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TITLE: Tumor-Secreted Autocrine Motility Factor (AMF): Causal Role in an Animal Model of Cachexia

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**14. ABSTRACT**
Cancer cachexia has three clinical features: 1) loss of appetite (anorexia), 2) nutritional mal-absorption, and 3) muscle and fat wasting caused by tumor-stimulated factors. This project focused on muscle wasting. A number of factors have been proposed to cause cancer cachexia. Lack of progress in the area is unfortunate, given the tremendous benefit patients with advanced cancer would receive from effective treatment of cachexia to improve quality of life and postpone mortality. We proposed that autocrine motility factor (AMF) is released into the bloodstream from cancer sites and stimulates muscle wasting. During the grant period we: 1) Established an animal model in which CHO/AMF tumors caused cachexia in mice; 2) Showed that injection of recombinant AMF protein caused significant weight loss in mice in 24 hours; 3) Solved the 3-D structures of mouse and human proteins; 4) Initiated characterization of the genes involved in muscle protein degradation in response to cachexia in the mouse model with CHO/AMF tumors.

**15. SUBJECT TERMS**
Cancer cachexia, proteasome, muscle wasting, phosphoglucose isomerase glucose 6-phosphate isomerase, autocrine motility factor
Tumor Secreted AMF: Causal Role in an Animal Model of Cachexia

FINAL REPORT, YEAR 03, SUPPLEMENT

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INTRODUCTION:

Overview: An extensive final report was filed one year ago, with a proviso for a requested (October 2005) 6-month no-cost extension. Thus, this is an addendum summarizing these final results. General Summary: Our original goal was to test critical whether AMF/PGI protein could cause cachexia. This was successfully accomplished. Additional goals were to establish an animal model for testing of the recombinant protein and to prepare various forms of purified recombinant AMF/PGI protein, mouse and human, for testing in the mouse model. These goals were also accomplished. The practical combination of these two parts, however, was not successful, and we concluded that administration of the recombinant protein to mice is useful only as a proof-of-principle. The final experiments in the no-cost period have completed testing of an alternate and more robust animal model, in which an AMF/PGI-secreting cell line (CHO-1C6) causes cachexia in mice carrying intramuscular tumors. Addendum summary: 1) CHO cells grown as bone metastases (regardless of AMF/PGI secretion) cause cachexia; 2) CHO cells that express AMF/PGI (1C6 clone) but not control CHO-K1 cells cause cachexia when grown as bone-contiguous intramuscular xenografts; 3) Subcutaneous 1C6 cells fail to cause cachexia. 4) Injection of purified recombinant AMF/PGI protein causes moderate, reversible weight loss in experimental animals in the presence of modest physiological stress (such as transient anesthesia for blood drawing) but not in unstressed animals. General conclusion: AMF-PGI is a secondary cachectic factor that causes statistically significant weight loss in two experimental settings: a) tumor growing in contact with bone cells in vivo; b) in animals with systemic physiological stress. However, the factor alone is not sufficient to cause cachectic weight loss.

Update on Final Report from August 2005. The original award was appropriately considered a high-risk project and funded for only two years. Work was substantially delayed by the relocation of the research team from the University of Texas to the University of Virginia and the long time to reestablish animal procedures and train new animal handlers. A number of lengthy previous reports have been provided, and the reviewers are spared their repetition.

BODY OF Addendum to FINAL REPORT

Timetable and SOW: Only Progress on Task 8 was not addressed in the 2005 Final Report.
Progress on Task 8: Cachexia model with mice carrying the CHO-1C6 tumor cell line or its control CHO-K1. The 1C6 cells secrete mouse AMF/PGI, making it impossible for us to compare in vivo the species-specific actions of mouse versus human AMF/PGI proteins as originally proposed. A human PGI-secreting cell line is not available. Details of this model were provided in the 2005 Final Report. To assure that there were not confounding effects of bone cell-produced factors on the cachexia model, the two CHO tumor lines (K1 control and 1C6) were superficially inoculated into the thigh muscles of groups of 12 Balb/C female nude mice. In this location the AMF/PGI secreting cells did not cause weight loss over 21 days; so there was no opportunity for PCR analysis of cachexia-associated marker genes in the proteasomal degradation pathway in samples of the mouse skeletal muscles. [This analysis was planned as part of the no-cost extension project.] However, the experiment provided useful final data on the requirement of bone cell factors (entirely unknown at this point) to act in concert with the AMF/PGI. The final animal experiment provided essential information to confirm our final conclusion: that AMF/PGI is a secondary causal factor of cachexia.

SUPPORTING DATA: since the final animal experiment did not result in cachexia the data are negative and full described in the previous paragraph without new figures here.

KEY RESEARCH ACCOMPLISHMENTS:

The eight major accomplishments were provided in the 2005 Final Report.

9) Demonstration that AMF/PGI causes statistically significant cachexia only in the context of other causes (whole animal physiological stress in the case of the injected recombinant factor or direct contact with bone cells probably of the osteoblastic/stromal cell lineage in the case of xenografted tumor cells). The two lines of evidence for the secondary role now allow us to publish our results in a clearly interpretable manner. The two final manuscripts are in preparation.

REPORTABLE OUTCOMES (since 8/05):

In addition to the twelve manuscripts previously published:

Two manuscripts published and two more submitted on additional mechanisms that could contribute to host responses (such as cachexia) to breast cancer bone metastases


4) Siclari VA, TA Guise TA, Chirgwin JM. Molecular Interactions between Breast Cancer Cells and the Bone Microenvironment Drive Skeletal Metastases. Submitted to Cancer Metastasis Rev 8/06


CONCLUSIONS

1) Purified mouse autocrine motility factor/phosphoglucose isomerase caused statistically significant weight loss (cachexia) by intraperitoneal injection at a dose that increased serum concentration of the factor. However, cachexia occurred only in anesthesia-stressed animals, suggesting that AMF/PGI alone was insufficient to cause weight loss. Thus, the main hypothesis of the original proposal was validated.

2) Xenograft models with CHO cells overexpressing mouse AMF/PGI showed that involvement of bone was necessary for the factor to cause cachexia. We speculate that tumor-bone interactions stimulate production of additional cytokines, although we did not detect increases in IL-6 or TNF-alpha in the mouse model.

3) Structures of the AMF/PGI proteins, including mouse and human proteins and the enzyme complexed with inhibitor were solved by x-ray crystallography and published. Mutant forms of the protein were prepared and a simple purification of endotoxin-free protein developed. The structural data are now complete for understanding the species-specific effects and their structural bases.

4) Progress was not made on elucidating a molecular basis for the contribution of AMF-PGI to cachexia. The high affinity receptor for AMF/PGI, called gp78 and sequenced 15 years ago (Watanabe et al, JBC 1991) has recently been expressed and functionally characterized in detail (Chen et al, 2006). The characterization is entirely incompatible with the encoded protein binding AMF/PGI and transducing the known responses to the ligand. Further work on AMF/PGI will require identification and molecular characterization of the presently unknown, true receptor.

REFERENCE ADDED FOR 2006: