The Function of Nuclear PEDF in Prostate Cancer Cells
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ABSTRACT
Despite clinically controlled growth of localized prostate cancer (PCa), metastatic PCA remains largely incurable, with rapid onset of lethality, even after intensive multimodal therapy. The development of new therapeutic alternatives with increased efficacy thus represents an urgent unmet need in PCA. The Pigment Epithelium-Derived Factor (PEDF) is a naturally expressed and secreted protein in the Serpin family that displays anti-angiogenic, anti-tumorigenic, and neurotrophic functions throughout the body. Recent findings from our laboratory demonstrated that PEDF is a therapeutic target for PCa. While PEDF secretion has been widely described as reduced in PCa when compared to normal cells, we surprisingly observed PEDF expression within the nucleus of PCa cells. The aim of this project is thus to characterize the function of nuclear PEDF in PCa cells. In these cells, we hypothesize that nuclear PEDF is functioning as a transcription factor. To test this hypothesis, we measured the expression of mRNA effectors of the PI3-Kinase/Akt pathway through Real-Time quantitative PCR analysis. We demonstrated that PEDF significantly inhibits the mRNA expression of the catalytic subunit of the PI3-Kinase, Akt1 and PDK1, identifying novel target genes for PEDF. As a next step, we will use chromatin immunoprecipitation to validate the binding of PEDF to the promoters of these genes of interest. We hope our research will advance the understanding of PCa and contribute to the development of improved treatment methods and therapeutic approaches for metastatic PCa patients.

INTRODUCTION
Metastatic PCa remains largely incurable, with rapid onset of lethality. Therefore, the development of new therapeutic alternatives with increased efficacy represents an urgent unmet need in PCa. The Pigment Epithelium-Derived Factor (PEDF) is a naturally expressed and secreted protein in the Serpin family that displays anti-angiogenic, anti-tumorigenic, and neurotrophic functions throughout the body. Recent findings from our laboratory demonstrated that PEDF is a therapeutic target for PCa. The objectives of this study are to further characterize the abilities of PEDF to alter gene expression levels resulting in anti-tumor effects in PCa cells. We hypothesize that PEDF is functioning as a transcription factor for the genes Akt1, PDK1, and Pi3Kinase in PCa cells. To address this hypothesis, we have been analyzing gene expression levels of these target genes after loss and gain of function treatments on PCa cells. We measured the expression of mRNA effectors of the PI3-Kinase/Akt pathway through Real-Time quantitative PCR analysis. As a next step, we will be using chromatin immunoprecipitation to validate the binding of the nuclear PEDF protein to the promoter site of these genes of interest. We expect this research will advance the understanding of PCAs and lead to new treatments to improve PCa outcomes.

HYPOTHESIS
PEDF functions as a transcription factor for the genes Akt1, PDK1, and Pi3Kinase in prostate cancer cells.

RESULTS

METHODS

Cells: Cultured in RPMI supplemented with 10% FBS at 37°C in 5% CO2.

Gain and Loss of Function: We used a transient transfection (DNA Serpin F1) and a stable transfection of PEDF DNA in PCa cells (P19). We also knocked down PEDF using Serpin F1 siRNA.

Whole cell extractions: Done using RIPA buffer.

Cytoplasmic & Nuclear extractions: were done using the NE-PER kit.

Western Blot: Proteins were separated by SDS-PAGE and transferred onto Immobilon®- Transfer membrane. After blocking in T-TBS containing 5% nonfat dry milk, the membranes were incubated with primary antibodies [anti-PEDF, anti-Lamin and anti-GAPDH]. The membranes were then stained with a horseradish peroxidase-conjugated secondary antibody. The signal was revealed using the Prime Western Blotting kit. After stripping, the membrane was re-probed for β-actin to assess loading.

RNA Extraction: Total RNAs were extracted using RNeasy extraction kit. RNA elution samples are then quantified using NanoDrop Spectrophotometer and size verified using 0.8% agarose gel. cDNA Synthesis & RT-qPCR: cDNA was synthesized using Thermo Scientific Verso cDNA synthesis Kit. We performed a one-step RT-qPCR iQ Universal SYBR Green Supermix. We used the housekeeping gene RPS15 as our control.

Statistical analysis: Significant differences were determined using Student’s t test (normal distribution) or Mann-Whitney U test (non-parametric distribution) for the studies with two independent groups. An ANOVA with Holm-Sidak’s test (normal distribution) or the Kruskal-Wallis ANOVA with Dunn’s test (nonparametric distribution) for the studies with multiple comparison were used (SigmaStat). Data are expressed as means ± standard deviation of three independent experiments. P ≤ 0.05 is considered statistically significant.

CONCLUSIONS
Upon analysis of our RT-qPCR data, we have made preliminary conclusions regarding the relationship between PEDF and our target genes. We have demonstrated that PEDF significantly inhibits the mRNA expression of the catalytic subunit of the PI3-Kinase, Akt1 and PDK1. This information has identified novel target genes for PEDF. Future objectives are to use chromatin immune-precipitation to validate the binding of PEDF to the promoter site of these genes of interest.

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