Microvesicle Induction of Prostate Specific Gene Expression in Normal Human Bone Marrow Cells

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Purpose: Transfer of genetic material from cancer cells to normal cells occurs via microvesicles. Cell specific phenotypes can be induced in normal cells by the transfer of material in microvesicles, leading to genetic changes. We report the identification and expression of prostate specific genes in normal human marrow cells co-cultured with human prostate cancer cells.

Materials and Methods: We harvested prostate tissue from 11 patients with prostate cancer. In 4 cases prostate tissue was co-cultured across from human marrow for 2 or 7 days but separated from it by a 0.4 μM polystyrene membrane. In 5 cases conditioned medium from patient cancer tissue was collected and ultracentrifuged, and microvesicles were collected for co-culture (3) and vesicle characterization (3). Explanted human marrow was harvested from cultures and RNA extracted. Real-time reverse transcriptase-polymerase chain reaction was done for select prostate specific genes.

Results: Marrow exposed to human prostate tumor or isolated microvesicles in culture in 4 and 3 cases, respectively, showed at least 2-fold or greater prostate gene expression than control marrow. In 1 case in which normal prostate was co-cultured there were no prostate gene increases in normal marrow.

Conclusions: Prostate cancer tumor cells co-cultured with human bone marrow cells induce prostate specific gene expression. The proposed mechanism of transfer of genetic material is via microvesicles. This represents an opportunity for novel therapeutic agents, such as antibodies, to block microvesicle release from cancer cells or for agents that may block cells from accepting microvesicles.

Key Words: prostate, prostatic neoplasms, phenotype, bone marrow, gene expression

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Abbreviations and Acronyms

BM = bone marrow

CFSE = carboxyfluorescein diacetate succinimidyl ester

CM = conditioned medium

CT = cycle threshold

DAPI = 4,6-diamidino-2-phenylindole

EBV = Epstein Barr virus

FITC = fluorescein isothiocyanate

KLK3 = kallikrein 3

PART = prostate androgen regulated transcript 1

PBS = phosphate buffered saline

PCA-3 = prostate cancer antigen 3

PCR = polymerase chain reaction

PSA = prostate specific antigen

PSCA = prostate stem cell antigen-A

RT = reverse transcriptase

STEAP = 6-transmembrane epithelial antigen of prostate

TMPRSS2 = transmembrane protease, serine 2—

UCF = ultracentrifuged

WBM = whole BM

PROSTATE cancer remains a major health problem, affecting more than 186,000 American males in 2008 with 27,000 deaths.[1] Therapy has centered on tumor hormone responsiveness and advances have been made but overall results are still discouraging.

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The mechanisms of local progression, recurrence and metastasis remain uncertain despite enormous work in this regard. A recent study indicated the secretion of oncosomes or microvesicles from the human prostate cancer cell lines DU145 and LNCap, and showed an association with a region of common chromosomal deletion in metastatic disease.[2] This study suggested that microvesicles shed from prostate cancer cells could alter the tumor microenvironment in a manner that may promote disease progression.

Microvesicles released from damaged or stimulated cell populations alter the phenotype of various target cells. They transfer CD41, integrins and CXCR4[3–5] as well as HIV and prions[6,7] between cells. Embryonic stem cell microvesicles reprogram hematopoietic stem/progenitor cells via the horizontal transfer of mRNA and protein.[8] Similarly tumor derived microvesicles carry several surface determinants and mRNA, and transfer some of these determinants to monocytes.[9] Apoptotic bodies from irradiated EBV carrying cell lines transfer DNA to various co-cultured cells and integrated but not episomal copies of EBV resulted in expression of the EBV encoded genes EBER and EBNAI in recipient cells at high copy number.[10] Extracts from T lymphocytes containing transcription factor complexes induced fibroblasts to express lymphoid genes.[11] In a recent study Deregibus et al extracted RNA from endothelial progenitor microvesicles and microarray, and performed data analysis.[12] They found a total of 298 transcripts, of which 183 were associated with Reference Sequence identifiers and the remaining UniGene, suggesting that the particles did not contain a random sample of cellular mRNA but rather a specific subset.

We previously used a model to investigate the capacity of normal murine lung to alter the genetic phenotype of normal murine marrow cells. In a cross-culture system in which marrow cells are cultured across from normal or irradiated lung we found that marrow cells expressed lung specific mRNA on real-time PCR for surfactants A, B, C and D, aquaporin 5 and Clara cell specific protein.[13] CM from lungs mediated similar genetic phenotypes in incubated marrow cells.[13] Lung CM, pelleted microparticles or microvesicles had high levels of lung specific mRNA. Incubation of marrow cells with fluorescence activated cell sorting isolated lung derived microvesicles also induced marked increases in lung specific mRNA and entry of microvesicles into marrow cells. Marrow cells co-cultured across from lung also showed an increased capacity to convert to lung cells after transplantation into irradiated hosts. Recent studies suggest that transcriptional mechanisms were involved in the finally observed genetic phenotype.[14] When rat lung was cultured opposite mouse marrow, induced surfactant mRNAs were mouse and rat, indicating that a rat transcription factor was transferred. Further study of murine lung derived microvesicles revealed protein and microRNA. Our working hypothesis is that lung specific mRNA and a protein transcription factor are transferred to cells via microvesicles. Also, microRNA is transferred, which may in turn modulate mRNA levels.

These observations on the transfer of genetic phenotype to marrow cells in mice along with observations of microvesicle derivation from cancer cells formed the basis of our studies of microvesicle evolution in excised human prostate cancer cells.

MATERIALS AND METHODS

Tumor Collection

According to the Rhode Island Hospital committee on protection of human subjects (institutional review board) consent was obtained for each patient. Tumor was surgically removed and processed. The sample was weighed and finely minced into approximately 1 cm^2 pieces.

BM Cells

BM was obtained from healthy volunteers with informed consent according to the institutional review board. BM cells (3×10^6 per well) were used 1) as WBM or 2) as Ficoll separated BM cells with mononuclear cells isolated using a Ficoll-Paque™ Premium density gradient according to manufacturer instructions or 3) ACL_2 separation. Red blood cells were lysed using BD Pharm Lyse™ lysing buffer according to manufacturer instructions.

Co-Culture

Experiments were done in 3×10^6 BM cells plated per well. Between 50 and 100 mg of minced tumor were placed in a 0.4 μm Millicell® culture plate insert with Dulbecco's modified Eagle's medium (Invitrogen™) supplemented with 10% fetal bovine serum (HyClone®), 1% penicillin/streptomycin (Invitrogen) and 20 ng/ml human stem cell factor (R & D Systems®). Control wells were cultured with equal numbers of BM cells plated without tumor. Co-cultures were maintained for 2 or 7 days in 5% CO_2 at 37C. For CM the tumor pieces were cultured without BM cells. After 7 days of culture CM was removed and further processed to isolate microvesicles.

Prostate Tumor Microvesicle Isolation

Microvesicles were isolated from CM after 2 or 7 days of culture. CM was centrifuged at 300 $\times$ gravity for 10 minutes at 4C. Supernatant was UCF at 28,000 $\times$ G for 1 hour at 4C, which was repeated. The resulting pellet was resuspended in PBS and an equal volume of the supravital red fluorescent cell membrane dye PKH26, diluted 1:250 in diluent C (Sigma®) and the cell cytoplasm supravital dye CFSE (Invitrogen) at a final concentration of 0.02 μM. Microvesicles were incubated for 15 minutes at 37C. An equal volume of 10% fetal bovine serum solution in PBS was added and samples were UCF as described. The UCF pellet was resuspended in growth medium and co-cultured with BM cells or further processed for electron microscopy.