FINAL REPORT
October 1, 2006

DETERMINATION OF SUPER MAMMARY LYMPH NODE PROTEINS AND GROWTH FACTOR ACTIVITY

Principal Investigator(s): Thomas R. Scott, Professor
trscott*@clemson.edu*
Department of Animal and Veterinary Sciences
123 P&A
Clemson University
Clemson, SC 29634-0311
(864) 656-4027/(864) 656-3131 FAX

Collaborators: Steve Ellis, Associate Professor
ellis*@clemson.edu*
Department of Animal and Veterinary Sciences
124 P&A
Clemson University
Clemson, SC 29634-0311
(864) 656-6969/(864) 656-3131 FAX

***Date Submitted**:* *March 28, 2005*

Objective (s):

Assess the efficacy of the supernatant proteins and isolated protein factors recovered from super mammary lymph nodes of dairy and beef cows in order to determine the usefulness of the proteins for biomedical and veterinary purposes.

Project Overview:

The protein containing supernatants from super mammary lymph nodes of dairy and beef udders will be used as serum replacements for cell culture experiments. The inclusion of these sources of proteins, that can support the growth of cell lines in culture, will lead to a huge market for biomedical and veterinary products. The cost of fetal calf serum or bovine calf serum runs $36-72/100 ml. Additionally, the cost of peptides and factors that have therapeutic uses for humans and animals can run hundreds to thousands of dollars per milligram or microgram. The recovery of these valuable proteins and factors from super mammary lymph nodes could provide a quick, less invasive, yet value added product for the processing and rendering industries.

Our laboratory group will test the efficacy of these supernatant proteins on the growth of important cell lines that are used in biomedical and veterinary testing for diagnosis of diseases and for production of valuable products (e.g., monoclonal antibodies, hormones, growth factors, etc.). Furthermore, the identification, through proteomics analysis, of individual proteins and factors in the supernatants will uncover several proteins that will be important for human and animal treatments and therapies.

Impacts and Significance:

Initial work on this project in the previous funding year (2004-05), began with collection of lymph nodes from a local abattoir, mincing/homogenization of the tissues, recovery of a crude extracts, determination of protein concentrations of the extracts, and preliminary studies with Mac-T cells. These studies showed that the then crude extracts contained about less than half the protein concentration of bovine growth serum (BGS, ~35 mg/ml). When compared in cell culture with Mac-T cells, the extract supported growth of the cells as well or better than BGS.

Work during this second year of funding has allowed our laboratory group to expand the used of the extracts to human breast cancer cells and equine chondrocytes. Furthermore, steps to improve the clarity of the extract samples have been incorporated into the preparation of lymph nodes. The latter has included both a heat inactivation step (60 C, 30-60 min) and an improved lymph node grinding and freeze drying approach. Both methods provide cleaner extract samples that contain less fat and tissue debris. Presently, the lymph nodes are first ground and freeze dried prior to a high speed centrifugation step and heat inactivation. Both the clarity and protein concentrations of the extracts have been improved with these preparations steps.

With breast cancer cell cultures, our laboratory is observing two different effects on the cancer cells depending on whether the extracts have been heat inactivated or not. When directly compared to BGS at matching protein concentrations in culture (3 mg/ml), it has been observed that crude extracts have an inhibitor effect on breast cancer cells while the heat inactivated extracts support the growth of breast cancer cells better than BGS. The next step in our studies will be to identify the protein factors that are removed from the crude preparations during heat inactivation. This will lead us to uncovering some of the potential inhibitor factors that down modulate the growth of the breast cancer cells. Also, cell lysates are being evaluated for the

activation states of selected signaling molecules (STAT3, caspase3, cyclin) to determine which signal pathways are being affected.

The equine chondrocyte studies have focused on supporting the growth of chondrocytes in culture as a means of assessing the potential therapeutic use of the extracts for joint problems in horses. It is believed that any therapeutic that can help rebuild chondrocytes in joints will help alleviate development of inflammation in joints. Preliminary work has shown that the extracts, both crude and heat inactivated, do support the growth of equine chondrocytes in culture. As the assay system is standardized for different chondrocyte sources (i.e., different horses) it will be possible to derive a range of appropriate doses to administer to lame horses. More replicated culture studies are being conducted presently to arrive at this range.

Publications:

None at this time, but three future M.S. theses will be written by students working with the extracts. These theses will be the bases for peer review journal articles in the future. Two patent applications on this technology are pending evaluation at this time.

Future Work:

The laboratory group is concentrating on the culture studies with both breast cancer cells and equine chondrocytes. The Mac-T cells have not proven to be a reliable cell for the types of growth studies we wish to undertake. With our move to breast cancer cells, we have found a type of cell that is more sensitive to the proteins and growth factors in the extracts. We are presently expanding the breast cancer assessments by adding two additional cell lines to the studies. The chondrocyte cultures will be repeated with match studies of chondrocytes culture simultaneously in order to observe horse-to-horse variability in responsiveness to the extract contents.

Acknowledgments:

The work on this project is being conducted by three M.S. students. Two students, Danelle Duffy (breast cancer cells) and Alison Reed (chondrocytes), are in their second year of graduate school and both anticipate graduation in May 2007. Sara Garrett is the third M.S. student who is just beginning her laboratory work with the additional breast cancer cell lines. Marcy Owens and Nancy Korn have provided technical assistance for this work.