

Summer Student Research Program

Project Description

FACULTY SPONSOR'S NAME AND DEGREE: Lizhao Wu, *PhD*

PHONE: (973) 972 - 3161

DEPARTMENT AND INTERNAL MAILING ADDRESS: G1218 UH Cancer Center

E-MAIL: wuli@umdnj.edu

PROJECT TITLE (200 Characters max):

E2F8 collaborates with retinoblastoma to prevent anemia

HYPOTHESIS:

E2F8 synergizes with Rb to prevent hemolysis

PROJECT DESCRIPTION (Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)

Loss of retinoblastoma (RB) tumor suppressor in humans leads to an inherited eye tumor, retinoblastoma. As the first tumor suppressor gene cloned from human cells, Rb has been extensively studied in both humans and mouse models. Various early in vitro and in vivo studies demonstrated that Rb exerts its tumor suppression function mainly by inhibiting distinct E2F activities. In fact, all human cancers analyzed so far carry genetic alterations in the Rb/E2F pathway, either due to mutations of Rb itself, or due to aberrations in factors that either regulate Rb activities or manifest Rb functions.

Mice deficient for Rb die in uterus at midgestation, accompanied by multiple cellular defects. One of the main defects is anemia, resulted from a failure in red blood cell differentiation. In addition, Mx1-Cre mediated deletion of Rb specifically in mouse hematopoietic stem cells (HSCs) leads to mild anemia, splenomegaly, much higher levels of extramedullary hematopoiesis due to substantial increases in erythroid progenitor cells in both spleen and bone marrows. In an effort to determine whether the role of Rb in the control of hematopoiesis depends on any E2F activities, we identified a surprising and specific synergy between Rb and one of the newly identified repressor E2Fs, E2F8 as mice with HSCs deficient for both Rb and E2f8 exhibit profound defects in hematopoiesis, including severe anemia. In addition, our preliminary data suggest that the severe anemia in the doubly mutant mice is likely due to hemolysis instead of ineffective erythropoiesis. To further understand how E2F8 synergizes with Rb to control hematopoiesis, we will use a conditional and inducible gene targeting system to generate mice that are deficient for Rb and E2f8 to further characterize the cellular and molecular mechanisms behind the severe anemia phenotype. Hematopoietic organs/cells from those compound knockout mice will be harvested at various time points for histological analysis by Hematoxylin and Eosin staining, evaluation of anemia by complete blood counts, cell lineage marker analysis by flow cytometry, evaluation of hemolysis by red blood cell lifespan assays, red blood cell fragility assays, and Prussian blue staining, as well as molecular analyses by real-time quantitative PCR and/or Western blots. The prospective student will be assisting a postdoctoral researcher to execute all the major experimental procedures described above, and may maintain a high degree of independency based on his or her experience.

SPONSOR'S MOST RECENT PUBLICATIONS RELEVANT TO THIS RESEARCH:

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Wenzel*, P., **L. Wu***, A. de Bruin, J. Chong, W. Chen, G. Dureska, E. Sites, T. Pan, A. Sharma, K. Huang, R. Ridgway, K. Mosaliganti, R. Sharp, R. Machiraju, J. Saltz, H. Yamamoto, J. C. Cross, M. L. Robinson, and G. Leone, 2007. Rb is critical in a mammalian tissue stem cell population. **Genes Dev.** 21: 85-97 (*equal contributions).

Wu, L., A. de Bruin, H. I. Saavedra, M. Starovic, Y. Yang, A. Trimboli, J. Opavska, P. Wilson, M. C. Ostrowski, J. C. Cross, M. Weinstein, T. J. Rosol, M. L. Robinson, and G. Leone, 2003. Extra-embryonic function of Rb is essential for embryonic development and viability. **Nature** 421: 942-947 (Cover highlight/News & Views 421: 903-904).

Saavedra, H. I., **L. Wu**, A. de Bruin, C. Timmers, T. J. Rosol, M. Weinstein, M. L. Robinson, and G. Leone, 2002. Specificity of E2F1, E2F2 and E2F3 in mediating phenotypes induced by loss of Rb. **Cell Growth Differ.** 13: 215-225.

IS THIS PROJECT SUPPORTED BY EXTRAMURAL FUNDS?

Yes or No

(IF YES, PLEASE SUPPLY THE GRANTING AGENCY'S NAME)

NIH/NCI

THIS PROJECT IS: Clinical Laboratory Behavioral Other

THIS PROJECT INVOLVES THE USE OF ANIMALS

PENDING APPROVED IACUC PROTOCOL # 09035

THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS

PENDING APPROVED IRB PROTOCOL # M

WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?

- *Isolation of genomic DNA from mouse tail tips
- *Mouse genotyping by polymerase chain reaction (PCR)
- *Harvesting various hematopoietic organs/cells, including blood, thymus, spleen, and bone marrow
- *Preparation of single cell suspensions from harvested hematopoietic organs
- *Flow cytometric analysis of single cell suspensions using various fluorescence labeled antibodies
- *Various assays to evaluation hemolysis
- *Real-time quantitative PCR analysis
- *Western blots
- *Data analyses for complete blood counts, flow cytometry, and real-time quantitative PCR