CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
David B. Cheng

Project Number
31906

Project Title
A Single Amino Acid Substitution Switches a Protein Specificity

Abstract

Objectives/Goals
The goal of my research project is to understand how protein specificity is determined. As one of the six Tumor Necrosis Factor Receptor Associated Factor (TRAF) family members, TRAF3 plays a critical role in regulating the non-canonical NF-kB pathway. TRAF3 mutations are associated with both human cancer and auto-immune diseases. The essential role of TRAF3 relies on its ability to specifically bind to NIK. Based on the sequence alignment and crystal structural studies, we found that tyrosine 441 of TRAF3 not only directly contacts with NIK but is also different in sequence from all other TRAF family members at the corresponding position. We hypothesized that tyrosine 441 of TRAF3 might be responsible for the binding and functional specificity of TRAF3.

Methods/Materials
To test our hypothesis, we took a gain of function approach. By using the PCR mutagenesis method, we have generated a point mutation in TRAF5 to create a TRAF5F410Y mutant, which substituted phenylalanine at the position 410 of TRAF5 (corresponding to the position 441 of TRAF3) with tyrosine. After cloning the TRAF5F410Y mutant cDNA into an expression vector, we transfected wild type TRAF3, wild type TRAF5 and TRAF5F410Y mutant into 293T cells and then compared their abilities to bind the NIK by GST pull down assays.

Results
In vitro binding assays indicated that while wild type TRAF5 did not bind to NIK, TRAF5F410Y mutant bound to NIK as strongly as TRAF3.

Conclusions/Discussion
Thus, we have demonstrated that a single amino acid substitution can switch the binding specificity of TRAF5 to that of TRAF3. Our studies may provide insight for drug design on TRAF proteins to treat cancers and inflammatory diseases.

Summary Statement
My research project is about the molecular mechanism responsible for the specific function of critical protein involved in cancer and autoimmune diseases.

Help Received
Mr. Larry Walker serves as the site coordinator and has helped me with some of the paperwork required for the science fair. Dr. Bahram Razani serves as my research advisor, teaching me all the techniques needed in my experiments. Ms. Anna Reichardt and Dr. Yaya Wang have helped me with some steps of