

## FINAL REPORT

Project Title: Lipid Metabolism in Liposarcoma: Project Number: SFA10-14  
A Novel Target for Therapeutic Intervention

1. Date project was initiated: 6/01/10
2. Period covered by this report: From 10/01/10 To 5/31/11
3. Publications, Abstracts, and Presentations:
  - a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry must include the author(s), article title, journal [book, editors(s), publisher, volume number, page number(s), and date.]
    - (1) Lay Press:
    - (2) Peer-Reviewed Scientific Journals:
    - (3) Invited Articles:
    - (4) Abstracts:
  - b. List presentations made during the last year (international, national, local societies, etc.). Use an asterisk (\*) if presentation produced a manuscript.



SFA Final Report

1. Date project was initiated: 5/1/2010
2. Period covered by this report: 5/1/10 – 5/31/10
3. Publications, abstracts, and presentations:

a. (2) Peer-reviewed journals

Nancy B. Kuemmerle, Evelien Rysman, Portia S. Lombardo, Alison J. Flanagan, Brea C. Lipe, Wendy A. Wells, Jason R. Pettus, Heather M. Froehlich, Vincent A. Memoli, Peter M. Morganelli, Johannes V. Swinnen, Luika A. Timmerman, Leila Chaychi, Catherine J. Fricano, Burton L. Eisenberg, William B. Coleman, and William B. Kinlaw, 2011. Lipoprotein Lipase Links Dietary Fat to Solid Tumor Cell Proliferation. *Molecular Cancer Therapeutics* 10(3):427-436.

(3) Invited Articles:

Nancy B. Kuemmerle and William B. Kinlaw, 2010. THRSP (thyroid hormone responsive). *Atlas of Genetics and Cytogenetics in Oncology and Haematology*.  
<http://atlasgeneticsoncology.org/Genes/THRSPID42555ch11q14.html>.

(4) Abstracts:

Nancy B. Kuemmerle, Evelien Rysman, Portia S. Lombardo, Alison J. Flanagan, Brea C. Lipe, Wendy A. Wells, Jason R. Pettus, Heather M. Froehlich, Vincent A. Memoli, Peter M. Morganelli, Johannes V. Swinnen, Luika A. Timmerman, Leila Chaychi, Catherine J. Fricano, Burton L. Eisenberg, William B. Coleman, and William B. Kinlaw, 2011. A role for lipoprotein lipase in fatty acid acquisition by breast, prostate, and liposarcoma tumors. Abstract #1256, American Association for Cancer Research Annual Meeting, 2011.

b. Presentations:

The abstract listed above was presented by me.

4. Brief list of keywords:

Liposarcoma  
Lipid metabolism  
Lipoprotein lipase  
Fatty acid synthase  
Fatty acid translocase  
CD36  
Immunohistochemistry

5. Please see attached file, SFAFinalReport.doc

6. Lay summary:

We have been studying proteins that are involved in the growth of liposarcomas. We did not see any differences in the sizes of these proteins in tumors versus adjacent, uninvolved fatty tissues. We did, however, note differences in the distribution of these proteins in tumor and fatty tissues. We used a tissue microarray that included specimens from 25 different liposarcomas to study the three mechanisms liposarcomas use to get fats to fuel their growth: the tumors can make their own fats using fatty acid synthase, which is controlled by the protein Spot14; they can break down lipoprotein particles from the diet using lipoprotein lipase; and they can take up fatty acids using lipoprotein lipase as a bridging molecule to enter the cell through a channel known as CD36, or fatty acid translocase. In most liposarcomas, CD36 is localized to the cell membrane. If we can inhibit CD36 or LPL, in addition to inhibiting fatty acid synthase or Spot14, we can shut down most of the pathways that liposarcomas use to get their energy for growth. We plan to work on small protein molecules, called peptidomimetics, that target LPL, Spot14, and CD36. We hope these peptidomimetics will be powerful but nontoxic inhibitors of the proteins liposarcomas need to grow.

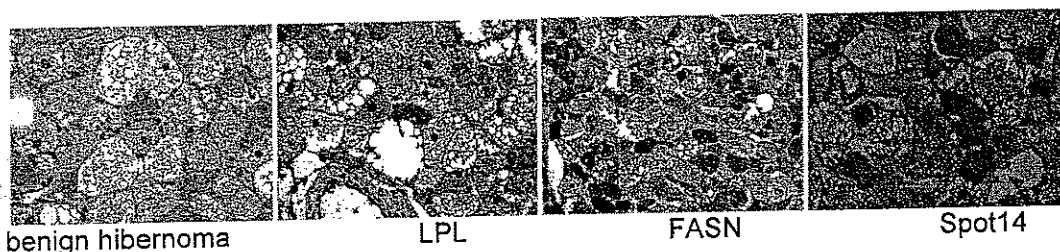
The objectives of this work were to identify and characterize proteins which were differentially expressed in liposarcomas and normal adipose tissue; and to study the relationships among the three pathways of lipid acquisition in liposarcoma cells. Proteins studied include fatty acid synthase (FASN); Spot14, a protein which drives expression of FASN; lipoprotein lipase (LPL), which is involved in hydrolysis and uptake of lipoprotein complexes from the diet; and CD36, also known as fatty acid translocase, which is a membrane channel through which fatty acids enter cells.

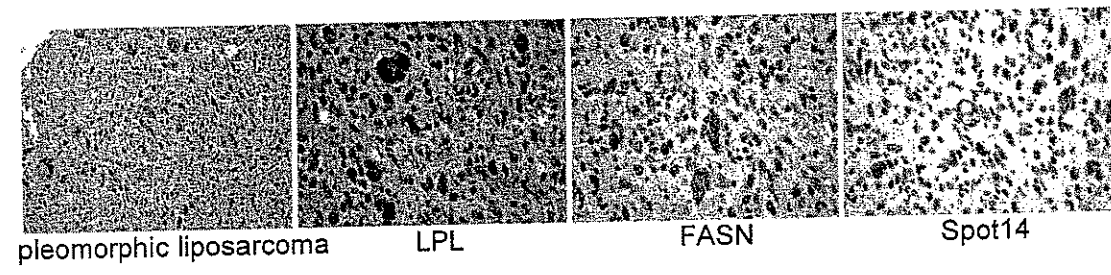
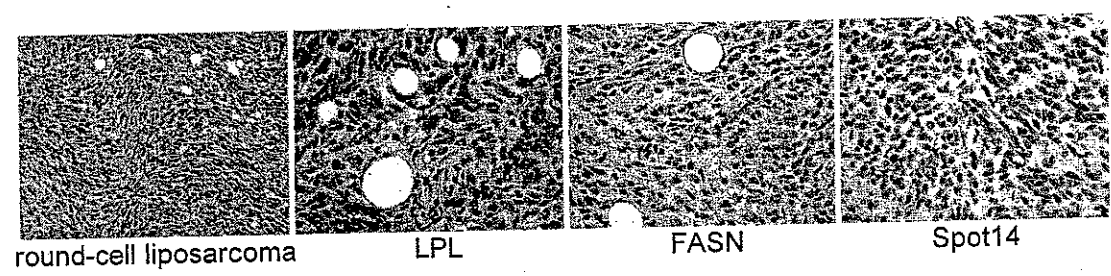
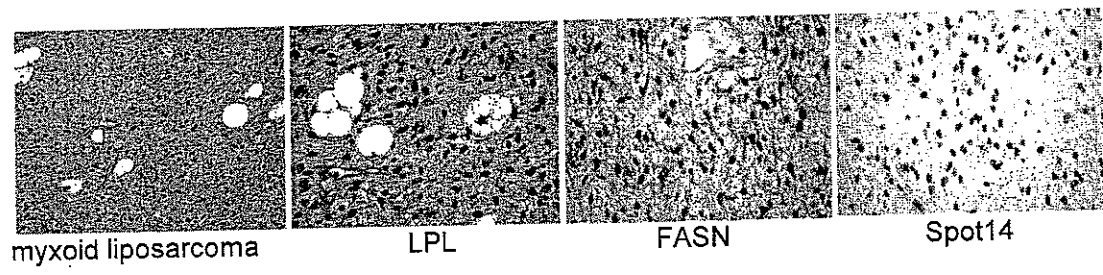
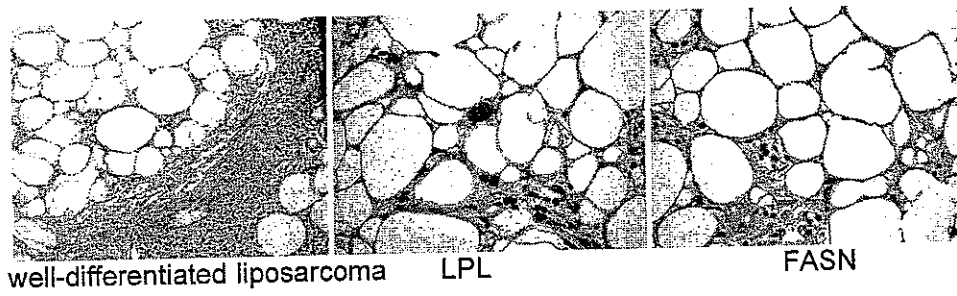
We have prepared lysates from four human liposarcomas and from uninvolved adipose tissue from the same patient. After denaturing these samples to release the various proteins from their complexes, total proteins were resolved through denaturing polyacrylamide gels. Using commercial antibodies directed against CD36 and FASN, as well as monoclonal antibodies against LPL and Spot14, we were able to identify abundant LPL, Spot14, FASN, and CD36 in lysates from tumors and uninvolved adipose tissue. We did not observe either size differences or significant quantitative differences in any of these proteins in liposarcomas vs. uninvolved adipose tissue.

However, study of a tumor microarray of 28 liposarcoma and adipose tissues prepared by pathologists at this institution revealed more specific information regarding these proteins. LPL exhibits both nuclear and cytoplasmic staining patterns in liposarcomas, lipomas, and normal adipose tissue. LPL staining is increased in liposarcomas in comparison to normal adipose tissue and most benign adipocytic tumors. Twenty-one of 25 liposarcomas demonstrated a staining pattern consistent with localization in the Golgi apparatus and in cell membranes; the others demonstrated diffuse cytoplasmic staining. LPL staining of a hibernoma, a benign tumor of brown fat, is increased compared to benign lipomas. High-grade liposarcomas (pleomorphic, dedifferentiated, and round-cell subtypes) tend to show similarly intense staining for LPL as low-intermediate grade liposarcomas (well-differentiated and myxoid subtypes). In addition to adipose cells, LPL staining is also identified in endothelium, skeletal muscle, renal tubules, and the intercalating ducts of salivary glands.

Nuclear staining for Spot14 is increased in liposarcomas compared to normal adipose tissue and benign adipocytic tumors. The staining intensity of Spot14 generally correlates with the LPL staining intensity.

Three separate patterns of fatty acid synthase staining are observed: diffuse cytoplasmic staining, sporadic cytoplasmic staining, or the absence of staining. When present, FASN staining is similar in intensity to the cytoplasmic staining of LPL in matched cases.



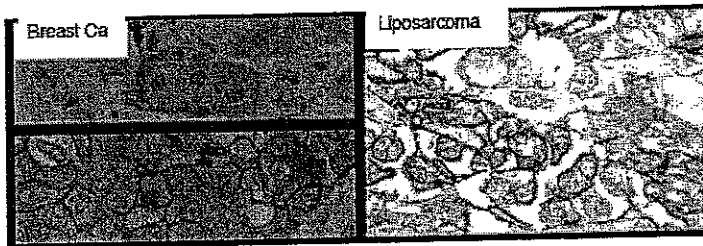


ABOVE: Staining and immunostaining of benign (hibernoma), low-intermediate grade liposarcomas (well-differentiated, myxoid), and high-grade liposarcomas (round-cell, pleomorphic). The left-most specimen in each panel is stained with haematoxylin and eosin; the remaining specimens are immunostained with antibodies to LPL, FASN, and Spot14, respectively. The haematoxylin counterstain is blue; successful immunostaining is brown.

As we observed in human breast tumors, CD36 in liposarcomas exhibits two staining patterns: diffuse cytoplasmic staining and staining localized to the cell membranes. This is one of our most exciting findings. It may be possible, by means of dietary agents or chemotherapy, to direct CD36 to either cytoplasmic localization or membrane association. If CD36 is cytoplasmic, it is not on the membrane surface, and it is thus unavailable for uptake of free fatty acids or lipoprotein particles. If, on the other hand, CD36 is exclusively membrane-bound, it would be an effective target for a competitive inhibitor of fatty acid and lipoprotein uptake, such as conjugated linoleic acid (CLA). CLA has been shown to slow the growth of human tumors; it is currently in clinical trial at

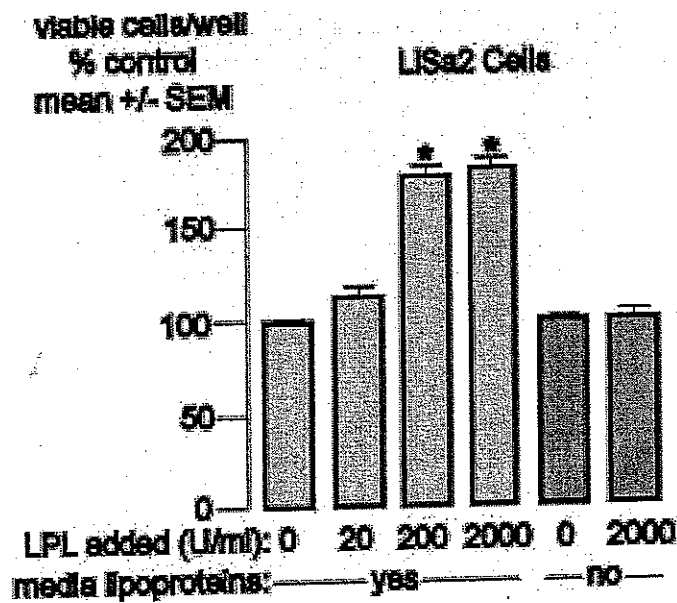
this institution. It is conceivable that targeting of CD36 in tumors would be less toxic and more efficacious than targeting LPL or FASN.

BELOW: Breast cancer tumors stained with a commercially available antibody to CD36 demonstrate diffuse cytoplasmic staining (upper frame) or staining localized to cell membranes (lower frame). A liposarcoma (right panel) exhibits staining localized to cell membranes.



Thus by immunohistochemistry, liposarcomas possess elements (Spot 14 and FASN) necessary for the tumors to acquire lipids via the synthetic pathway as well as those necessary for cellular uptake of lipids from the diet, namely LPL and the membrane channel CD36, the fatty acid translocase.

In order to investigate whether the expression of LPL in liposarcomas reflected a functional significance, we provided LiSa-2 liposarcoma cells with LPL from a bacterial source. In the presence of triglyceride-rich lipoprotein particles, the growth of the LiSa-2 cells was significantly greater than control cells without exogenous LPL or lipoprotein particles.



ABOVE: LPL stimulates tumor cell growth in the presence of lipoproteins. LiSa-2 liposarcoma cells were seeded (24k cells/well) into 24-well plates, and 200  $\mu$ l media containing complete or lipoprotein-depleted fetal calf serum plus the indicated concentrations of bacterial LPL were added the next morning and replaced at 24 h intervals. Viable cells/well were estimated after 72 h using the MTT assay (mean  $\pm$  SEM normalized to the control groups, n = 6; \*p < 0.05 compared to control).

This work demonstrates growth of liposarcoma cells is enhanced in the presence of both LPL and lipoproteins. Importantly, it corroborates that LPL provides a link between dietary lipids and cell proliferation. Previous work from this laboratory demonstrated inhibition of LiSa-2 liposarcoma cell growth. Thus targeting LPL itself would be expected to greatly reduce liposarcoma viability.

In summary, we have employed a tissue microarray constructed at this facility to study expression and localization of proteins involved in lipid metabolism in liposarcoma. This investigation has enabled us to identify CD36 as a potential target for intervention in these tumors. Additionally, we have characterized the role of LPL and exogenous lipids in tumor proliferation, and we have demonstrated that exogenous LPL in the presence of lipoproteins enhances growth of liposarcomas. Building upon previous work from this laboratory, we plan to study dietary interventions including CLA and to develop peptidomimetic compounds to inhibit CD36 and LPL.