

“Geminin Mutant Reveals the Mechanism to Inhibit DNA Re-replication”**Marissa Suchyta, University of Chicago Laboratory High School, Chicago, IL – 2009 National Individual Finalist**

Abstract: It is essential that a dividing cell replicates its genetic material accurately, completely, and only once. Re-replication of DNA would result in genetic instability and uncontrolled cell proliferation, the fundamental abnormality in all types of cancer. The protein Geminin prevents a second round of DNA synthesis by binding and inhibiting Cdt1, an essential replication protein. The mechanism by which Geminin inhibits Cdt1 is poorly understood. We identified a mutant of Geminin that binds to Cdt1 but does not inhibit Cdt1 activity. Protein induced from this mutant gene was purified to 90% purity using affinity chromatography. When added to *Xenopus* egg extracts, this mutant protein did not inhibit DNA replication as wild-type Geminin did. Re-replication assays, performed by adding BrdUTP to egg extracts with the Geminin mutant or wild-type proteins, also demonstrated that the mutated Geminin caused increased amounts of DNA re-replication. This study shows that expression of the Geminin mutant causes massive over-replication of the DNA. It was hypothesized that Geminin binds to Cdt1 in a non-inhibitory fashion during assembly of the pre-replication complex, and that when the origin fires Geminin changes conformation and binds in an inhibitory fashion. Drugs that inhibit Geminin's action may cause cancerous cells to over-replicate their DNA and die by apoptosis.

Mentor: Dr. Thomas McGarry**“Histone Deacetylase-1 (HDAC-1) Increases β -cell Proliferation in 832/13 Cells and Rat Islets”****Lanair Lett, North Carolina School of Science and Mathematics, Durham, NC – 2009 National Individual Finalist**

Abstract: Previous studies on the β -cell transcription factor Nkx6.1 have suggested that histone deacetylase-1 (HDAC-1) plays some role in β -cell proliferation. This study focuses on understanding how HDAC-1 alone affects β -cell proliferation and the function of mature β -cells. In this study we demonstrated that β -cells overexpressing the gene for HDAC-1 (via transduction with adenoviruses containing HDAC-1 cDNA) increased proliferation six-fold. Furthermore, we demonstrated that siRNA mediated suppression of HDAC-1 in the β -cell results in decreases proliferation. Finally, we demonstrated that primary rat islets treated with AdCMV-HDAC-1 had a doubling in proliferation as compared to untreated islets or islets treated with a control adenovirus (AdCMV-GFP). Importantly, the induction of proliferation did not correlate with a decrease in glucose-stimulated insulin secretion. By proving that HDAC-1 can increase proliferation of β -cells we have shown that it has a potential in developing future therapeutic methods for treating diabetes, the fifth leading cause of death in America. One such method, islet transplantation, is currently infeasible because of the amount of pancreatic tissue it requires to produce the necessary amount of β -cell mass. If β -cell proliferation can be induced in a pancreas prior to transplantation, the amount of tissue necessary for the procedure would decrease and the feasibility and efficiency of the procedure would be greatly increased.

Mentor: Dr. Jeffery Tessem

“The Localization and Function of Rabex-5”

Christine Lai and Diyang Tang, Acton-Boxborough Regional High School, Acton, MA – 2008 National Team Finalists

Abstract: Endocytosis, which is the process of taking up molecules, such as growth factor receptors from the cell surface, is a fundamental cellular mechanism for regulating cell growth and survival. Many studies have implicated Rab5 as a master regulator of endocytosis and shown that it affects the internalization of a variety of growth factor receptors such as EGF (epidermal growth factor). However, little has been done on how Rab5 itself is regulated in the cell. Our research investigates Rabex-5, a known *in vitro* activator (guanosine nucleotide exchange factor) of Rab5. By immunofluorescence microscopy, we showed for the first time that endogenous Rabex-5 has a spatial association with Rab5 in cultured retinal pigment epithelial (RPE) cells. Using the same technique, we also found that Rabex-5 resides on the early endosome most of the time and has little association with the late endosome. Finally, we showed that endogenous Rabex-5 has a spatial association with labeled EGF in cultured RPE cells at the early endosome. Thus, our data suggest that Rabex-5 might regulate the early stages of endocytosis through regulation of Rab5.

Mentor: Dr. David Lambright, Meng-tse Lee

“Transformation of Herceptin (Trastuzumab) Sensitive SKBR3 Breast Tumor Cells into Herceptin Resistant cells by Transfection with t-Darpp DNA”

Sarah Waliany, Flintridge Preparatory School, La Canada Flintridge, CA and Shelina Kurwa, Westridge School for Girls, Pasadena, CA – 2007 National Team Finalists

Abstract: Purpose: To show that t-Darpp DNA can induce Herceptin-resistance in previously sensitive Her-2 overexpressing SKBR3 breast cancer cells. Methodology: Two t-Darpp DNA fragments (3' end and 5' end) were combined to create a complete t-Darpp DNA strand by digestion, gel purification, ligation, and transformation. The complete t-Darpp DNA strand was transfected into Herceptin-sensitive Her-2 positive SKBR3 cells (from a company that sells established cell lines) without t-Darpp. These were then cultured with 0, 0.2 and 1.0 μ M of Herceptin for 7, 14 and 21 days. An SRB Assay measured the protein biomass to determine cell growth. Western Blotting measured pAkt and Akt expression in the cells. Results: Exposure to Herceptin led to reduction in cell growth in cells without t-Darpp, whereas growth was much greater in the cells transfected with t-Darpp. Unlike the control cells, the experimental cells expressed pAkt after Herceptin treatment. Conclusion: This is the first study that shows that Herceptin-sensitive breast cancer cells become resistant through transfection with t-Darpp. The clinically important result was that t-Darpp positive cells may proliferate more in the presence of Herceptin.

Mentor: Dr. Susan Kane

“Inhibition of VEGF Decreases Photodynamic Therapy-Induced Angiogenesis, and Reduces Tumor Regrowth of Nude Mice Bearing U87 Human Glioma”

Christopher Ding, Adams High School, Rochester Hills, MI and James Jiang, Troy High School, Troy, MI – 2007 National Team Finalists

Abstract: The current prognosis for patients with malignant gliomas remains poor. Photodynamic therapy (PDT) has been clinically developed as an adjuvant local therapy for brain tumors. Nevertheless, the efficacy of PDT treatment of glioma remains unsatisfactory. In addition to an incomplete killing of tumor cells, a reason for the failure of PDT as an effective treatment of glioma may be attributed to PDT-induced angiogenesis. We propose to test the novel hypothesis that angiogenesis in non-tumor tissue induced by PDT independent of tumor, contributes to tumor growth. Therefore, therapeutic intervention to inhibit angiogenesis in adjacent non-tumor tissue will diminish the likelihood of tumor regrowth from residual tumor cells. Here, we test this hypothesis in a model of PDT-treated nude mouse bearing human glioma. Our data show that inhibition of VEGF signaling post-PDT treatment significantly reduces subsequent angiogenesis in brains of nude mice. We also report that inhibition of VEGF signaling in conjunction with PDT treatment significantly reduces tumor regrowth. Our results suggest that concomitant anti-angiogenic therapy represents an effective method of enhancing PDT therapy of brain tumor.

Mentor: Dr. Michael Chopp

“Bone Growth in Zebrafish Fins Occurs via Multiple Pulses of Cell Proliferation”

Isha Himani Jain, Freedom High School, Bethlehem, PA – 2007 National Individual Winner

Abstract: Fin length in the zebrafish is achieved by the distal addition of bony segments of the correct length. Genetic and molecular data provided evidence that segment growth utilizes a single pulse of growth, followed by a period of stasis. Examination of cell proliferation during segment growth was predicted to expose a graphical model consistent with a single burst of cell division (e.g. constant, parabolic, or exponential decay) during the lengthening of the distal-most segment. Cell proliferation was detected either by labeling animals with BrdU (during S-phase) or monitoring histone3-phosphate (mitosis). Results from both methods revealed that the number of proliferating cells fluctuates in apparent pulses as a segment grows (i.e. during the growth phase). Thus, rather than segment size being the result of a single burst of proliferation, it appears that segment growth is the result of several pulses of cell division that occur about every 60 microns (average segment length ~ 250 microns). These results indicate that segment lengthening requires multiple pulses of cell proliferation.

Mentor: Dr. M. Kathryn Iovine