

2012

Characterization of a Hyperthermophilic Redox Protein, Rubredoxin, as a Potential Targeted Cancer Therapeutic

Siri Lakshmi Chirumamilla

Philadelphia College of Osteopathic Medicine, sirich@pcom.edu

Follow this and additional works at: <http://digitalcommons.pcom.edu/biomed>

 Part of the [Medical Biochemistry Commons](#), [Medical Pharmacology Commons](#), and the [Oncology Commons](#)

Recommended Citation

Chirumamilla, Siri Lakshmi, "Characterization of a Hyperthermophilic Redox Protein, Rubredoxin, as a Potential Targeted Cancer Therapeutic" (2012). *PCOM Biomedical Studies Student Scholarship*. Paper 48.

This Thesis is brought to you for free and open access by the Student Dissertations, Theses and Papers at DigitalCommons@PCOM. It has been accepted for inclusion in PCOM Biomedical Studies Student Scholarship by an authorized administrator of DigitalCommons@PCOM. For more information, please contact library@pcom.edu.

Abstract

Characterization of a hyperthermophilic redox protein, rubredoxin, as a potential targeted cancer therapeutic

Siri Lakshmi Chirumamilla

M.S., May 2012

Philadelphia College of Osteopathic Medicine

Francis E. Jenney, Jr, Thesis Adviser

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 remains for antineoplastic drugs that target cancer cells and leave normal cells unharmed, with little to no systemic toxicity. The search for a targeted cancer therapeutic is necessary and urgent, and *Pyrococcus furiosus* 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 bound by the sulfurs of four cysteine residues, which contribute to the redox activity of this protein. Rubredoxin from the hyperthermophile *Pyrococcus furiosus* is thermostable and appears to have low immunogenicity. The focus of this project was to incorporate tumor specific binding sequences at the central loop, express, and purify these recombinant rubredoxin proteins. Next, the wild-type and recombinant rubredoxins were characterized based on absorption spectra, thermostability, metal content, and antibody affinity. Lastly, the effect of wild-type and recombinant rubredoxins was assessed on cancer cells *in-vitro*.

The *Pyrococcus furiosus* rubredoxin gene was manipulated via site-directed mutagenesis to incorporate two test epitopes (E-tag and RGD-tag) at the central loop (between D20 and N21 position). The mutant proteins (D20-Etag and D20-RGD) were purified and analyzed using absorption spectroscopy, thermostability, SDS-PAGE, electrospray ionization mass spectroscopy (ESI-MS),

and inductively coupled mass spectrometry (ICP-MS). Binding studies for the D20-Etag mutant were done using a dot blot. Lastly, integrin-stimulated Jurkat cancer cells were incubated with wild-type rubredoxin, D20-Etag, D20-RGD, and the cells were assayed for apoptosis via gel electrophoresis at 24 and 48 hour time points.

The E-tag epitope 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. There was an apoptotic effect of D20-RGD rubredoxin on the Jurkat cell line. These results provided a further understanding and appreciation of rubredoxin as a potential targeted therapeutic to cancer cells.