TITLE: Characterization of a small iron protein, *Pyrococcus furiosus* rubredoxin, as a potential cancer drug delivery system

AUTHORS: Siri L. Chirumamilla[1]*, David Blum[2], Ray L. Mernaugh[2], Francis E. Jenney Jr.[1]

AFFILIATIONS: [1]Basic sciences, GA Campus Philadelphia College of Osteopathic Medicine, Suwanee, GA, and [2]Vanderbilt University School of Medicine, Nashville, TN

ABSTRACT:

Background: Cancer is an elusive neoplastic disease that claims the lives of many people around the world every year. Though treatments have become more specific to the different types of cancer, the need for antineoplastic drugs that target cancer cells and leave normal cells unharmed, with little to no systemic toxicity remains, and rubredoxin might be such a tool. Rubredoxin is a small (53 amino acids), water soluble, non-heme iron electron transfer protein that contains an iron atom cofactor, which can be substituted with various cytotoxic transition metals such as nickel and cobalt with little or no effect on the protein. Rubredoxin from the hyperthermophile *Pyrococcus furiosus* is thermostable and appears to have low immunogenicity. The focus of this project is to incorporate tumor-specific binding sequences at several modifiable sites on the protein as well as substitute the iron-center with cytotoxic metals. Once a stable rubredoxin containing these characteristics is created, its effects and efficacy will be studied on specific cancer cells *in vitro*.

Methods and results: Site-directed mutagenesis was used to incorporate a test epitope (E-tag) at the D20 position and an RGD-sequence at the carboxyl terminus respectively. The mutant proteins were purified using anion-exchange DEAE, size-exclusion G-75 Sephadex, and ceramic hydroxyapatite column chromatography. Proteins were analyzed using absorption spectroscopy, bicinchoninic acid (BCA) assay, SDS-PAGE, and electrospray ionization mass spectroscopy. Binding studies for the D20-Etag mutants will be done using dot blot and western blotting. The metal content of the mutants was assessed using inductively-coupled plasma mass spectrometry. Lastly, integrin-stimulated Jurkat cancer cell lines will be incubated with wild-type rubredoxin, D20-Etag, D20-RGD, and the effect of these mutants will be assessed using western blotting and DNA fragmentation.

Conclusions: The epitope E-tag was successfully incorporated between the D20 and N21 amino acid residues using site directed mutagenesis. The D20-Etag and D20-RGD mutant rubredoxin proteins were successfully expressed, purified, and analyzed. Currently, the iron-center is being substituted with different metals. Also, the effect of wild-type rubredoxin, D20-RGD, D20-Etag on integrin-stimulated Jurkat cells is being studied. This set of experiments will provide a further understanding and appreciation of rubredoxin as a potential targeted drug delivery system to cancer cells.