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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

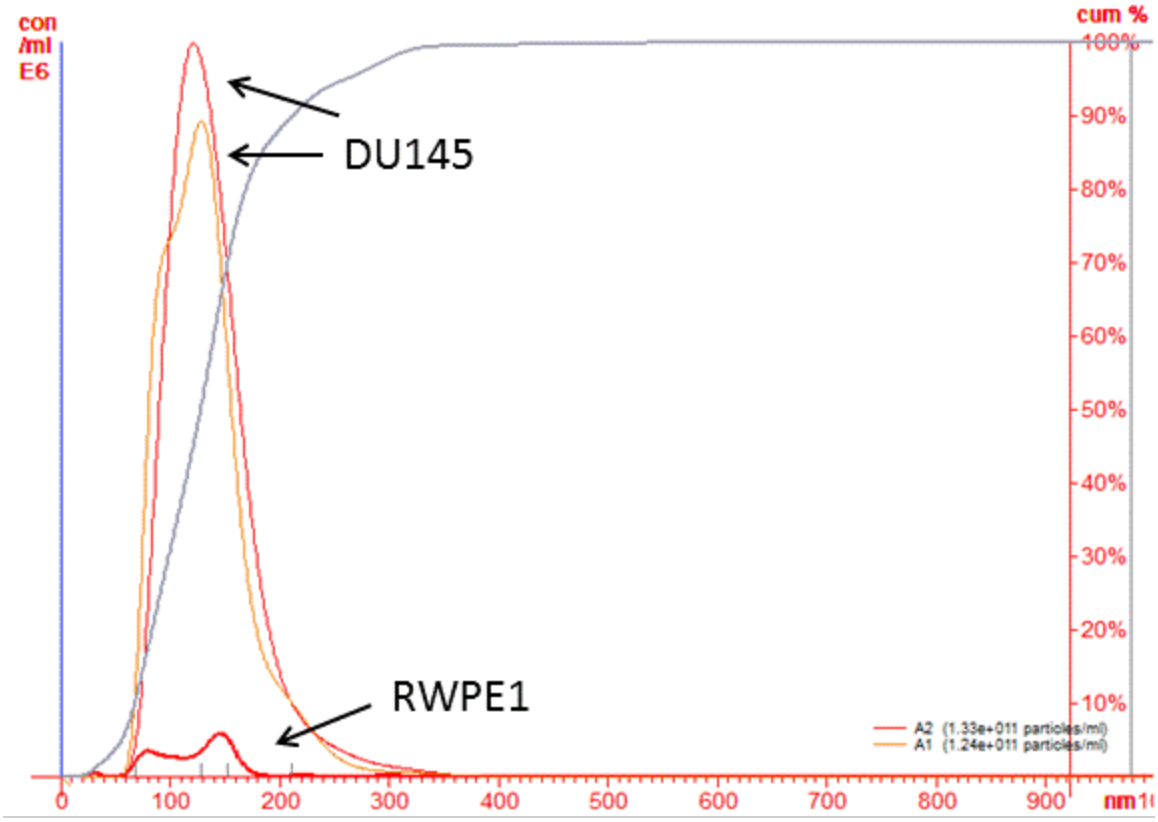

Figure 1: NanoSight analyses of exosomes from RWPE1 and DU145 cells

From the above analyses, we concluded that: 1) Non-tumorigenic RWPE1 cells produced 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. Currently we are exploring alternative approaches to obtain exosomes from normal prostate epithelial cells.

One alternative approach is to extract exosomes from normal prostate tissues and then evaluate their potential tumor suppressive activities as proposed. The use of tissues has several advantages over cultured prostate epithelial cells. The exosomes isolated from tissues reflect more accurately what went on in vivo. The tissues will be made available by our institutional tissue banking services.

Another alternative approach we are exploring is to directly determine the components of exosomes from non-tumorigenic and normal prostate epithelial cells as well as those from prostate cancer cells (Aim 2 directly). Then the components identified unique to exosomes from non-tumorigenic epithelial cells will be reconstructed and evaluated for tumor suppressive activities (Aim 3).

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

The studies will be initiated once we obtain enough exosomes from non-tumorigenic prostate epithelial cells.

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 be initiated.

KEY RESEARCH ACCOMPLISHMENT and REPORTABLE OUTCOMES

Although we have made some original discoveries regarding the different productivities of exosomes from RWPE1 and DU145 cells, further studies are needed for a research paper.

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 studies will determine whether those exosomes from non-tumorigenic cells have tumor suppressing activities and whether we can engineer those tumor suppressive exosomes for therapeutic applications.

APPENDICES

N/A

SUPPORTING DATA

Embedded in the reporting body

REFERENCES

1. Fevrier B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. Current opinion in cell biology 2004;16(4):415-21.

2. Record M, Subra C, Silvente-Poirot S, Poirot M. Exosomes as intercellular signalosomes and pharmacological effectors. Biochemical pharmacology;81(10):1171-82.

3. Skog J, Wurdinger T, van Rijn S, *et al.* Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nature cell biology 2008;10(12):1470-6.

4. Nilsson J, Skog J, Nordstrand A, *et al.* Prostate cancer-derived urine exosomes: a novel approach to biomarkers for prostate cancer. British journal of cancer 2009;100(10):1603-7.

5. Tauro BJ, Greening DW, Mathias RA, *et al.* Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods (San Diego, Calif;56(2):293-304.