Mesenchymal Stem Cells Regulate the Epigenome of Breast Cancer Cells to Facilitate Dormancy in Bone Marrow

Alejandra Ferrer[1,2], Garima Sinha[1,2], Oleta Sandiford[1,2], Yahaira Naaldijk[2], Sorosh Tourzani, Jean-Pierre Etchegaray[3], Pranela Rameshwar, PhD[2]
Rutgers School of Graduate Studies[1], Department of Medicine[2], Rutgers University-Newark[3]

Introduction

- Breast cancer (BC) is the most common cancer among women. Despite early diagnosis and aggressive treatment, BC remains a clinical problem. Clinical and experimental evidence indicates that BC cells (BCCs) can home to different distant organs, including the preferred organ, bone marrow (BM). Infiltration of BCCs into the BM can occur years before clinical detection as well as anytime during progression.
- BCCs survive in the BM by adopting dormancy and exhibiting cancer stem cell (CSC) properties, allowing them to resist conventional treatments. Dormant BCCs are the source of cancer relapse and tumor initiation at tertiary sites. In addition, dormant BCCs share properties with hematopoietic stem cells (HSCs), making difficult the development of therapeutic strategies since they can potentially harm the hematopoietic system and affect patient prognosis. Hence, it is important to study the processes by which BCCs establish dormancy and a CSC phenotype in the BM to effectively eradicate these cells from the system.
- Intercellular communication between the BCCs and BM microenvironmental cells supports the establishment of BCC dormancy in BM. Intercellular interactions can be contact-dependent, via gap junctional intercellular communication (GJIC), and contact-independent, including soluble and insoluble factors, such as cytokines and microvesicles such as exosomes, respectively.
- Alterations of the epigenome of BCCs are a potential avenue through which BM microenvironmental cells, such as mesenchymal stem cells (MSCs), mediate BC dormancy and recurrence, due to the ability for such changes to impart cellular plasticity. The proposed study will identify the role of mesenchymal stem cells (MSCs) in mediating epigenetic changes in BCCs.

MSC-derived exosomes promote BCC stemness

Experimental Model: Primed vs. Naïve MSC-derived exosomes

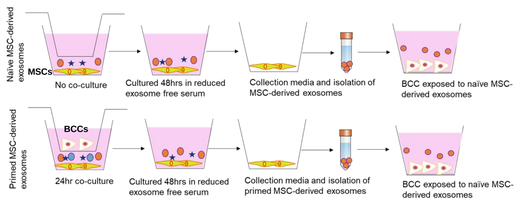


Fig. 1: BCCs and MSCs were co-cultured using the transwell method. This system allows communication via secretome (i.e. soluble and insoluble factors) without physical contact between cells. A separate set of BCCs were exposed to MSC-derived exosomes to assess changes in cell cycle and stemness

MSCs release a different set of exosomes upon exposure to BCCs that promote stemness

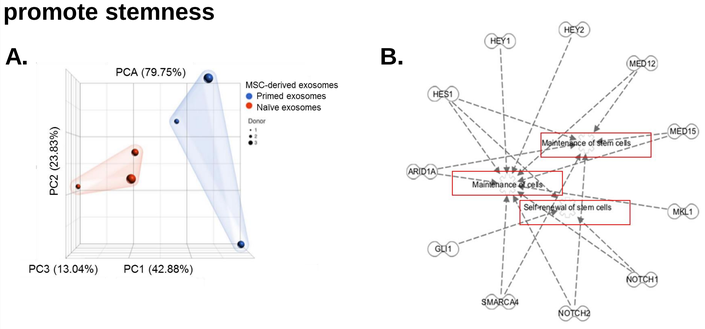


Fig. 2: A. Principal component plot (PCA) from RNA-seq performed on naïve and primed MSC-derived exosomes. **B.** Ingenuity pathway Analysis (IPA) showing that BCCs transition into a CSC-like phenotype.

Epigenetics: potential avenue for BCC dormancy

RNA-seq reveals that MSCs produce and release 5 epigenetic mediators in the primed condition

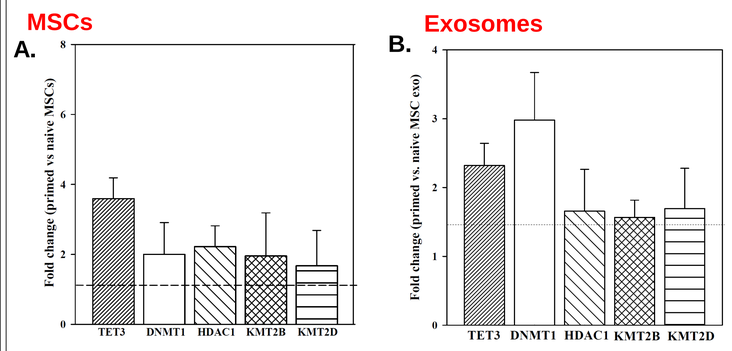


Fig 3. A. RNA-seq depicting the fold change (>1.5 cut-off) in expression of five epigenetic mediators in MSCs that were exposed to BCCs relative to naïve MSCs (pvalue<0.05 for each gene). **B:** RNA-seq showing the fold change (>1.5) of the five epigenetic mediators in MSC-derived exosomes upon exposure to BCCs.

Survival rate of BC patients with mutations on epigenetic mediators

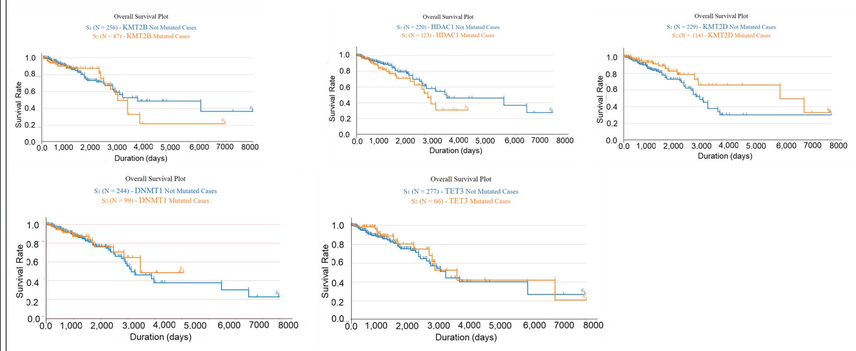
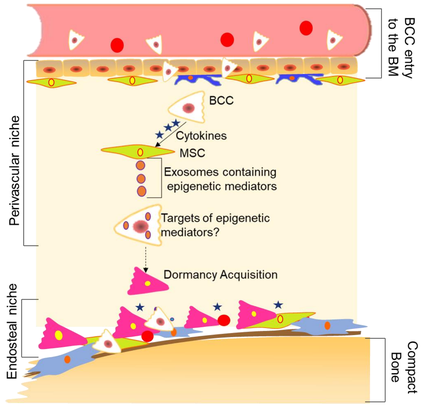


Fig 4. Data undermined from The Genome Atlas Program (TCGA) database depicting that BC patients with mutations on epigenetic mediators have poor prognosis.

Hypothesis



MSCs mediate epigenetic changes in BCCs to promote and sustain dormancy as well as to transition the BCC subsets into a CSC-like phenotype

Fig 5. Overarching hypothesis of thesis project. BCC-derived cytokines dictate release of epigenetic mediators from MSC via exosomes. The mediators induce changes in the epigenome of BCCs towards dormancy and a CSC phenotype.

Expression of epigenetic regulators in MSCs

Expression of epigenetic regulators in MSCs is induced 12hrs after exposure to BCCs

Experimental Design

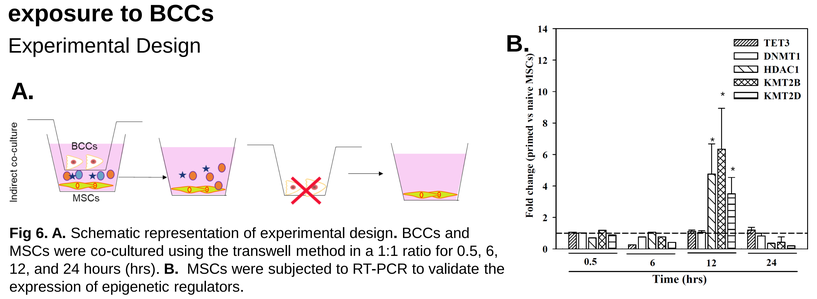
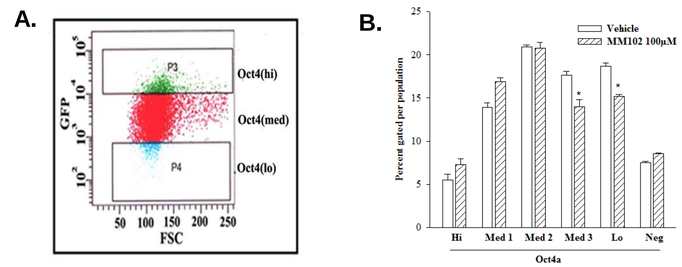


Fig 6. A. Schematic representation of experimental design**.** BCCs and MSCs were co-cultured using the transwell method in a 1:1 ratio for 0.5, 6, 12, and 24 hours (hrs). **B.** MSCs were subjected to RT-PCR to validate the expression of epigenetic regulators.

Inhibition of epigenetic regulators impact BCC subsets

Inhibition of histone methylation decreases Oct4 lo population



Inhibition of DNA methylation increases Oct4 hi BCCs

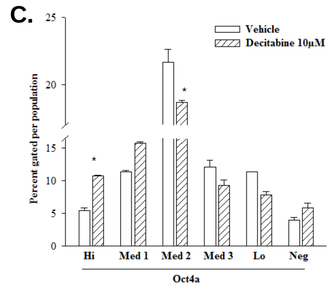


Fig 7. A. Gating scheme that allows for stratification of BCC subsets. BCCs were stably transfected with an Oct4a-GFP reporter. Oct4a is a core stem cell gene. **B.** BCCs were treated with 100μM of MM102 (inhibitor of histone 3 lysine 4 (H3K4) methylation) for 48hrs. **C.** BCCs were exposed to decitabine (10μM) for 48hrs and were subjected to flow cytometry to identify BCC subsets.

Future Directions

1. Understand how MSC-derived epigenetic mediators affect BCC transition into dormancy
2. Identify targets of MSC-derived epigenetic mediators on BCCs and interrogate their function in BCC dormancy
3. Dissect how BCCs dictate changes in MSCs that result in dormancy acquisition

References

Walker ND, et al. *Cancer Lett*. 2016.
Aguirre-Ghiso JA, Sosa MS. *Annu Rev Cancer Biol*. 2018.
Talmadge JE. *Cancer Res*. 2007.
Taback B, et al. *Cancer Res*. 2003.
Kopp H-G, et al. *Anticancer Res*. 2011.
Bliss SA, et al. Cancer Res. 2016.
Patel SA, et al. *Sci Rep*. 2012.
Dhawan A, Friedrichs J, Von Bonin M, Bejestani EP, et al. *Carcinogenesis*. 2016.
Lim PK, et al. *Cancer Res*. 2011.
Walker ND, et al. *Cell Death & Disease*. 2019.
Allis CD, Jenuwein T. Nat Rev Genet. 2016.
Crea F, et al. *Trends Mol Med*. 2015.