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Julie Nguyen
Chapman University, nguye481@mail.chapman.edu

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The Role of Exosomes in Prostate Field Cancerization

Julie Nguyen, Marco Bisoffi
Chapman University Schmid College of Science and Technology, Orange, CA 92866. Biochemistry and Molecular Biology

INTRODUCTION
Statistics of prostate cancer:
- 80% men before the age of 80 are diagnosed
- Incidence: 233,000 men are diagnosed each year
- Mortality: ~30,000 men die each year (one death every 17 minutes)

Definition of field cancerization (or field effect): Molecular alterations (genetic/biochemical) in structurally intact cells residing in histologically normal tissues adjacent to tumors. This may represent a state of premalignancy before histologically change. The etiology of field cancerization formation remains unknown.

Clinical significance of field cancerization: Increase of the clinically informative tissue area in prostate tissues, for example, for the reduction of false negative detection rate (diagnosis) in biopsies.

STUDY OBJECTIVE
We have previously shown that Early Growth Response 1 (EGR-1), Macrophage Inhibitory Cytokine 1 (MIC-1), Platelet Derived Growth Factor A (PDGF-A), and Fatty Acid Synthase (FASN) are markers of prostate field cancerization. The OBJECTIVE of this study was to explore the role of exosomes in mediating field cancerization by analyzing known cancerous biomarkers such as EGR-1, MIC-1, PDGF-A, and FASN in non-cancerous prostate cells.

METHODS
Exosome isolation: Exosomes were isolated by sequential ultracentrifugation from LNCaP and PC-3 human prostate cancer cells. Their protein concentrations were determined by Bradford Assay and their visualization was conducted by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by Coomassie brilliant blue staining.

Western Blot: Exosomes were isolated from cancerous LNCaP and PC-3 cells. RWPE-1 cells were treated with exosomes from LNCaP and PC-3 cells for 24 hours. After the incubation period, the cells were lysed and separated by size using gel electrophoresis. The proteins on the gel were transferred onto a membrane. The membrane was treated with primary antibodies for EGR-1, FASN, and MIC-1 for 24 hours. After washing, the membrane was treated with secondary antibodies for 1 hour. The bands on the membrane were visualized using Chemiluminescence.

qRT-PCR: RNA from non-cancerous RWPE-1 cells treated with exosomes from LNCaP and PC-3 cells for 24 hours was isolated using silica-based column chromatography. Expression of EGR-1, MIC-1, PDGF-A, and FASN was determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) using specific primers, normalization to TATA binding protein (TBP), and the delta-delta CT method.

RESULTS

Exosome Isolation

Figure 1: Approximately 3-5 micrometers of exosome protein content from LNCaP and PC-3 cells were size-separated by SDS-PAGE and Coomassie stained. The numbers to the left indicate the position in kiloDalton (kD) of the standard protein markers.

Protein Expression of EGR-1, FASN, MIC-1 and β-Actin with varying exosome treatment

Figure 2: β-Actin protein expression for RWPE-1 cells treated with no exosomes, denatured exosomes, and LNCaP exosomes by Western Blot analysis. The β-Actin bands are consistent, indicative of equal protein loading.

Figure 3: EGR-1, FASN, and MIC-1 protein expression for RWPE-1 cells treated with no exosomes, denatured exosomes, and LNCaP exosomes by Western Blot analysis.

mRNA Expression of EGR-1

Figure 4: mRNA expression of EGR-1 by qRT-PCR analysis in RWPE-1 cells treated with exosomes (exos) from LNCaP and PC-3. The asterisk on top of the bars represent statistical significance at p ≤ 0.05 with respect to control (RWPE-1 + no exos).

CONCLUSION AND FUTURE STUDIES
- The Western Blot experiment shows that exosomes released by prostate cancer cells repress the expression of markers of field cancerization in non-cancerous prostate epithelial cells.
- The qRT-PCR experiments show that exosomes released by prostate cancer cells are able to induce the expression of markers of field cancerization in non-cancerous prostate epithelial cells.
- Exosome-mediated induction of field cancerization in the prostate offers a potential etiologic explanation for its formation, which to date, remains elusive.
- Exosomes may thus be potent biomarkers for the presence of prostate cancer in false-negative biopsies, or early indicators of further imminent prostate cancer formation (tumorigenesis) in as yet unaffected areas of the prostate.
- In addition, exosomes could be targets for preventative intervention against tumorigenesis.
- Future studies include; (i) Confirmation of marker induction/repression; (ii) detection of exosomes in human tissues (cancer and tumor-adjacent).

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