Pauline and Irving Tanner Dean’s Scholar Program
2017 Summer Research Grant Application

Title: **“Effect of miRNA-Containing MBVs on Cancer Cell Proliferation”**

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Research Advisor: Dr. Stephen F. Badylak
Abstract:
The proposed Tanner Dean’s project for the summer of 2017 is for the continuation of my earlier work on the characterization of MBVs (Matrix-Bound Nanovesicles). This earlier work was completed during my summer internship at the University of Pittsburgh Cancer Institute under the supervision of Dr. Stephen F. Badylak, a world-renowned leader in tissue engineering/regenerative medicine. Biological scaffold (bioscaffold) materials composed of extracellular matrix (ECM) derived from biological or commercial sources have a wide range of applications such as: tissue repair, immunotherapy, cancer treatment, and many more. Recently, Dr. Badylak’s group has identified the presence of MBVs within ECM bioscaffolds. The MBVs are tiny fluid-filled sacs that bud off from a cell’s outer surface and allow cell communication by transferring proteins, RNAs and other "cargo" from one cell to another through ECM. I, along with other team members, have further characterized that MBV cargo must be responsible for responses against tumor growth and demonstrated that these MBVs contain small RNA molecules in size from 25-250 bp known as microRNA (miR). The laboratory further established that UBM (urinary bladder ECM matrix)-derived MBVs contain miR-143/145, while dermis-derived MBVs contain miR-145 and no miR-143.

In light of the preliminary data, I propose to explore how miR composition of UBM- and dermis-derived MBVs would affect cancer growth in vivo since miR-143 and miR-145 have been reported to suppress cancer cell growth. The molecular mechanism of miR-143 driven suppression of cancer cell growth is under investigation, however, in some cases they are shown to work by inhibiting or targeting glycolysis, COX-2, or N-RAS, while miR-145 driven suppression is resulted by targeting Sox9/aducin 3 and connective tissue growth factor. As part of my hypothesis, I will test the cell viability (normal cells vs. tumor cells) in the presence or absence of UBM- and dermis-derived MBVs and determine how the cargo of the two MBVs (miR-143/145 cluster) suppresses cancer cell growth. For this project, I will be using several tumor cell lines along with normal cell lines to be provided by Dr. Badylak’s laboratory. The data generated under this study will then be used for both therapeutic and diagnostic purposes.
Biographical Sketch:

EDUCATION:
North Allegheny High School: Graduated valedictorian class of 2016
Cornell University: College of Arts and Sciences, double majoring in Biology and English, Harold Tanner Dean’s Scholar, expected graduation—2020

RESEARCH EXPERIENCE:
Ever since my friend’s mother, a neurologist, talked to our kindergarten class about biology, I felt a subtle interest in biology that began to grow as I became familiar with science and eventually research. Visiting my parents’ research labs, I liked pretending to do research, playing with pipets and lab coats. In middle school, I presented posters on experiments I conducted at my house, but as high school came, I wanted to become more involved and do more involved research. With age, my curiosity grew more into understanding human disease process and its cure or prevention. I discovered a research summer camp for high school students during ninth grade and decided to apply. Accepted to the University of Pittsburgh Cancer Institute International Summer Academy (UPCI), I was truly introduced to the world of research. That summer, I learned about cancer biology and conducted a statistical analysis-based project on HPV awareness with Dr. Linda Robertson during the eight-week program. However, I wanted to do more research and wanted to try research in a wet lab, so I reapplied to the program the next year after tenth grade and conducted research with Dr. Robert Binder in his tumor immunology lab, working with natural killer (NK) cells and heat shock proteins (HSPs). The experience was very rewarding, but I was assigned my project when I truly wanted to create my own project. Returning to the program the following year, I was given the chance to work with Dr. Stephen F. Badylak and loved the cutting-edge research his lab was doing; however, I also received the opportunity to conduct my own research project that compelled me to return to his lab ever since. I work with the extracellular matrix (ECM) to find novel immunotherapeutic techniques to combat glioblastoma. For past two summers I have continued to work with my mentor Dr. George Hussey in Dr. Badylak to further our cancer research.

PUBLICATION:
Hussey G, Mehdi Z, Badylak SF et al., Development of Biologic Scaffolds from Human Glioma Tumors as an Organotypic Model to Study Disease Pathogenesis – 20th Annual Regenerative Medicine Workshop; Mar 16 - 19, 2016; Hilton Head Island, South Carolina (Abstract) (Paper Under Preparation)

HONORS and AWARDS:
- 4 years of oncological research with over 1,400 hours
- Awarded best speaker and best poster at UPCI Academy - 2015
- 2016-participated in CURB research poster forum at Cornell University and presented research at National Collegiate Research Conference (NCRC) 2017 at Harvard University
• 2012-2015: 4 times 1st place awards at Pennsylvania Junior Academy of Science (PJAS)
• 2016: Competed at National Science Bowl Competition, Top 16 nationally
• National Merit Finalist and Merit Scholar; AP National Scholar
• Gold Medal for Outstanding Young Citizens Scholarship and Outstanding Asian American Scholarship
Statement of Purpose:

Background:
Cancer is a leading cause of death in the US and across the world (World Cancer Report 2014). It is a complex disease caused by abnormal cell growth affecting any tissue or organ in a living body. A cancer causing tumor (malignant) is the one which spreads from one part of the body or tissue to another through metastasis and could be terminal if not detected early and not treated aggressively. Over 100 types of cancers affect humans and in 2017, more than 1.6 million people will be diagnosed with cancer in the US (National Cancer Institute, NIH). Cancer is a genetic or environmental disease which is caused by defects in DNA resulting in uncontrollable cell growth, either due to genetic predispositions or due to irradiation or other environmental factors that affect the body’s DNA (Vogelstein and Kinzler 2004; NTP 11th Report on Carcinogens, 2004). Due to extensive research over the past few decades, tremendous progress has been made in understanding the genetics of cancer after human genome sequencing. As of now 36 plus cancer causing genes (oncogenes) have been identified (Bishop 1994; Vysotskaia, et al. 2017; Wooster et al. 1994). In this grant I am proposing to target these oncogenes and/or their partners by Matrix-Bound Nanovesicles (MBVs) carrying microRNAs (miRNAs) (Fig. 1).
Preliminary Data and Related Studies:

Recently, the presence of MBVs within extracellular matrix (ECM) bioscaffolds is identified by Dr. Badylak’s group at the University of Pittsburgh (Huleihel et al. 2016). The MBVs are tiny fluid-filled sacs that bud off from a cell’s outer surface and allow cell communication by transferring RNAs, proteins, lipids, and other cargo from one cell to another through the ECM (Fig. 1). The Badylak lab has found that the MBV cargo contains small RNA molecules, ranging in size from 25-250 bp. We further established that UBM (urinary bladder matrix)-derived MBVs contain miR-143/145, while dermis-derived MBVs contain miR-145 and no miR-143.

The miR-143/145 cluster is a part of a group of RNA, called micro RNA, that are short, noncoding RNAs ranging in size from 18-24 nucleotides (nt) and regulate gene expression by binding to mRNA and either degrading mRNA or inactivating it (Fig. 2A). These miRNAs are expressed within cells and can be transported through MBVs within the ECM. Over the past decade, over 700 human miRNAs have been identified and are estimated to target approximately 60% of protein-coding genes, affecting a number of cellular processes such as differentiation, proliferation, and apoptosis (Ambros 2004). Because miRNAs are involved in the regulation of a number of key physiological processes and intracellular signaling pathways, they have been extensively studied in cancer pathogenesis (Fig. 2B) (Bloomston et al. 2007; Lee et al. 2007;
Loterman et al. 2008). In our ECM-based lab, we find that regulatory molecules can have dramatic effects on tissue types because of the ECM-cell interaction that is governed by the concept of dynamic reciprocity, where the cells affect the ECM and in turn the ECM and its components affect the cells. Therefore, we are researching the potentiality of MBVs as a means of immunotherapy of cancer.

**Figure 2:** A: Cartoon depicting the production and function of Micro RNA s (miRNAs). B: Cartoon depicting the role of bunch of Micro RNA s (miRNAs) in various cancers

**Hypothesis:** Since miRNAs have been established as an important player in cancer pathogenesis, we hypothesize that miRNA composition of MBVs will have differential effects on proliferation of cancer cells in culture.

**Specific Aims:** Explore how miRNA composition of UBM MBVs and dermis-derived-MBV would affect proliferation of different cancer cells utilizing several cancer cell lines available in Dr. Badylak's lab.

**Experimental Design:**

1) **Production of MBVs:** MBVs from two sources, skin and urinary bladder, will be used for this project. These ECMs are routinely prepared in Dr. Badylak's lab using their standard protocol (Reing et al. 2010; Mase VJ Jr. et al. 2010).

1.1 **Dermal ECM:** Full-thickness skin will be harvested from market-weight (~110 kg) pigs (Tissue Source Inc.), and the subcutaneous fat and epidermis will be removed by mechanical delamination. This tissue will then be treated with 0.25% trypsin (Thermo Fisher Scientific) for 6 hours, 70% ethanol for 10 hours, 3% H$_2$O$_2$ for 15 min, 1% Triton X-100 (Sigma-Aldrich) in 0.26% EDTA/0.69% tris for 6 hours with a solution change for an additional 16 hours, and 0.1% peracetic acid/4% ethanol (Rochester Midland) for 2 hours. Alternate wash with water and
phosphate-buffered saline (PBS) will be performed between each chemical change. All chemical exposures will be conducted under agitation on an orbital shaker at 300 rpm. Dermal ECM will then be lyophilized and milled into particulate form using a Wiley Mill with a #40 mesh screen.

1.2 Urinary Bladder Matrix: Porcine urinary bladders from market-weight animals will be acquired from Tissue Source, LLC. Briefly, the tunica serosa, tunica muscularis externa, tunica submucosa, and tunica muscularis mucosa will be mechanically removed. The luminal urothelial cells of the tunica mucosa will be dissociated from the basement membrane by washing with deionized water. The remaining tissue, consisted of basement membrane and subjacent lamina propria of the tunica mucosa, will be decellularized by agitation in 0.1% peracetic acid with 4% ethanol for 2 hours at 300 rpm. The tissue will then be extensively rinsed with PBS and sterile water. The UBM will then be lyophilized and milled into particulate form using a Wiley Mill with a #60 mesh screen.

1.3 Enzymatic digestion of ECM samples and MBV isolation: ECMs will be enzymatically digested to release MBVs. Briefly, ECM samples will be lyophilized and ground into a powder using a Wiley Mill with #40 or #60 mesh screen. Each samples (5 mg dry weight) will then be digested with either proteinase K (0.1 mg/ml) in buffer [50 mM tris-HCl (pH 8) and 200 mM NaCl] for 24 hours at room temperature, collagenase (0.1 mg/ml) in buffer [50 mM tris (pH 8), 5 mM CaCl2, and 200 mM NaCl] for 24 hours at room temperature, or pepsin (1 mg/ml) in 0.01 M HCl for 24 hours at room temperature. Enzymatically digested ECM will then be subjected to successive centrifugations at 500g (10 min), 2500g (20 min), and 10,000g (30 min) to remove collagen fibril remnants. Each of the above centrifugation steps will be performed thrice. The fiber-free supernatant will then be centrifuged at 100,000g (Beckman Coulter Optima L-90K ultracentrifuge) at 4°C for 70 min. The 100,000g pellets will be washed and suspended in 500 µl of PBS and passed through a 0.22-µm filter (Millipore).

2) Effect of MBVs on Cancer Lines:

2.1 Cell culture: Cancerous and normal cells (Glioma, Skgt-4, FLO-1, OE19, HEla, CHME5 – human microglia, N1E-115 – neural stem cell, and primary dermal fibroblast) will be grown in their respective culture media in the presence of antibiotic combination to avoid contamination as per manufacturer’s guidelines at 37°C in 5% CO2. These cell lines are routinely maintained in Dr. Badylak’s lab.
2.2 Effect of MBVs on Cell Proliferation: 5x104 cells/well will be plated in 96-well tissue culture plates and after 24 hrs of incubation at 37\(^\circ\) C, media will be removed and replaced with fresh media containing MBVs. After 24 hrs of treatment, cell proliferation will be detected using MTT assay kit where MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] is converted into formazan crystals by living cells, which determines mitochondrial activity. After 24 hrs of treatment, the media will be removed and replaced with 100 \(\mu\)L media and 13 \(\mu\)L MTT solution, and incubated for a further 3 hours. Then, all but 25 \(\mu\)L media will be removed, and 50 \(\mu\)L DMSO per well will be added to dissolve the formazan precipitate. The developed color will be read at a 570 nm. Data will be plotted as percent viability compared to control (untreated cells).

Statistical Analysis: All the experiments will be performed in triplicate. ANOVA and student t-test will be used to determine the significance between experimental and control groups.

Trouble Shooting: I don’t anticipate any major difficulty in achieving my objective of this grant since Dr. Badylak’s lab has all the resources I will need for the successful execution of my project.

Bibliography:


Bishop DT. BRCA1, BRCA2, BRCA3 ... a myriad of breast cancer genes. Eur J Cancer. 30A:1738-9 (1994).


