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Cytochrome c

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13. ABSTRACT (Maximum 200 Words) The purpose of this research project is to investigate the interaction between heat shock protein 27 (hsp27) and cytochrome c in the inhibition of apoptosis. The scope of the study will include: measuring the binding of hsp27 to cytochrome c in vivo, determining why hsp27 binds to cytochrome c and determining the fate of the hsp27:cytochrome c complex. Major findings to date include: cellular survival cures to cytochrome c and molecular chaperone assays with hsp27 and citrate synthase and alpha-glucosidase. A no-cost one year time extension was granted to complete the goals of the project.				
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INTRODUCTION:

This annual report is for the period June 2001 – May 2002. The objectives of the purposed study are to (1) measure the binding of hsp27 to cytochrome c *in vivo*, (2) determine why hsp27 binds to cytochrome c and (3) determine the fate of the hsp27:cytochrome c complex. We have made significant progress on the stated objectives but have not yet achieved them, so we asked for and received a one year, no-cost, time extension to complete the objectives.

BODY:

The first objective, measure the binding of hsp27 to cytochrome c *in vivo*, is progressing well. We have made extracts of cells induced to undergo apoptosis. We have also successfully conducted Western blot analysis of *in vitro* immunoprecipiated samples of hsp27 and cytochrome c. We are now ready to begin the immunoprecipitation of the *in vivo* samples. To demonstrate that cytochrome c can interact with hsp27 *in vivo*, we have added cytochrome c to whole cells and determined that cells that constitutively express hsp27 survive at a much greater frequency than cells that did not express hsp27. We believe (and are in the process of demonstrating) that cytochrome c enters the cell and triggers apoptosis.

The second objective, determine why hsp27 binds to cytochrome c is in progress. We have begun using a DHFR refolding model and have determined the conditions that are necessary to thermally denature DHFR. We have also used citrate synthase and alpha-glucosidase as model systems as well.

The last objective, determine the fate of the hsp27:cytochrome c complex, is also showing good progress. We have initially focused on using a chromatography approach to help identify the hsp27:cytochrome c complex. We are using the apoptosome as a model system to determine if hsp27 can prevent its formation. We have determined the optimum conditions for the column and are now ready to begin a time course study.

KEY RESEARCH ACCOMPLISHMENTS:

Cellular Survival Curves to Cytochrome c.

Hsp27 Molecular Chaperone assays with Citrate Synthase and Alpha-Glucosidase

Determination of Column Conditions for Apoptosome Detection

REPORTABLE OUTCOMES:

Scientific talk "The role of heat shock proteins in the inhibition of apoptosis" given at the 2002 Radiation Research Society Meeting in Reno, NV that outlined our progress on this project.

Poster session "Heat Shock Protein 27 Inhibits Apoptosis in Human Breast Cancer cells by Binding to Cytochrome c" given at the 2002 Era of Hope U.S. Army Breast Cancer Research Conference in Orlando, Fl., that outlines our progress to date.

CONCLUSIONS:

More time is needed to achieve the objectives. We believe that we will be able to achieve the three goals by the end of the time extension. The data generated by these studies should be sufficient for two publications as well as a competitive grant application to the U.S. Army to extent these studies.

REFERENCES: None

APPENDICES: None