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PRINCIPAL INVESTIGATOR: Miranda Fong

CONTRACTING ORGANIZATION: Beckman Research Institute of the City of Hope
Duarte, CA 91010-3012
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Miranda Fong

E-Mail: mifong@coh.org

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Beckman Research Institute of the City
of Hope
1500 E. Duarte Rd
Duarte, CA 91010

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14. ABSTRACT
Reprogrammed glucose metabolism due to increased glycolysis and glucose uptake is a hallmark of cancer. We show that cancer cells can suppress glucose metabolism in endothelial, lung fibroblasts, and brain astrocyte niche cells through secreting exosomal miR-122, a miRNA whose level in the circulation predicts metastasis of breast cancer. MiR-122 suppresses glucose uptake and metabolism primarily through down-regulating the glycolytic enzyme pyruvate kinase M2 (PKM2). Our results demonstrate that cancer cells are capable of influencing how niche cells metabolize glucose through exosome secretion of miR-122 and the consequent down-regulation of glucose metabolic enzymes in niche cells leading to reduced glucose utilization.

15. SUBJECT TERMS
breast cancer, exosome, miRNA, glucose metabolism, PKM2, GLUT1, niche adaption

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1. **INTRODUCTION:**

Our preliminary data indicates that 1) miR-122 is highly secreted by BC cells and its level in the circulation is a marker for predicting metastatic progression in early-stage BC patients; 2) miR-122 down-regulates the glycolytic gene *PKM* and oxidative phosphorylation gene *citrate synthase (CS)* through targeting a conserved site in the 3'UTR; 3) suppression of PKM2 expression by miR-122 reduced the level of the GLUT1 causing reduced glucose uptake; and 4) anti-miR-122 therapy suppressed metastasis in a xenograft mouse model of human BC. Therefore, we hypothesize that BC-derived exosomal miR-122, in part by targeting PKM and/or CS, reprograms systemic glucose utilization in niche cells for the creation of a favorable pre-metastatic environment to facilitate BC progression and metastasis. In depth functional analysis of cancer-secreted miR-122, which contributes to the adaptations of cancer-hosting niche, will provide novel information in the dynamic environmental crosstalk between cancer and host during disease progression. Furthermore, this study will demonstrate the feasibility of targeting miRNAs to block the intercellular communication at an early stage and prevent cancer-directed reprogramming of pre-metastatic niches. Our future objectives are to validate the miR-122 pathway in glucose metabolism in primary BC, to identify patients that may benefit from anti-miR-122 treatment strategies, to understand the system-wide effects of BC-secreted miR-122 on energy metabolism reprogramming, and to identify how metastatic niche adaption occurs by circulating tumor cells.

2. **KEYWORDS:** breast cancer, exosome, miRNA, glucose metabolism, PKM2, GLUT1, niche adaption

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**
  - Major Task 1: Exosome treatment of lung fibroblasts, brain astrocytes, and endothelial cells (niche cells)
    - Milestone Achieved: identification of specific gene targets of miR-122 and mechanism of glucose allocation in various niche cells. 100% completed.
  - Major Task 2: Exosome injection in NSG mice
    - Milestone Achievement: Identification of miR-122 induced metastatic niche selection by circulating tumor cells. 0% completed.
  - Major Task 3: Orthotopic xenograft tumors expressing high miR-122 and effectiveness of anti-miR-122 intervention

- Milestone Achievement: Characterization of effects of breast cancer- secreted miR-122 on systemic glucose allocation reprogramming; 1-2 peer-reviewed article. 0% completed.

- **What was accomplished under these goals?**
  - 1) Major activities include determining the effect of extracellular miR-122 by isolating extracellular vesicles (EV) by ultacentrifugation from MCF10A/vec (control cell line), MCF10A/miR-122 (miR-122 specific over-expressing cell line), and breast cancer cell line MDA-MB-231(which has high miR-122) for treating lung fibroblasts, brain astrocytes, and HMVEC cells. EV were labeled with DiI to measure EV uptake in niche cells (Fig. 1a, 2a). EV were also isolated from the aforementioned cell lines and used to treat NSG mice i.v (Fig. 4). 2) The specific objective was to examine the effect of extracellular miR-122 on PKM2, CS, GLUT1 expression (Fig. 1b-e, 2b-e, 3a-c). Glucose metabolism was examined in EV-fed niche cells by 2-NBDG uptake and media metabolite measurement (Fig. 1f-g, 2f, 3d-e). EV-treated mice were injected with 2-NBDG to measure glucose uptake (Fig. 4a-c). Cell type specific markers were used to identify astrocytes (GFAP) and fibroblasts (FSP-1). Human specific CD63 was used as an exosome marker. MiR-122, PKM, and GLUT1 expression was also measured (Fig 4d-e). 3) Results indicate that extracellular miR-122 reduces PKM RNA and protein (Fig. 1b-e, 2b-e, 3a-c) which can be alleviated by treating the cells with anti-miR-122 oligos in fibroblasts, astrocytes, and HMVEC. The reduction of GLUT1 as a by-production of reduced PKM resulted in reduced glucose uptake measured by 2-NBDG uptake and media metabolite analysis (Fig. 1f-g, 2f, 3e). Restoration of PKM or GLUT1 restored glucose uptake (Fig. 1h-i). To determine if the reduction of PKM or GLUT1 alone was sufficient to reduce glucose uptake, siRNA against PKM2 or GLUT1 was used in lung fibroblasts and cells were treated with 2-NBDG. Knock-down of either PKM2 or GLUT1 was sufficient to reduce glucose uptake (Fig. 1j). Additionally when conditioned media was collected from fibroblasts transfected with siRNA and fed to cancer cells, the additional glucose in the media resulted in an increase of cancer cell proliferation measured by BrdU incorporation (Fig. 1k). NMR analysis in HMVEC cells showed a reduction

of glucose, pyruvate, and lactate upon treatment with high miR-122 EV (Fig. 3d), demonstrating that miR-122 down-regulates glucose metabolism.

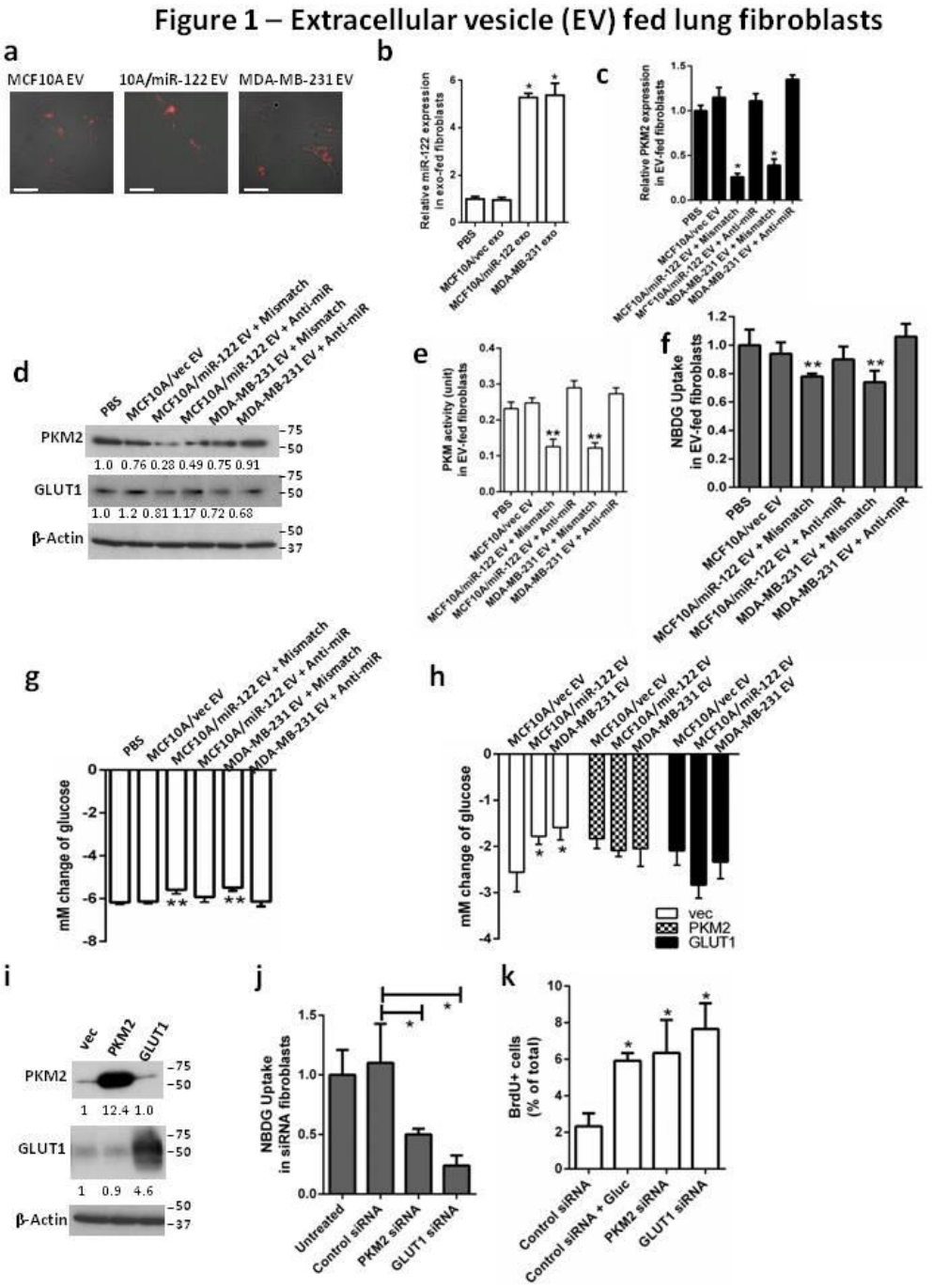

Figure 1. Cancer-secreted miR-122 downregulates glucose uptake in lung fibroblasts. (**a**) Uptake of DiI-labelled exosome-containing EVs. Bar equals 60 μm. (b) Level of miR-122 by RT-qPCR. (c) Level of PKM2 by RT-qPCR. (d) Western blot analysis (with marker size indicated in kDa), (e) PKM activity assay using 5 μg of proteins. (f) 2-NBDG uptake. (g) Change of glucose in the CM. (h) Change of glucose in the CM of fibroblasts with restored PKM2 or GLUT1 expression. (i) Western blot of fibroblasts for (h). (j) 2-NBDG uptake in siRNA-transfected fibroblasts. (k) CM was collected from siRNA-transfected fibroblasts cultured for 72 h. The CM was then fed to MDA-MB-231-HM cells before proliferation was assessed by BrdU-incorporation at 72 h. * $p < 0.05$, ** $p < 0.01$ for all panels derived from Kruskal-Wallis test. Data are represented as mean ± SD in all panels except (a, d, i).

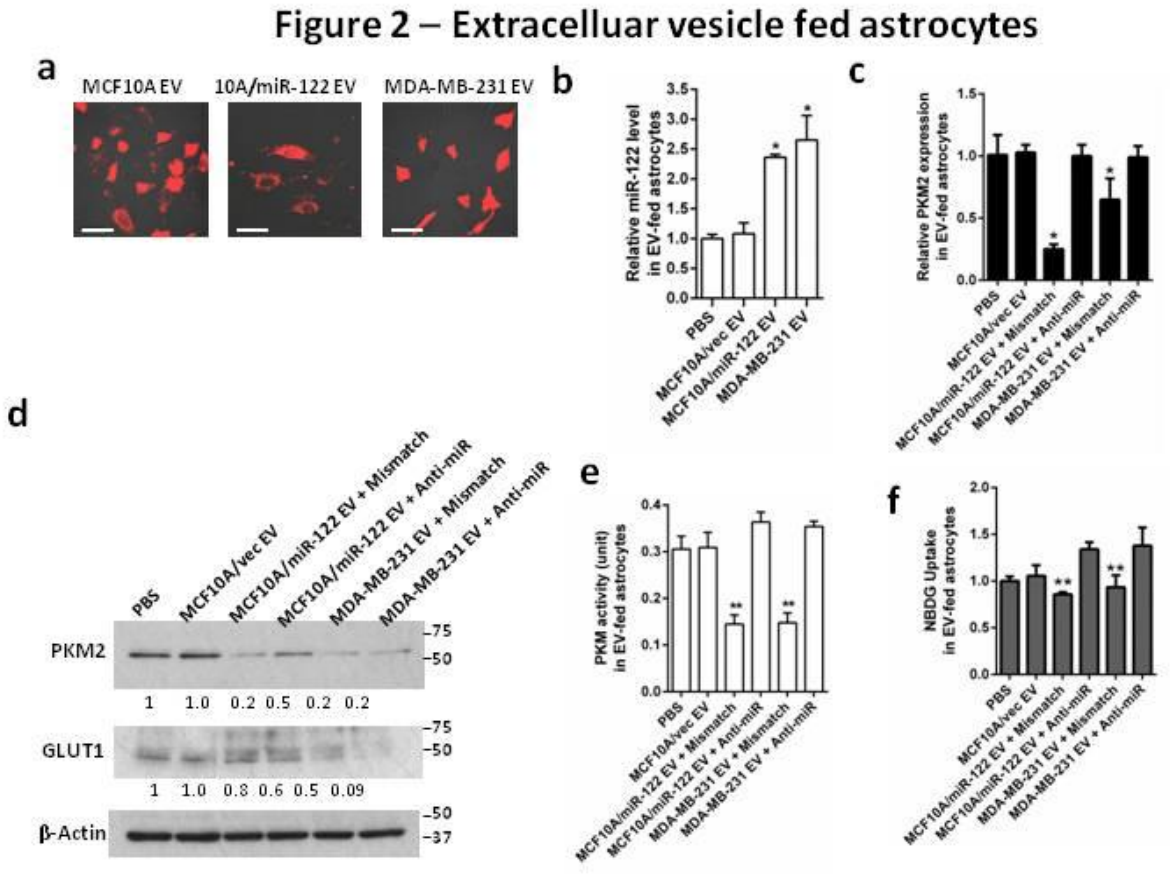

Figure 2. Cancer-secreted miR-122 downregulates glucose uptake in astrocytes. (**a**) EV uptake by astrocytes. Bar equals 60 μm. (b) Level of miR-122 by RT-qPCR. (c) Level of PKM2 by RT-qPCR. (d) Western blot analysis (with marker size indicated in kDa), (e) PKM activity assay using 10 μg of proteins. (f) 2-NBDG uptake. * $p < 0.05$, ** $p < 0.01$ for all panels derived from Kruskal-Wallis test. Data are represented as mean ± SD in all panels except (a, d).

Figure 3 – Extracellular vesicle fed HMVEC

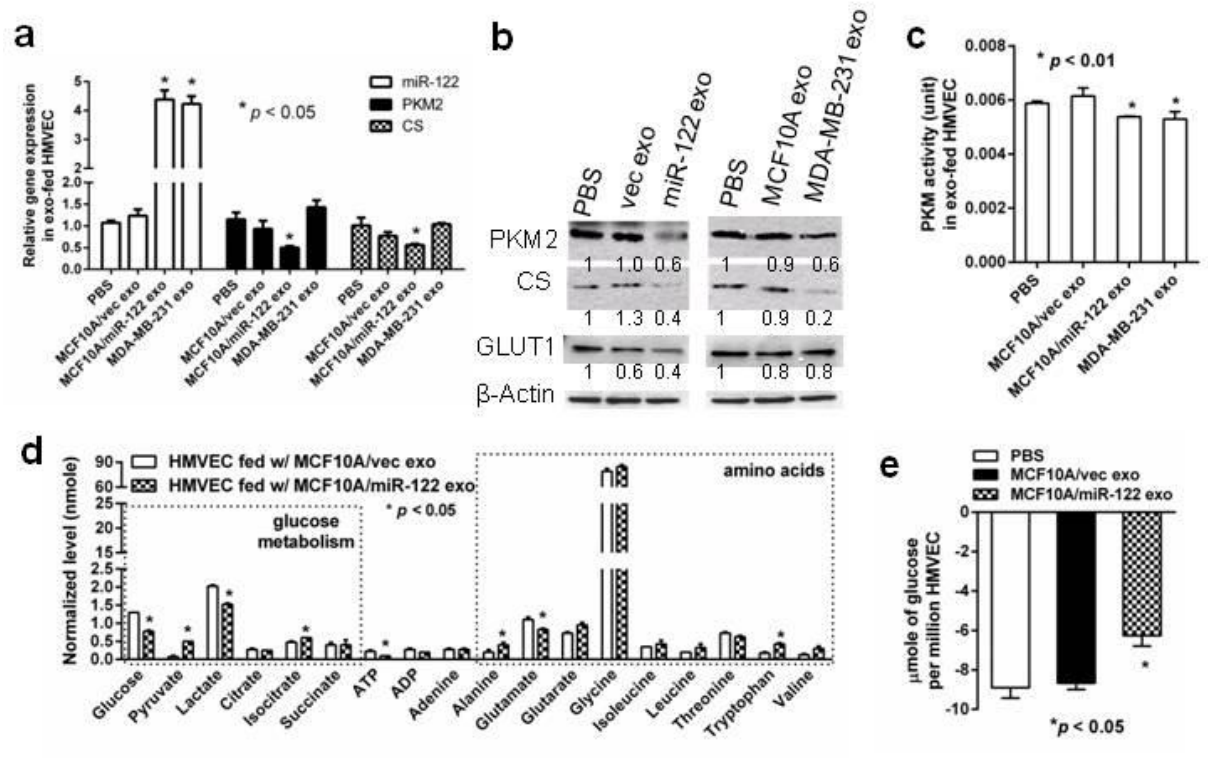

Figure 3. Cancer-secreted miR-122 down-regulates glucose uptake in HMVEC. (a) Levels of miR-122, PKM2, and CS by RT-qPCR. (b) Western blot analysis of PKM2, CS, and GLUT1. (c) PKM activity assay using 5 µg of proteins. (d) NMR analysis of intracellular metabolites. (e) Change of glucose in the CM.

- **What opportunities for training and professional development has the project provided?**
  - I have had training activities including one-on-one work with my mentor. For the first year of this award, I presented at the bi-monthly department seminar series and two internal poster sessions designated to showcase research at City of Hope.
- **How were the results disseminated to communities of interest?**
  - Nothing to report.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - Mice will be treated with extracellular vesicles high in miR-122 (derived from MCF10A/miR-122 and MDA-MB-231 cells) bi-weekly for 3 weeks before analysis. Glucose uptake in the brain and lung (the comparable organs identified from the *in vitro* work) will be analyzed by NBDG uptake, as well as gene expression of PKM and GLUT1 by RT-qPCR. Additionally, after exosome conditioning, mice will receive an intracardiac injection of MDA-MB-231 cells to mimic the metastatic phase of tumor progression. This will allow us to determine if alternation of glucose metabolism in the pre-metastatic niche contributes to circulating tumor cell colonization and metastatic formation.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  - Nothing to report.
- **What was the impact on other disciplines?**
  - Nothing to report.
- **What was the impact on technology transfer?**
  - Nothing to report.
- **What was the impact on society beyond science and technology?**
  - Nothing to report
  -

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**
  - Nothing to report.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - Nothing to report.
- **Changes that had a significant impact on expenditures**
  - Nothing to report.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
  - Nothing to report.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
- **Journal publications.**
  - Nothing to report
- **Books or other non-periodical, one-time publications.**
  - Nothing to report
- **Other publications, conference papers, and presentations.**
  - City of Hope Annual Poster Collaboration, 2014 (Local)
  - Research Staff Organization (RSO) Retreat Poster Session, 2014 (Local)
  - Department of Cancer Biology Seminar Series, 2014 (Local)
- **Website(s) or other Internet site(s)**

  Nothing to report
- **Technologies or techniques**

  Nothing to report
- **Inventions, patent applications, and/or licenses**

  Nothing to report.
- **Other Products**

  Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	Miranda Fong
Project Role:	PI
Nearest person month worked:	12
Contribution to Project:	No change
Funding Support:	None

Name:	Emily Wang
Project Role:	Co-mentor
Nearest person month worked:	12
Contribution to Project:	No change

Name:	Susan Kane
Project Role:	Co-mentor
Nearest person month worked:	12
Contribution to Project:	No change

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - Nothing to Report
- **What other organizations were involved as partners?**
  - Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS:** Nothing to report

9. **APPENDICES:**

Poster Abstract:

Reprogrammed glucose metabolism due to increased glycolysis and glucose uptake is a hallmark of cancer. We show that cancer cells can suppress glucose metabolism in endothelial, lung fibroblasts, and brain astrocyte niche cells through secreting exosomal miR-122, a miRNA whose level in the circulation predicts metastasis of breast cancer. MiR-122 suppresses glucose uptake and metabolism primarily through down-regulating the glycolytic enzyme pyruvate kinase M2 (PKM2). Our results demonstrate that cancer cells are capable of influencing how niche cells metabolize glucose through exosome secretion of miR-122 and the consequent down-regulation of glucose metabolic enzymes in niche cells leading to reduced glucose utilization. We hypothesize that this mechanism allows for cancer cells to have glucose more readily available to sustain their rapid proliferation when competing among other cell types in the niche. Our in vivo data indicates that miR-122 intervention can reduce metastasis to the brain and lung.