treatments for GBM by taking into account the unique biologic attributes of the therapeutically problematic GSC population.

Hippocampal Shape Abnormalities of Childhood-Onset Schizophrenia Patients and their Healthy Siblings. Sarah Johnson. Lidz Prize in Psychiatry. Reprinted with permission from Elsevier: Johnson SLM, Wang L, Alpert KI, Greenstein D, Clasen L, Lalonde F, Miller R, Rapoport J, Gogtay N. Hippocampal Shape Abnormalities of Patients with Childhood-Onset Schizophrenia and their Unaffected Siblings. J Am Acad Child Adolesc Psychiatry. 2013;52(5):527-36.

Objective: The hippocampus has been implicated in the pathogenesis of schizophrenia, with hippocampal volume deficits being a consistently reported abnormality in schizophrenia, although the sub-regional specificity of the deficits has not been characterized. We explored the nature and developmental trajectory of sub-regional shape abnormalities of the hippocampus in patients with childhood-onset schizophrenia (COS), their healthy siblings, and healthy volunteers.

Method: Two hundred fifty-five anatomic brain MRI scans were obtained from 103 patients with COS, 169 scans from their 79 healthy siblings, and 255 scans from 101 age- and gender-matched healthy volunteers, across ages 9 to 29 years. The hippocampus was segmented using FreeSurfer automated image analysis software, and hippocampal shape was evaluated by comparing subjects at over 6,000 vertices on the left and right hippocampal surfaces. Longitudinal data were examined using mixed model regression analysis.

Results: Patients with COS showed significant bilateral inward deformation in the anterior hippocampus. The healthy siblings also showed a trend for anterior inward deformation. The three groups did not differ in the trajectory of hippocampal shape or volume changes over time. Inward deformations in the anterior hippocampus were positively related with positive symptom severity, while outward surface displacement was positively related to overall functioning.

Conclusions: This is the first and largest longitudinal three-way analysis of sub-regional hippocampal shape abnormalities in patients with COS and their healthy siblings, compared to healthy controls. The abnormalities seen in the anterior hippocampus of patients with COS possibly suggest disturbed frontal-hippocampal connectivity. A trend toward mild overlapping shape abnormalities in the healthy siblings suggests a more subtle, subregionally specific neuroanatomical endophenotype.

Bisphenol-A Exposure in Utero Programs Adult Estrogen Responsiveness in the Uterus and Mammary Glands. Elisa Jorgensen. The Peter F. Curran Prize.

Bisphenol-A (BPA) is an environmentally ubiquitous estrogen-like endocrine-disrupting compound. Exposure to BPA in utero has been linked to female reproductive disorders, including endometrial hyperplasia, endometriosis, fibroids, and breast cancer. Estrogens have a role in the etiology of each of these conditions. Here we hypothesize that BPA exposure in utero leads to changes in developmental programming of estrogen responsive gene expression. Additionally, we examine methylation status of several altered genes with known estrogen response elements.

Eight pregnant CD-1 mice were continuously treated with BPA (5 mg/kg/day) or vehicle via osmotic minipump on days 9 to 18 of gestation. Two weeks after birth, the uteri of half the female offspring were isolated, and RNA was extracted. At 6 weeks, the remaining female offspring were oophorectomized, then at 8 weeks were treated with a single IP injection of 300 ng estradiol (E2) or vehicle, and the uteri were removed for RNA and DNA isolation. Total RNA was labeled and hybridized to a mouse BeadChip WG-6 expression microarray (Illumina). Genes showing statistically significant change (> 2-fold) versus control were verified using real time RT-PCR. Genomic DNA was enriched using MeDIP, then labeled and hybridized to a mouse Roche/NimbleGen 720K CpG Promoter Methylation Array.

At 2 weeks, global gene expression was remarkably similar among the control and BPA exposed groups. Of a total 45,000 genes examined, only 18 (10 upregulated and eight down-

regulated) showed changes in expression of two-fold or greater. After estrogen exposure at puberty (8 weeks), the expression profile was markedly changed. At baseline, a total of 365 genes (77 upregulated and 288 downregulated) showed altered expression in BPA-exposed offspring. With E2 treatment, expression of another 316 genes (90 up and 226 down) showed altered E2 response in BPA-treated offspring; this included several genes that were not previously regulated by estrogen (e.g., *Fzd10, Gdf10*) or that demonstrated an exaggerated response to estrogen treatment (*Ret, Wif1, S100a8*). Expression changes of those genes with the greatest fold change were verified by real-time RT-PCR. Of these genes with significantly altered expression at puberty, 208 also showed aberrations to the normal pattern of methylation, including changes to at least 14 genes with known ERE's (e.g., *G6pdx, Stat5a, Scd1*).

Significant changes in gene expression were observed in the uteri of mice exposed to BPA as a fetus; however, these differences became apparent only after endogenous estrogen exposure at puberty or with estrogen treatment. Gene-environment interactions driven by BPA alter the normal developmental programming of estrogen responsive gene expression in the uterus. Hyper-responsiveness to estrogens is a potential mechanism to explain the increased incidence of estrogen-related disorders seen after exposure to endocrine disruptors. Many of these changes may be mediated by alterations to genome-wide methylation patterns.

Male-Partner Participation in the Prevention of Mother-to-Child HIV Transmission in South Africa. Kevin Koo. David and Harriet Seligson Thesis Prize. Sponsored by the Department of Laboratory Medicine.

Programs for the prevention of mother-to-child HIV transmission (PMTCT) in sub-Saharan Africa have focused overwhelmingly on women, to the unintended exclusion of their male partners. Greater involvement of men may improve HIV-related outcomes through increased adherence to PMTCT, but a stronger understanding of the factors impeding male-partner HIV testing and disclosure is needed. A cross-sectional study using mixed methods was conducted in Tshwane, South Africa. In-depth interviews were held with men whose partners were recently pregnant while PMTCT was in effect, and focus group discussions were conducted with health care workers and community representatives. Of 124 men who participated, 94 percent believed male HIV testing was important, but 40 percent had never been tested. Of those tested, only 31 percent were tested during the pregnancy, while 37 percent were tested afterward. A man's likelihood of getting tested during his partner's pregnancy was associated with prior discussion with his partner about testing, knowing she had already been tested, and her disclosure of the test result (all $p < 0.05$). Qualitative analysis revealed formidable structural and psychosocial barriers to men's participation: the perception of PMTCT as not "male-friendly," a narrow focus at clinics on HIV testing instead of health promotion, and few opportunities for fathers to share decision-making roles. Three-quarters of the fathers reported that they would accept an invitation from their partners to attend the clinic for PMTCT; based on emergent themes, six partner invitation cards were designed, and 158 men and 409 women evaluated the cards. One invitation card depicting the themes of fatherhood and the baby was selected by 41 percent of men and 31 percent of women as the most likely for women to give to their male partners and the most successful at encouraging men to be tested. This is the first study to address barriers to HIV testing in PMTCT from the perspective of male partners. Improved partner communication and clinic attendance using a partner invitation card intervention could facilitate more robust male-partner participation and help PMTCT programs in resource-poor settings to achieve their full potential.

Improvement of Asthma Control and Inflammation in Pediatric Patients Undergoing Adenotonsillectomy. Jonathan Levin. John P. Peters Prize.

Observational studies have suggested improvement in asthma control after adenotonsillectomy, but longitudinal studies that correlate the effect of the procedure on markers of airway inflammation with changes in asthma control are limited. We conducted a longitudinal, observational study on 130 pediatric patients undergoing adenotonsillectomy, including 66 with

asthma and 64 control subjects. Asthma Control Test (ACT) scores, chitotriosidase (CHIT1) activity, and YKL-40 (CHI3L1) levels in the circulation were measured at the time of surgery and at a 6-month follow-up visit, and genotypes of chitinase family proteins were measured at baseline. Gene expression data was analyzed from blood, tonsil, and nasal epithelial tissue samples at baseline and in the blood at follow-up by microarray analysis. Mean ACT scores improved by 3 points ($p < 0.001$) after 6 months. Eighty-five percent of children with poorly controlled asthma demonstrated an increase in ACT score of at least three points or a decrease in Emergency Department/Urgent Care visits, oral corticosteroid courses, or rescue short acting bronchodilator usage. Serum chitinase activity decreased significantly in children with asthma ($p < 0.01$), but not in children without asthma ($p = 0.83$) undergoing tonsillectomy. Higher chitinase activity levels at baseline were associated with improved asthma control following surgery in all children with asthma ($p < 0.01$) and in the subgroup of children with poorly controlled asthma ($p < 0.05$). Subjects with asthma had a higher allele frequency of the CHIT1 mutation ($p < 0.02$). Gene expression analysis identified a number of inflammatory genes differentially expressed in children who had improved asthma control that were not changed in children without improved control and control subjects. Of particular interest was SerpinB2, a plasmin activation inhibitor previously implicated in asthma, significantly downregulated after surgery compared to baseline in children with improved control. This data suggests that adenotonsillectomy improves asthma control by modulation of airway inflammation. Elevated serum chitinase activity may be a clinically useful determinant to identify patients with poorly controlled asthma that will benefit from the procedure.

Computer-Assisted Detection of Proximal Arterial Stenosis on Doppler Ultrasound. John Millet. The Miriam Kathleen Dasey Award and the Dr. Marvin Moser Prize.

The primary aim of this study was to determine if use of a novel computer-generated quantitative measure, effective acceleration time (effAT), can improve diagnostic accuracy for detecting a proximal arterial stenosis on spectral Doppler ultrasound.

This was a retrospective, case-control study, whereby aortic stenosis (AS) was used as a model to detect distal tardus parvus (TP) physiology. Patients with echocardiography-confirmed AS ($n = 132$; 60 mild, 44 moderate, 28 severe) and matched controls ($n = 48$) who underwent carotid ultrasound within 90 days were identified through a diagnostic imaging database at a single medical center. A custom-built computerized spectral analysis program generated effAT values for spectral Doppler waveforms in the extracranial carotid arteries, and a receiver operating characteristic (ROC) analysis was performed to determine the optimal median effAT cutoff value to detect AS. Two radiologists, blinded to subject disease status, reviewed all carotid sonograms for presence of TP waveforms. Inter-rater variability was measured, and the accuracy of the radiologists to detect AS with and without use of the effAT cutoff was calculated.

There were no significant differences between cases and controls with regards to age, sex, body mass index, or ejection fraction. Accuracy of radiologist detection of AS via waveform interpretation ranged from 43 to 61 percent. Inter-rater agreement in the detection of TP waveforms was 76 percent (136/180 cases, $K = 0.44$, $p < 0.001$). ROC analysis revealed an optimal effAT cutoff of ≥ 48 ms to detect AS with a corresponding area under the curve of 0.77 (95 percent CI: 0.75-0.84). Use of the effAT cutoff independent of radiologist waveform interpretation demonstrated an accuracy of 72 percent.

Radiologist detection of a proximal arterial stenosis though visual interpretation of spectral Doppler waveform morphology is limited by low accuracy and moderate inter-observer variability. Use of a computer-generated median effAT cutoff value markedly improves diagnostic accuracy and eliminates observer variability.

Intraocular Pressure, Aqueous Humor Dynamics, and Fibrosis using a Novel Glaucoma Drainage Pathway. Julius Oatts. Association for Academic Surgery-Novartis Research Award.

The purpose of this study was to compare fibrosis, aqueous humor dynamics, and intraocular pressure (IOP) of two suprachoroidal shunts that are part of a new class of glau-

coma drainage devices. After *in vitro* testing, 20 rabbits were implanted with either a gold shunt (GS, GMSplus+, Solx) or polypropylene shunt (PS, Aquashunt, OPKO). Ten eyes received mitomycin C (MMC) and triamcinolone. Peak and trough IOP were monitored with a pneumatonometer and tono-pen through 15 weeks. Aqueous humor dynamics were evaluated fluorophotometrically and tonographically. Fibrosis was quantified using ImageJ. *In vitro* growth was similar. *In vivo*, both shunts were devoid of foreign body reaction but exhibited fibrosis, and GS showed vascularization. There was no significant difference in aqueous or uveoscleral flow. Preoperative morning IOP was 23.7 ± 2 mm Hg and evening IOP was 26.5 ± 2 mm Hg ($p = 0.000$). Morning IOP was decreased through 15 weeks and evening IOP through 8 weeks in all groups. The morning IOP decrease was most profound at 15 weeks in PS (41 percent) compared to GS (18 percent). Antifibrotics initially enhanced but eventually diminished shunt performance. At 15 weeks, thickness of scleral fibrosis was greater in GS (246 ± 47 μ;) and PS (188 ± 47 μ, $p = 0.285$) compared with GS+MMC (109 ± 26 μ, $p = 0.023$ to GS) and PS+MMC (48 ± 30 μ, $p = 0.028$ to PS). In a rabbit model, suprachoroidal polypropylene and gold shunts allow access to a new drainage pathway with different IOP profiles that can be modified with antifibrotics.

Childhood Cancer in the Cinema: How the Celluloid Mirror Reflects Psychosocial Care. Jovana Pavisic. Nicholas J. Giarman Prize.

Film is a powerful medium for sharing illness narratives and can exert a significant influence on public medical discourse. The childhood cancer narrative has more recently emerged on the screen, and these images have yet to be analyzed. This study aims to evaluate the childhood cancer experience in commercially produced, readily available films with a character with childhood cancer, with a particular focus on psychosocial care. Twenty-nine films were reviewed, using quantitative and qualitative content analysis to identify the medical and psychosocial characteristics of the cinematic childhood cancer experience. Psychosocial support was rated on a five-point scale (0-4) based on the availability and efficacy of support characters in the categories of non-professional internal (e.g., parent), non-professional external (e.g., friend), professional medical (e.g., oncologist), and professional psychosocial (e.g., social worker) supports. Main themes were identified and described, and relevant scenes were extracted into an educational DVD. Film depicts an unrealistic, bleak picture of childhood cancer, with a 66 percent mortality rate among the 35 characters evaluated. A range of psychosocial stressors are reflected that are consistent with those experienced in reality. Psychosocial support is limited to resources already available to families prior to the cancer diagnosis: the average support rating across all 29 films is 2.4 for non-professional internal and external supports, 1.6 for professional medical supports, and 0.3 for professional psychosocial supports. Seven themes emerged on the screen: disruption, social impact, psychological impact, physical toll, struggle/war/fight, coping, and barren landscape. Images of an isolated family courageously battling cancer alone with limited support from a treatment team solely dedicated to medical care is emphasized. In conclusion, cinema highlights the struggle between life and death in pediatric cancer, but minimizes the importance of the psychosocial dimension of care, which can perpetuate the stigma that exists around psychosocial interventions. These films, and the included DVD, can be used to encourage discussion among medical providers about how to optimize psychosocial care in pediatric oncology so that such care is not abandoned in actual practice as it is, for entertainment purposes, on the screen.

Biological and Clinical Markers of Neuronal Injury in Primary and Chronic HIV-1 Infection. Michael Peluso. William U. Gardner Prize.

The use of antiretroviral therapy (ART) has shifted the neurological manifestations of HIV-1 infection toward mild but debilitating HIV-associated neurocognitive disorder

(HAND). Through two studies, we sought to characterize neuronal injury during primary and chronic HIV infection and to describe its relationship with HAND.

The aim of the first study was to quantify cerebrospinal fluid (CSF) and neuroimaging biomarkers of neuronal injury in primary HIV infection (PHI). We compared CSF levels of neurofilament light chain (NFL), tau, and amyloid proteins in 92 subjects with PHI and 25 controls and examined relationships with disease progression and neuroinflammation, neuropsychological testing, and proton-magnetic resonance spectroscopy (MRS). We hypothesized that PHI is characterized by increased CSF NFL that correlates with neuronal inflammation and that tau and amyloid levels are normal in PHI. NFL was elevated in PHI ($p = 0.0004$) and correlated with CSF neopterin ($r = 0.38$, $p = 0.0005$), IP-10 ($r = 0.39$, $p = 0.002$), WBCs ($r = 0.32$, $p = 0.004$), and CSF:plasma albumin ratio ($r = 0.60$, $p < 0.0001$). NFL correlated with decreased N-acteylaspartate and glutamate in the anterior cingulate, frontal white matter, and parietal gray matter ($r > 0.30$, $p < 0.05$). Beta-amyloid was elevated in PHI ($p = 0.0005$) and correlated with time infected ($r = 0.34$, $p = 0.003$). Neither marker correlated with neuropsychological abnormalities. T-tau and amyloid precursor proteins did not differ between groups.

The aim of the second study was to characterize HIV-infected patients with neurosymptomatic CSF "escape," defined as detectable CSF HIV RNA in the setting of treatment-suppressed plasma levels or CSF RNA >1 log higher than plasma RNA. We conducted a retrospective case series of virologically controlled HIV-infected patients on ART with progressive neurological abnormalities who were determined to have CSF "escap at 4 urban medical centers in the United States and Europe. We recorded levels of CSF HIV RNA and inflammatory markers, clinical signs and symptoms, and magnetic resonance imaging (MRI) findings. We hypothesized that individuals with this condition would have inflammation in CSF and MRI studies, that CSF virus would be resistant to the ART regimen, and that symptoms would improve when ART was changed based upon central nervous system (CNS) drug penetration and resistance genotyping. Ten patients presented with sensory, motor, and cognitive abnormalities. Median CSF HIV RNA was 3900 copies/mL; median plasma HIV RNA was 62 copies/mL. Median CD4+ T cell count was 482 cells/mm3. All patients had been controlled <500 copies/mL for median 27.5 months and 5/10 had been suppressed <50 copies/mL for median 19.5 months. Patients were on a stable ART regimen for median 21 months. All had CSF pleocytosis or elevated CSF protein; 7/8 had MRI abnormalities; and 6/7 harbored CSF resistance mutations. Following optimization of ART, 8/9 patients improved clinically.

Although these processes occur at distinct time points in the disease, both neuronal injury during PHI and the development of symptomatic CSF "escape" in chronic, well-treated infection are associated with, and possibly caused by, mechanisms involving immune activation and inflammation within the CNS. The inflammatory milieu induced by the activity of HIV in invading cells and triggering an immune response has important implications throughout the time course of infection, and may be particularly important for understanding the pathophysiology of HAND.