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Molecular Insights into Prostate Field Cancerization: Telomere Length, EGR--1 Expression, and Regulation of MIC--1, PDGF--A, and FAS

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Molecular Insights into Prostate Field Cancerization

Telomere Length, EGR-1 Expression, and Regulation of MIC-1, PDGF-A, and FAS

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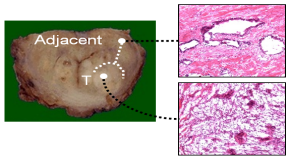
BACKGROUND

Demographics of prostate cancer:

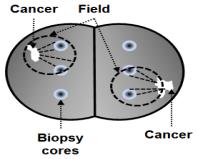
- **80% men before the age of 80 are diagnosed**
- **233,000 men are diagnosed each year**
- **30,000 men die each year (one death every 16 minutes)**

DEFINITION of field cancerization: Molecular alterations (genetic/biochemical) in structurally intact cells residing in histologically normal tissues adjacent to tumors. This may represent a state of pre-malignancy before histologically change.

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.



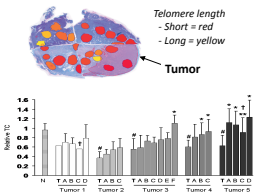
Tissue resection and H&E histology of tumor (T) and histologically normal adjacent prostate tissue removed at 1 cm from the tumor margin.



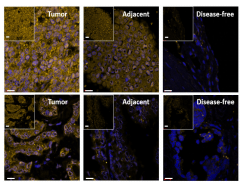
Concept of the increased clinically informative area as it pertains to prostate biopsies for confirmatory diagnosis of cancer.

We have previously shown field cancerization in histologically normal tissues 1 cm adjacent to prostate tumor margins:

- ***Telomere attrition (genomic instability).***
- ***Altered expression of the transcription factor early growth response 1 protein (EGR-1), the secreted pro-survival factors macrophage inhibitory cytokine 1 (MIC-1) and platelet derived growth factor A (PDGF-A), and the lipogenic enzyme fatty acid synthase (FAS).***



Telomere length (color coded) in whole mount prostates. Telomere length increases with increasing distance (A-F) from a tumor.

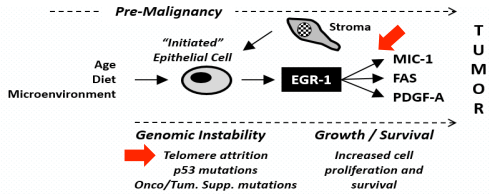


EGR-1 expression determined by immunofluorescence in tumor, tumor adjacent histologically normal (field cancerized), and disease-free prostate tissues.

HYPOTHESIS

The molecular mechanisms and pathways leading to the molecular alterations characteristic for prostate field cancerization remain unknown. WE HYPOTHESIZE THAT:

Micro-environmental factors induce field cancerization through genomic instability (telomere attrition), p53 mutations, induction of EGR-1 and expression of MIC-1, PDGF-A, and FAS.



Hypothesized model for molecular prostate field cancerization
Red arrows denote points covered in the present work

OBJECTIVE

To test steps of the hypothesized model for molecular prostate field cancerization. In particular, we addressed herein the following questions:

- **Does EGR-1 regulate the expression of MIC-1, PDGF-A, and FAS?**
  RATIONALE: EGR-1 is a key transcription factor.
- **Does N,N'-bis [2-(1-piperidino)ethyl]-3,4,9,10-tetracarboxylic diimide (PIPER) induce EGR-1 expression?**
  RATIONALE: PIPER inhibits the enzyme telomerase that maintains telomere length.

METHODS

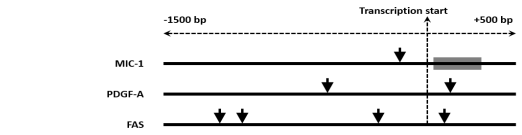
Computational EGR-1 transcription factor binding site analysis **was performed using the Tfsitescan software (http://www.ifti.org/cgi-bin/ifti/Tfsitescan.pl) and genomic sequences for EGR-1, MIC-1, PDGF-A, and FAS (*Homo sapiens* chromosomes 19, 7, and 17, respectively) were retrieved from the GRCh38 Primary Assembly of the Gene database available at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/).**

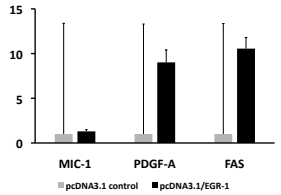
EGR-1 regulation of MIC-1, PDGF-A, and FAS **was determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) in non-cancerous RWPE-1 human prostate epithelial cells transfected with pcDNA3.1/EGR-1 plasmid. Data was normalized to TATA binding protein.**

Long-term effect of PIPER on EGR-1 expression **in PC-3 human prostate cancer cells was assessed by chemiluminescence Western blot analysis using antibodies specific for EGR-1 and β-actin (for normalization).**

RESULTS

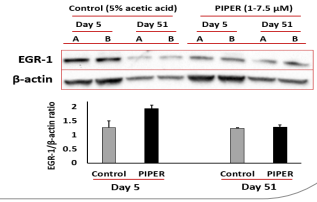
EGR-1 transcription factor binding site analysis of genomic DNA sequences from -1500bp to +500bp relative to the transcription initiation site of MIC-1, PDGF-A, and FAS.





Effect of ectopic expression of EGR-1 on MIC-1, PDGF-A, and FAS mRNA expression (y-axis) in RWPE-1 cells. Cells were transfected for 24 hours. *The ΔΔCt method was and normalization to TATA binding protein were used.*

Effect of long-term (51 days) exposure to 1-7.5μM PIPER on EGR-1 protein expression in PC-3 cells. *Ratiometric densitometry was used to determine expression.*



CONCLUSIONS

- **Genomic sequences upstream of the transcription initiation sites of MIC-1, PDGF-A, and FAS contain EGR-1 recognition sequences.**
- **Ectopic expression of EGR-1 induces mRNA expression of MIC-1, PDGF-A, and FAS elements factors. *Transfection with the pcDNA3.1 control plasmid resulted in highly variable background expression.***
- **While the telomerase inhibitor PIPER at sub-lethal doses may induce EGR-1 protein expression short-term (5 days), it does not do so long-term (51 days). *It remains to be shown whether telomere length was affected during this time.***

These preliminary results warrant further studies towards testing the hypothesized pathway of molecular field cancerization in prostate tissues.