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PRINCIPAL INVESTIGATOR: Daotai Nie

CONTRACTING ORGANIZATION: Southern Illinois University School of Medicine, Springfield, IL 62794-9626

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Daotai Nie

email: dnie@siumed.edu

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Southern Illinois University School of Medicine
Springfield, IL 62794
dnie@siumed.edu

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14. ABSTRACT

One of the overarching challenges of PCRP is to develop effective treatments for advanced prostate cancer. As nano-meter sized vesicles released by many cell types, exosomes serve as vehicles for long range intercellular communications, with the bioactive contents of exosomes as the messengers. It is hypothesized that normal prostate epithelial cells secrete exosomes to keep neighboring epithelial cells from undergoing uncontrolled growth. On the other hand, due to the altered contents of exosomes, those from prostate cancer cells (tumor exosomes) no longer have tumor suppressive functions. If this hypothesis is proven true, the tumor suppressive exosomes can be characterized and reconstructed as therapeutic agents used for the treatment of prostate cancer. To develop this concept, exosomes will be isolated from normal prostate epithelial cells by differential centrifugations or affinity purifications and evaluated for tumor suppressing activities against various prostate cancer cells (Aim 1). Then the components of the tumor suppressing exosomes will be separated, identified and characterized using biochemical, molecular, and analytical methods and compared with those found in tumor exosomes (Aim 2). Then the exosomes will be "additively manufactured" by recombining the identified components in parts or as a whole, and evaluated for their therapeutic utilities against prostate cancer (Aim 3). The proposed studies address the focus area of therapy. In short term, the studies will identify potential tumor suppressing exosomes that can inhibit prostate cancer. In the long term, the studies will lead new approaches to harness the tumor suppressive powers of normal prostate epithelial and to develop new biological therapeutics for treatment of prostate cancer.

15. SUBJECT TERMS
exosomes, therapeutics, prostate cancer, tumor growth

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Introduction

Background: Exosomes are nano-meter sized vesicles released by many cell types (1). Comprised of lipids, proteins, coding and non-coding RNAs, exosomes serve as cargo carriers used for long range intercellular communications (2). Tumor exosomes can facilitate tumor progression (3). Urinary exosomes are suggested as a potential prostate cancer biomarker (4). This proposal explores a novel idea that exosomes secreted by non-cancerous prostate epithelial cells have tumor suppressing activities through their tumor suppressing cargo. If this hypothesis is proven true, the tumor suppressive exosomes can be characterized and reconstructed as therapeutic agents used for the treatment of prostate cancer.

Hypothesis/Rationale/Purpose: Released by many cell types, exosomes serve as vehicles for long range intercellular communications, with the bioactive contents of exosomes as the messengers. It is hypothesized that normal prostate epithelial cells secrete exosomes to keep neighboring epithelial cells from undergoing uncontrolled growth. On the other hand, due to the altered contents of exosomes, those from prostate cancer cells (tumor exosomes) no longer have tumor suppressive functions. The purpose of this grant is: 1) to identify and characterize tumor suppressing exosomes produced by normal prostate epithelial cells, and 2) to reconstruct the tumor suppressing exosomes as novel treatment of prostate cancer.

Objectives:
Aim 1: Exosomes will be isolated from normal prostate epithelial cells and evaluated for tumor suppressing activities (Aim 1).

Aim 2: The components of the tumor suppressing exosomes will be separated, identified and characterized (Aim 2).

Aim 3: The exosomes will be "additively manufactured" by recombining the identified components in parts or as a whole, and evaluated for their therapeutic utilities (Aim 3).

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BODY OF REPORT

Scientific portion:

Aim 1: Exosomes will be isolated from normal prostate epithelial cells and evaluated for tumor suppressing activities (Aim 1).

RWPE1 cells were cultured in KGM medium. DU145 cells were cultured in RPMI-1640 media with 10% FBS. Samples of 9.5 ml of KGM and 11 ml of RPMI media were used for exosome isolation using ExoQuick-TC, according to the protocols from the manufacturer (SBI).

Visible pellets were present from the samples conditioned from DU145 cells, but not from RWPE-1 cells. The pellets from DU145 cells were re-suspended in 0.5 ml of PBS, while those from RWPE1 cells in 50 microliters of PBS. The levels of exosomes isolated, as measured by OD280 (NanogDrop), were much higher in the conditioned media from DU145 cells than those from RWPE1 cells (Around 100 fold difference). The concentrations are:

DU145-1: 0.755 mg/ml, total volume 0.5 ml.

DU145-2: 0.910 mg/ml, total volume 0.5 ml.

RWPE1-1: 0.272 mg/ml, total volume 0.05 ml

RWPE1-2: 0.089 mg/ml, total volume 0.05 ml

Exo-Cet measurement also confirmed that DU145 cells produced far more exosomes than RWPE-1 cells.

NanoSight analysis revealed that exosomes from DU145 cells were mainly in the range of 100 ~ 120 nm (Fig 1). Interestingly exosomes from RWPE1 cells presented bimodal distribution in terms of sizes, with first peak around 80 nm and the second peak around 150 nm (Fig 1). The numbers of exosomes as measured by NanoSight are listed below for different samples:

DU145-1: 1.24×10^8 per microliter

DU145-2: 1.34×10^8 per microliter

RWPE1-1: 6.82×10^6 per microliter

RWPE1-2: 5.8×10^6 per microliter

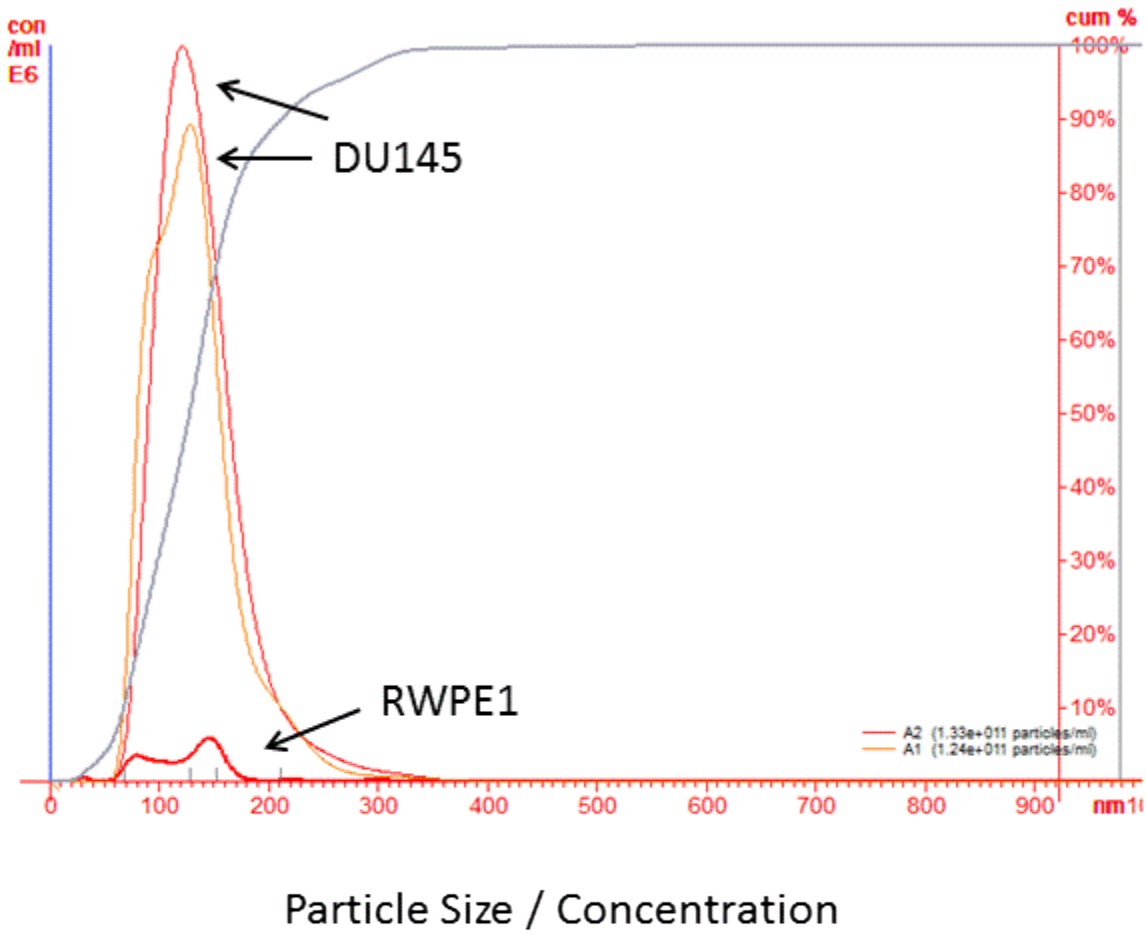


Figure 1: NanoSight analyses of exosomes from RWPE1 and DU145 cells

From the above analyses, we concluded that: 1) Non-tumorigenic RWPE1 cells far fewer exosomes when compared to prostate cancer DU145 cells (ranging from 30 to 100 folds less); 2) Exosomes from RWPE-1 cells are different from those from DU145 cells in terms of sizes. Due to the low production of exosomes from RWPE1 cells, it is extremely difficult to obtain enough exosomes from non-tumorigenic epithelial cells for evaluation of their potential anti-tumor activities.

One approach is to isolate exosomes from normal prostate tissues and evaluate their potential tumor suppressive activities. Institutional IRB has been applied and approved. We are currently acquiring tissue specimens from the prostate (Normal adjacent to tumors, and tumors) for exosome research.

We took another different approach since it has been reported that the exosomes from normal prostate contain different cargos of miRNAs.

Aim 2: The components of the tumor suppressing exosomes will be separated, identified and characterized (Aim 2).

Exosomes contain lipids, RNAs and proteins. The miRNAs are potentially biologically active. As the first step to identify the exosome components that can be potentially tumor suppressive, the miRNAs from normal prostate exosomes are analyzed for their enrichment in exosomes and their potential activities. The miRNA 200c and miRNA205 have been identified as potentially tumor suppressive.

Aim 3: The exosomes will be "additively manufactured" by recombining the identified components in parts or as a whole, and evaluated for their therapeutic utilities (Aim 3).

To engineer exosomes with miRNA200c and miRNA205 enriched, we used XMIRXpress vector recently made available through SBI.

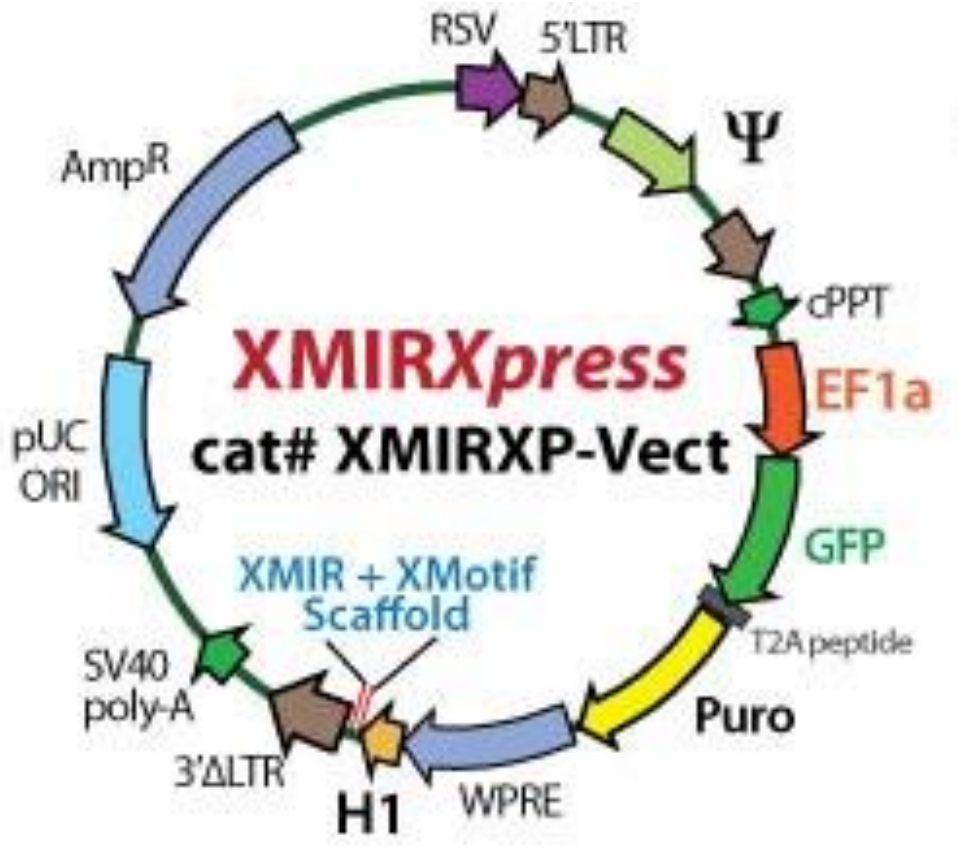
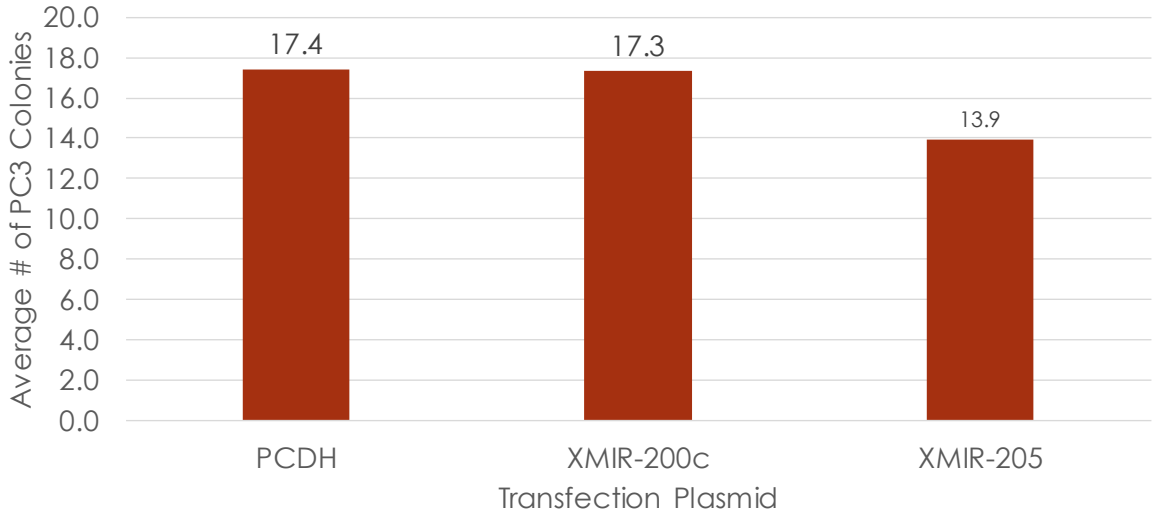


Figure 2. XMIRXpress vector map for engineering exosomes with different Xmir (SBI).

Using this vector, we attempted to engineer exosomes with potential tumor suppressive activities. In a proof of concept experiment, 293T cells were transfected with XMIRXpress vectors which can express miRNA200C or miRNA205 and exosomes produced by transfected cells were evaluated for their abilities to suppress prostate cancer cell growth.



PCDH Standard Deviation: 4.1 colonies
XMIR-200c Standard Deviation: 4.7 colonies
XMIR-205 Standard Deviation: 3.2 colonies

P-value: 0.012361
T-test of means between PCDH, XMIR-205

Figure 3. Suppression of prostate cancer cell colony formation by engineered exosomes with Xmir-205.

As shown in Figure 3, exosomes engineered with XMIR-205 significantly reduced the colony formation of PC3 cells ($p < 0.01236$). On the other hand, exosomes engineered with XMIR-200c presented minimal tumor suppressive activities. The data suggest that we can alter the contents of exosomes for therapeutic purposes. Currently we are seeking additional funding to produce engineered, tumor suppressive exosomes in a large scale for pre-clinical evaluations in animal models.

KEY RESEARCH ACCOMPLISHMENT and REPORTABLE OUTCOMES

Key findings:

1. Non-tumorigenic prostate epithelial cells produced much less exosomes than prostate cancer cells;
2. Engineered exosomes with miRNA205 presented tumor suppressive activities.

Publications: Further experiments are needed for a research paper.

Grant obtained: We obtained a Team Science Grant, which includes clinician and basic scientists in the project, from Simmons Cancer Institute to further study the differences exosomes isolated from prostate cancer patients and normal controls.

Conclusions and significance (So what?):

The studies have found that non-tumorigenic prostate epithelial cells produced far fewer exosomes when compared to prostate cancer cells. Further, engineered exosomes with XMIR205, but not XMIR200c, presented tumor suppressive activities. Large scale production and purification of the engineered tumor suppressive exosomes are needed for further evaluation in preclinical models.

APPENDICES

N/A

SUPPORTING DATA

Embedded in the reporting body

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