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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.

**OBJECTIVE**

To test steps of the hypothesized model for molecular prostate field cancerization. In particular, we addressed 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-tetraacyloxy 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 TSSelan software (http://www.ico.org/cgi-bin/rft/TSSelan.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 (RT-PCR) in non-cancerous RWPE-1 human prostate epithelial cells transfected with pCNA3.1/EGR1 plasmid. Data was normalized to TATA binding protein.

**RESULTS**

- 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 used to normalize 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 pCNA3.1 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.

**Molecular Insights into Prostate Field Cancerization**

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

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**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.

**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 pCNA3.1 control plasmid resulted in highly variable background expression.
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