Summer Student Research Program
Project Description

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PROJECT TITLE (200 Characters max):

"Role of the Fibroblast Growth Factor 21 (FGF21) in Tumor Microenvironment"

HYPOTHESIS:

"FGF21 plays a role in the crosstalk between adipocytes and breast tumor cells".

PROJECT DESCRIPTION **(Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)**

Epidemiological studies have suggested a close link between obesity and breast cancer. There is an urgent need to investigate the potential pathways linking obesity and breast cancer to have an early diagnosis in patients and optimize the chance of cure. Obesity is an established risk factor for the development of postmenopausal breast cancer and is an independent prognostic factor for the development of distant metastases and death after the diagnosis of breast cancer. Triple Negative Breast Cancer (TNBC) is an aggressive disease subtype that has a poor prognosis and there are inadequate therapies to prevent recurrence in those patients.

Fibroblast Growth Factor 21 (FGF21) belongs to the FGF sub family of endocrine factors, acts as an endocrine factor with important roles in regulating local and systemic metabolic homeostasis of lipid, glucose and energy metabolism. High serum levels of FGF21 were found in obese individuals, subjects with metabolic syndrome, type 2 diabetes mellitus and coronary heart disease. Up to date, the clinical implication of FGF21 in cancer was not elucidated.

We have recently reported that FGF21 levels were significantly increased in clear cell renal cell carcinoma (ccRCC) patients compared with healthy controls. Moreover, there was an association between the increased serum FGF21 levels and the shorter disease free survival in a cohort of 98 ccRCC patients, after adjustment for other predictors of outcome. Our results suggest that higher FGF21 serum level is an independent prognostic biomarker, associated with worse free-disease survival.

There is no information available on the role of FGF21 in mammary tumor initiation and progression; further studies are needed to elucidate the mechanisms behind the increased serum FGF21 levels in cancer patients. The elevated FGF21 levels observed in these patients could be

due to a high FGF21 synthesis by the tumor cells or the stress caused microenvironment metabolic disorders. Our preliminary studies have shown that the level of circulating FGF21 is also potentially useful as a high sensitive biomarker for early diagnosis of breast patients. We have found increased serum FGF21 levels since the early stages of breast cancer but no differences were observed among stages. These findings suggest that the initiation of the tumor itself could be considered as a stressful condition that induces an increased FGF21 secretion by hepatocytes or/and adipocytes.

In order to elucidate the role of FGF21 in tumor growth and microenvironment; in our studies, we will use the E0771 mammary cancer cells and the 4T1 mammary aggressive "triple negative" tumor model which resembles breast cancer in patients. In addition, we will use the 3T3-L1 cells to elucidate the involvement of the FGF21 secreted by the adipocytes. We believe that our studies will serve as a preliminary groundwork for a novel target in the treatment of metastatic ER negative cancer.

Our overall hypothesis is that **"FGF21 plays a role in the crosstalk between adipocytes and breast tumor cells".** Based on our clinical results in cancer patients, we are proposing to elucidate the molecular mechanism by which FGF21 is produced and affects the mammary tumor microenvironment. To this end, the student will have to implement the following plan:

1. To culture E0771 and 4T1 mammary tumor cells and collect conditioned medium (CM).
2. To differentiate of 3T3-L1 adipocytes.
3. To characterize CRISPR/Cas9 modified FGF-21KO- tumor cells vs. parental cells.
4. To evaluate by western blot, ELISA and PCR some critical molecules in tumor microenvironment. For example: Adipokines: leptin, adiponectin, TGF-beta, IL-8, FGF21, VEGF. TAMs receptors; Prostaglandins and Inflammatory: COX-2, mPGES, 15-PGDH, NFkb, PGE2 levels, TNF-α, IL-6, IL-8, IL1β. ECM: MMP-9, MMP-2, Collagens I, IV and VI, Fibronectin, E-Cadherin.
5. To co-culture 3T3-L1 murine adipocytes with mammary tumor cells (4T1 or E0771) or treated the tumor cells with the CM from 3T3-L1 adipocytes to evaluate cell proliferation, adhesion, migration and invasion properties.

SPONSOR'S PUBLICATIONS RELEVANT TO THIS RESEARCH:

1. Knott ME, Minatta JN, Roulet L, Gueglio G, Pasik L, Ranuncolo SM, Nuñez M, Puricelli L, **De Lorenzo MS*.** Circulating Fibroblast Growth Factor 21 (FGF21) as diagnostic and prognostic biomarker in Renal Cancer. *J Mol Biomark Diagn. 2016 Jun; 1(Suppl 2). pii: 015. Epub 2016 May 20.* ***Corresponding author*.**

2. Kimani SG; Kumar S.; Davra V.; Chang Y-J.; Kasikara C.; Geng K.; Tsou W-I.; Wang S.; Hoque M.; Boháč A.; Lewis-Antes A.; **De Lorenzo MS**.; Kotenko SV.; Birge RB. Normalization of TAM post-receptor signaling reveals a cell invasive and metastatic signature for Axl tyrosine kinase. *Cell Commun Signal. 2016 Sep 6; 14(1):19. doi: 10.1186/s12964-016-0142-1.*

3. Baljinnyam E., Umemura M., Chuang C., **De Lorenzo MS.**, Iwatsubo M., Chen S., Goydos JS., Ishikawa Y., Whitelock JM. and Iwatsubo K. Epac1 increases migration of endothelial cells and melanoma cells via FGF2-mediated intercellular communication. *Pigment Cell Melanoma Res. 27(4): 611-20, 2014.*

4. De Lorenzo MS., Chen W, Baljinnyam E, Carlini MJ, La Perle K, Bishop SP, Wagner TE, Rabson AB, Vatner DE, Puricelli LI, Vatner SF. "Reduced malignancy as a mechanism for longevity in mice with adenylyl cyclase type 5 disruption". *Aging Cell 13(1):102-10, 2014.*

5. Masanari U., Baljinnyam E., Feske S., **De Lorenzo MS.**, Xie LH., Fujita T., Yokoyama U., Chen S., Goydos JS., Ishikawa Y. and Iwatsubo K. Store-operated Ca^{2+} entry (SOCE) regulates cell proliferation and migration of melanoma via extracellular signal-regulated kinase (ERK) signaling. *PLoS One. 9 (2): e89292; 2014.*

6. Yan L, Gao S, Ho D, Park M, Ge H, Wang C, Tian Y, Lai L, **De Lorenzo MS**, Vatner DE and Vatner SF. Calorie restriction can reverse, as well as prevent, aging cardiomyopathy. *Age (Dordr) 35(6):2177-82, 2013.*

7. Yan L, Park JY, Dillinger JG, **De Lorenzo MS**, Yuan C, Lai L, Wang C, Ho D, Tian B, Stanley WC, Auwerx J, Vatner DE, Vatner SF. Common Mechanisms for Calorie Restriction and AC5 Knockout Models of Longevity. *Aging Cell 11(6):1110-20, 2012.*

8. Banke NH, Yan L, Pound KM, Dhar S, Reinhardt H, **De Lorenzo MS**, Vatner SF, Lewandowski ED. Sexual dimorphism in cardiac triacylglyceride dynamics in mice on long term caloric restriction. *J Mol Cell Cardiol., 52(3):733-40, 2012.*

9. De Lorenzo MS*, Baljinnyam E., Vatner DE., Abarzúa P., Vatner SF. and Rabson AB. Caloric Restriction Reduces Growth of Mammary Tumors and Metastases. *Carcinogenesis 32(9):1381-7, 2011.* ***Corresponding author.***

10. Baljinnyam E., Umemura M., **De Lorenzo MS.**, Xie LH., Nowycky M., Iwatsubo M., Chen S., Goydos JS. and Iwatsubo K. Gβγsubunits inhibit Epac-induced melanoma cell migration. *BMC Cancer, 11(1):256, 2011.*

11. Baljinnyam E., Umemura M., **De Lorenzo MS**. and Iwatsubo K. Epac1 Promotes Melanoma Metastasis via Modification of Heparan Sulfate. *Pigment Cell Melanoma Res. 24(4):680-7, 2011.*

12. Carlini MJ, **De Lorenzo MS** and Puricelli L. Cross-talk between tumor cells and the microenvironment at the metastatic niche. *Current Pharm Biotechnology, 12 (11): 1900-1908, 2011*.

13. Baljinnyam E, **De Lorenzo MS**, Xie LH, Iwatsubo M, Chen S, Goydos JS, Nowycky MC, Iwatsubo K. Exchange protein directly activated by cyclic AMP increases melanoma cell migration by a Ca2+-dependent mechanism. *Cancer Res.; 70(13):5607-17*, 2010.

14. Muller A., **De Lorenzo M.**, Dannenberg A. and Mulhall JP. The development of an in vivo model for the assessment of cigarette smoking-associated erectile dysfunction. *Journal of Men' Health; 7(3): 315-316, 2010.*

15. Catanzaro D.F., Zhou Y., Chen R., Yu F., Catanzaro S.E., **De Lorenzo M.S.**, Subbaramaiah K., Zhou X.K., Pratico D., Dannenberg A.J. and Weksler B.B. Potentially Reduced Exposure Cigarettes Accelerate Atherosclerosis: Evidence for the Roles of Nicotine. *Cardiovasc Toxicol. 7(3):192-201, 2007.*

16. Moraitis D., Du B., **De Lorenzo M.S.**, Boyle J.O., Weksler B.B., Cohen E.G., Carew J.F., Altorki N.K., Kopelovich L., Subbaramaiah K. and Dannenberg A.J. Levels of COX-2 are increased in the oral mucosa of smokers. Evidence for the role of EGFR and its ligands. *Cancer Research, 65: 664-670, 2005.*

17. Port J.L., Yamaguchi K., Du B., **De Lorenzo M.S.**, Chang M., Heerdt P.M., Kopelovich L., Altorki N.K., Subbaramaiah K. and Dannenberg A.J. Tobacco Smoke Induces CYP1B1 in the Aerodigestive Tract. *Carcinogenesis, 25: 2275-2281, 2004.*

18. De Lorenzo M.S., Farina H.G., Alonso D.F. and Gomez D.E. Role of protein kinase C-dependent signaling pathways in the antiangiogenic properties of nafoxidine. *Anticancer Research, 24: 1737-1743, 2004.*

19. De Lorenzo M.S., Yamaguchi K., Subbaramaiah K. and Dannenberg A.J. Bryostatin-1 Stimulates the Transcription of Cyclooxygenase-2. Evidence for an AP-1-dependent Mechanism". *Clinical Cancer Research, 9: 5036-5043, 2003.*

20. De Lorenzo M.S., Ripoll G.V., Yoshiji H., Yamazaki M., Thorgeirsson U.P., Alonso D.F., Gomez D.E. Altered tumor angiogenesis and metastasis of B16 melanoma in transgenic mice overexpressing tissue inhibitor of metalloproteinases-1. *In Vivo, 17: 45-50, 2003.*

21. Yoshii J., Yoshiji H., Kuriyama S., Ikenaka Y., Noguchi R., Okuda H., Tsujinoue H., Nakatani T., Kishida H., Nakae D., Gomez D.E., **De Lorenzo M.S.**, Tejera A.M. and Fukui H. The copper- chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells. *International Journal of Cancer, 94: 768-773, 2001.*

22. De Lorenzo M.S., Lorenzano Menna P., Alonso D.F. and Gomez D.E. In vitro activity of a Solanum tuberosum extract against mammary carcinoma cells. *Planta Medica, 67: 164-166, 2001.*

23. De Lorenzo M.S., Alonso D.F. and Gomez D.E. Nafoxidine modulates expression of matrix-metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in endothelial cells. *Anticancer Research, 20: 395-400, 2000.*

24. Gomez D.E., **De Lorenzo M.S.**, Alonso D.F., Andrade Z.A. Expression of metalloproteinases (MMP-1, MMP-2, and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) in schistosomal portal fibrosis. *American Journal of Tropical Medicine and Hygiene, 61(1): 9-13, 1999.*

25. Davel L.E., Puricelli L.I., Vidal M.C., **De Lorenzo M.S.**, Sacerdote de Lustig E., Bal de Kier Joffe E.D. Soluble factors from the target organ enhance tumor cell angiogenesis: role of laminin SIKVAV sequence. *Oncology Reports, 6: 907-911, 1999.*

26. Alonso D.F., Farina H.G., Skilton G., Gabri M.R., **De Lorenzo M.S.** and Gomez D.E. Reduction of mouse mammary tumor formation and metastasis by lovastatin, an inhibitor of mevalonate pathway of cholesterol synthesis. *Breast Cancer Research and Treatment, 50: 83-93, 1998.*

27. Alonso D.F., Skilton G., **De Lorenzo M.S.**, Scursoni A.M., Yoshiji H. and Gomez D.E. Histopathological findings in a highly invasive mouse mammary carcinoma transfected with human tissue inhibitor of metalloproteinases -1. *Oncology Reports, 5: 1083-1087, 1998.*

28. Alonso D.F., **De Lorenzo M.S.**, Tejera A.M. and Gomez D.E. Fibrin formation induced by tumor procoagulants enhances urokinase activity produced by mammary carcinoma cells. *Oncology Reports, 5: 209-212, 1998.*

29. Puricelli L., **De Lorenzo M.S.**, Eiján A.M., Malagrino H., Kes S., Pasik L., Matos E., Bal de Kier Joffé E. and Casabé A. Increased levels of urinary basic fibroblast growth factor in bladder cancer patients. *Oncology Reports, 4: 619-622, 1997.*

30. Alonso D.F., Skilton G., Farina H.G., **De Lorenzo M.S.** and Gomez D.E. Modulation of growth and urokinase secretion by vasopressin and closely related nonapeptides in metastatic mouse mammary tumor cells. *International Journal of Oncology, 10: 375-379, 1997.*

- **Recent Abstracts (by chronological order)**

1. "Immunomodulatory functions of TAM receptors in the Tumor Microenvironment". Davra V., Kumar S., Kasikara C., Geng K., **De Lorenzo MS.** and Raymond B. Birge. *Keystone Symposia, January 2017.*

2. Circulating fibroblast growth factor 21 (FGF21) as diagnostic and prognostic biomarker in renal cancer. Knott ME., Minatta JN., Roulet L., Gueglio G., Pasik L., Ranuncolo SM., Nuñez M., Puricelli L. and **De Lorenzo MS.** *American Association for Cancer Research Meeting, April 2016.*

3. Factor de Crecimiento de Fibroblastos 21 sérico (FGF21s) como biomarcador diagnóstico y pronóstico en pacientes con Carcinoma Celular Renal de células claras (CCRcc). Knott ME., Minatta JN., Roulet L., Gueglio G., Pasik L., Brezinski M., Malagrino H., Pallotta MG., Lastiri J., Ranuncolo SM., Nuñez M., Puricelli L. and **De Lorenzo MS.** *Argentinean Association for Clinical Oncology, July 2015.*

4. Levels of Fibroblast Growth Factor 21 (FGF21) in serum as diagnostic biomarker in patients with breast cancer. Knott ME., Ranuncolo SM., Nuñez M., Armanasco E., Puricelli LI. and **De Lorenzo MS**. *American Association for Cancer Research Meeting, April 2015.*

5. Fibroblast Growth Factor 21 in serum is a biomarker of diagnosis in patients with renal cell carcinoma (CCR). Knott ME., Pallota MG., Gueglio G., Quiroga NG., Malagrino H., Brzezinski M., Ranuncolo SM., Puricelli LI. and **De Lorenzo MS**. *Argentinean Society for Clinical Investigation, November 2014.*

6. "Combination of Metformin plus Orlistat Prevents Tumor Progression: Novel role of the Metabolic Hormone Fibroblast Growth Factor 21 (FGF21)". Sivaram S., Baljinnyam E., Iwatsubo K., Puricelli LI. and **De Lorenzo MS.** *American Association for Cancer Research Meeting, April 2014.*

7. "Inhibition of Mammalian Sterile 20-like Kinase 1 Rescues Cardiomyopathy through Protection from Myocyte Necrosis as well as Apoptosis". Lee GJ, **De Lorenzo MS.**, Vatner SF., Vatner DE., Yan L. *Experimental Biology Meeting, April 2014.*

IS THIS PROJECT SUPPORTED BY EXTRAMURAL FUNDS?
Yes ☐ or No ☒
(IF YES, PLEASE SUPPLY THE GRANTING AGENCY'S NAME)

THIS PROJECT IS: ☐ **Clinical** ☒ **Laboratory** ☐ **Behavioral** ☐ **Other**

THIS PROJECT IS CANCER-RELATED ☒
Please explain Cancer relevance

In our studies, we will use the 4T1 mammary aggressive "triple negative" tumor mouse model which resembles breast cancer in patients. We believe that our studies will serve as a preliminary groundwork for the potential application in the prevention of recurrence of local and metastatic ER negative tumors after the initial adjuvant therapy.

Summer Student Research Program
Project Description

THIS PROJECT IS HEART, LUNG & BLOOD- RELATED ☐
Please explain Heart, Lung, Blood relevance

THIS PROJECT EMPLOYS RADIOISOTOPES ☐

THIS PROJECT INVOLVES THE USE OF ANIMALS ☐
PENDING ☐ **APPROVED** ☐ **IACUC PROTOCOL #**

THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS ☐
PENDING ☐ **APPROVED** ☐ **IRB PROTOCOL # M**

THIS PROJECT IS SUITABLE FOR:

UNDERGRADUATE STUDENTS	☐	ENTERING FRESHMAN	☐
SOPHMORES	☐	ALL STUDENTS	☒

THIS PROJECT IS WORK-STUDY: **Yes** ☐ **or** **No** ☒

THIS PROJECT WILL BE POSTED DURING ACADEMIC YEAR FOR INTERESTED VOLUNTEERS: **Yes** ☐ **or** **No** ☒

WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?

The student will establish the differentiation of pre-adipocytes into adipocytes and perform cell co-culture experiments with 4T1 and E0771 mammary tumor cells to analyze different molecules in the CM and cell lysates. The student will analyze these molecules using ELISA kits, western blot, enzyme activity kits or q-PCR. The student will acquire laboratory experience and learn several basic molecular and biochemical techniques. Look relevant bibliography and prepare analytic tables comparing different research made on this subject. To be able to think and develop the own ideas related to the project. The student will also learn the importance of discovering new molecular targets to translate basic research findings into potential pharmacological approaches to combat cancer disease.