PCA3 NON-CODING RNA MODULATES THE EXPRESSION OF TMPRSS2:ERG FUSION AND OSTEOPONTIN ISOFORMS IN PROSTATE CANCER CELLS

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ABSTRACT

Prostate cancer antigen 3 (PCA3) is a prostate specific long noncoding RNA (lncRNA) overexpressed in prostate cancer (PCa) tissues in relation non-neoplasic tissues, while is not expressed in any other tumor type analyzed. Our group previously demonstrated that PCA3 expression is regulated by the androgen receptor (AR) signaling. Also, we found that PCA3 knockdown significantly inhibited the expression of AR target genes, besides negatively modulating PCa cell survival. Osteopontin (OPN) and TMPRSS2 (T2) genes are among gene products able to modulate PCa cell survival and controled by AR signaling. TMPRSS2 (T2) is responsive to androgen gene, prostate specific, and the T2:ERG gene fusion is the most common gene rearrangement in the PCa, corresponding to 90% of the fusions present in this tumour. These gene products suffer alternative splicing and their resulting isoforms have been reported as modulators of cell survival. OPN has three splicing isoforms named OPNa, OPNb and OPNc, while the T2:ERG fusion has at least 17 splicing variants, including as T2:ERG3 and T2:ERG8. In this context, the present work aimed to investigate whether PCA3 can modulate the expression of OPN and the T2:ERG fusion splicing isoforms and how these variants can be associated to PCa cell survival.

KEYWORDS: PCA3, PCa, Osteopontin, T2:ERG, Splicing variants.

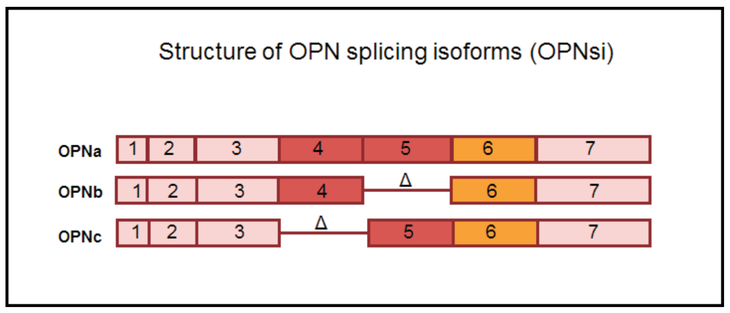

Figure 01. Osteopontin splicing isoforms. OPNa isoform is the complete splice variant, while OPNb does not contain exon 5 and OPNc lacks exon 4.

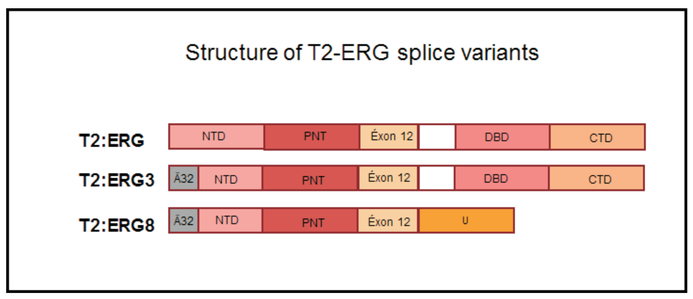

Figure 02. TMPRSS2:ERG splicing isoforms. T2:ERG full lenght variant contain N-terminal domain (NTD), Pointed domain (PNT), DNA-binding domain (DBD) and C-terminal domain (CTD). T2:ERG3 isoform lacks 32 amino acids in NTD, and T2:ERG8 a truncated form lacking DBD/CTD, which was replaced by sequences resulting in the addition of 70 unique (U) amino acids.

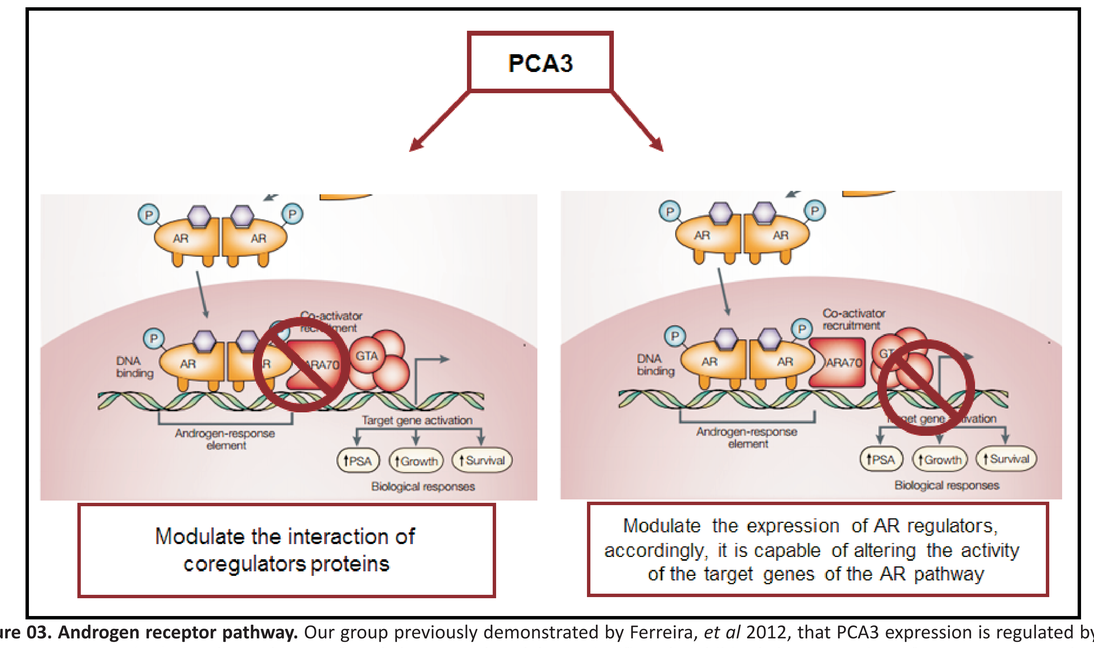

Figure 03. Androgen receptor pathway. Our group previously demonstrated by Ferreira, *et al* 2012, that PCA3 expression is regulated by the androgen receptor (AR) signaling. Also, we found that PCA3 knockdown significantly inhibited the expression of AR target genes, besides negatively modulating PCa cell survival.

METHODOLOGY

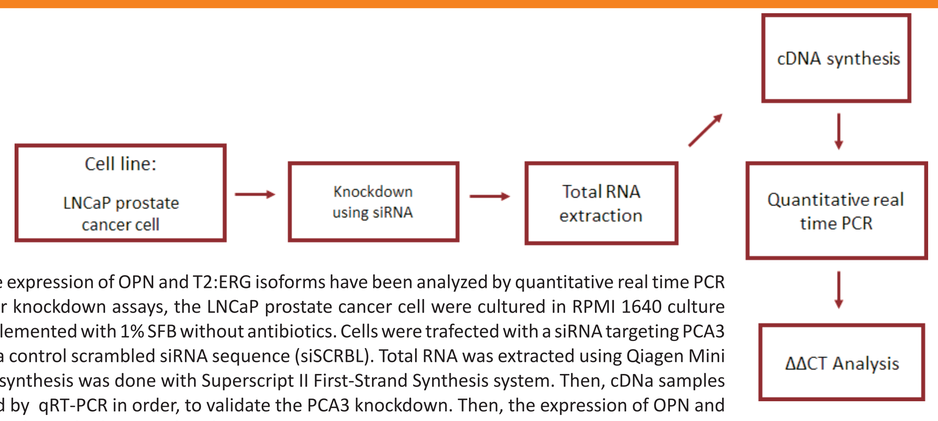

Figure 04. The expression of OPN and T2:ERG isoforms have been analyzed by quantitative real time PCR (qRT-PCR). For knockdown assays, the LNCaP prostate cancer cell were cultured in RPMI 1640 culture medium supplemented with 1% SFB without antibiotics. Cells were trafected with a siRNA targeting PCA3 (siPCA3) and a control scrambled siRNA sequence (siSCRBL). Total RNA was extracted using Qiagen Mini kit and cDNA synthesis was done with Superscript II First-Strand Synthesis system. Then, cDNa samples were analyzed by qRT-PCR in order, to validate the PCA3 knockdown. Then, the expression of OPN and T2:ERG isoforms have also been analyzed.

RESULTS

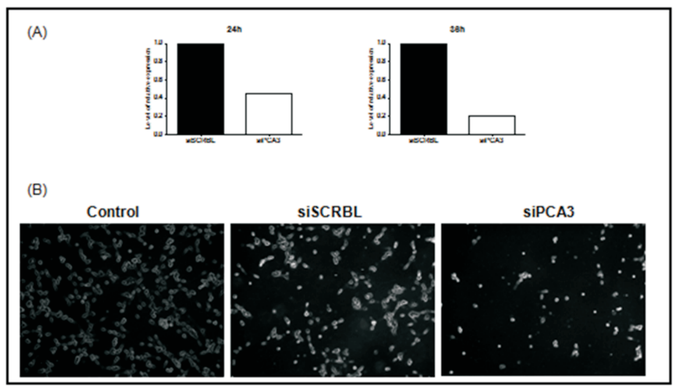

Figure 05. **Effect of siPCA3 knockdown on LNCaP cell viabillity.** A) We evaluate the effect of siPCA3 knockdown after 24h and 36h of cell transfection. Transcriptional evaluation of OPN and T2:ERG splicing isoforms have been performed in 24h after siRNA transfection. B) In response to LNCaP transfection with siPCA3 there was a significant decrease on cell viability, when compared to siSCRBL-transfected cells.

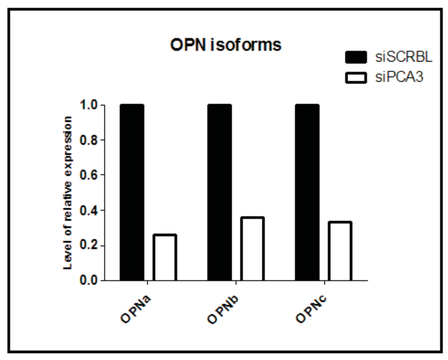

Figure 06. **Relative expression levels of OPN isoforms in response to PCA3 knockdown. .** The transcriptional levels of the OPN isoforms were analyzed by qRT-PCR, using oligonucleotides specific for these isoforms and GAPDH was used as constitutive expression gene. The results presented were performed in duplicate and representative of 1 assay, using the siSCRBL as the reference sample (reference value = 1). In response to PCA3 knockdown, we observed a significant decreased on the expression of the three OPN isoforms.

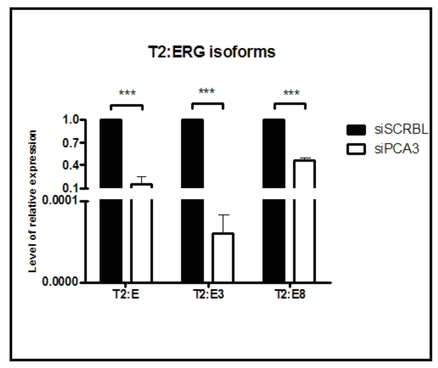

Figure 07. **Relative expression levels of T2:ERG isoforms in response to PCA3 knockdown .** The transcriptional levels of the T2:ERG isoforms were analyzed by qRT-PCR, using oligonucleotides specific for these isoforms and GAPDH was used as constitutive expression gene. The results presented were performed in duplicate and are representative of 3 independent assays, using the T2:ERG as reference sample (reference value = 1). In response to PCA3 knockdown, we observed a significant decrease on the expression levels of the three T2:ERG isoforms. The P values refer to the comparison between the level of transcriptional expression of T2:ERG and T2:ERG3 and T2:ERG8 isoforms. *** P <0.001.

CONCLUSIONS

We found that in response to PCA3 knockdown, all three OPN isoforms significantly decreased their expression levels, as well as all the three T2:ERG variants. Among these isoforms, OPNc and the T2:ERG3 variants were most downregulated. These data provide early evidence regarding the putative role of PCA3 as modulator of T2:ERG and OPN splicing isoforms and that PCA3 roles on modulating survival can involve the gene targest.

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