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Knockdown of antioxidant enzyme Sulfiredoxin impairs viability, migration capacity and survival of castration-resistant prostate cancer cells

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Introduction

Prostate cancer (PCa) has the second highest rate of incidence and death in men, especially because of the metastatic and castration-resistant stage, which presents a large genetic heterogeneity that difficult the cure. New specific molecular targets are been investigated inside the precision medicine to increase treatment efficiency. Previously, we identified the antioxidant enzyme Sulfiredoxin (Srx) as upregulated in a subgroup of PCa patients with worse prognoses and lower survival rates. Considering the therapeutic potential of this enzyme, our aim was to investigate the functional effects of Srx knockdown in prostate cells, as well as to describe the regulatory pathways involved with Srx expression and PCa progression.

Materials and Methods

We used four prostate cell lines representing tumor advancing: one normal-epithelial (PNT-2), one cancerous and androgen-sensitive (LNCaP), and two cancerous and castration-resistant (DU145 and PC-3). First, the mRNA to Srx was silenced by siRNA (siSrx) in all prostate cells during 24, 48, and 72h. Then, the cellular viability and migration capacity of cells were analyzed by MTT and Wound Healing assay, respectively. Also, using non-silenced and silenced cells, we investigated the gene expression of canonical (SRXN1, NRF2, KEAP1, AP1, PRXD1-4) and non-canonical (DJ1, ERK1/2, p38 MAPK, GSK3β) Srx master regulators, as well as proliferation (MKI67) and apoptosis (BAX, BCL2) markers.

Results

As results, considering 72h of Srx silencing, the cellular viability of both PNT-2 (Figure 1A) and LNCaP (Figure 1B) reduced in about 18%. Interestingly, the viability of DU145 decreased significantly in 41% (Figure 1C), and PC-3 in 49% (Figure 1D).

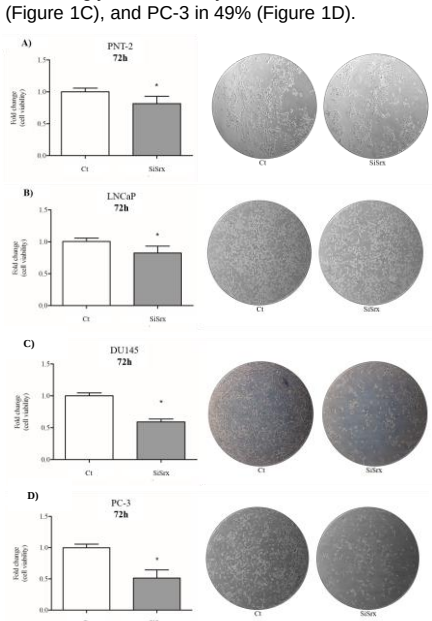

Figure 1. Graphs and representative images presenting the viability of PNT-2 (A), LNCaP (B), DU145 (C) and PC3 cells (D) without (Control, Ct) and with attenuation of Srx (SiSrx) after 72h of exposition to siRNA-mediated silencing. Graphs data are expressed as fold change related to Ct. ∗Denotes statistical significance with $p < 0.0001$.

Srx knockdown also impairs the migration capacity of PCa cells. After 72h of Srx abrogation, the area coverage by PNT-2 (Figure 2A, 2B) and LNCaP (Figure 2C, 2D) during Wound Healing assay reduced in about 6%; DU145 (Figure 2E and 2F) and PC-3 (Figure 2G and 2H) had a significant reduction in the coverage area of 16.5% and 34.9%, respectively.

Results

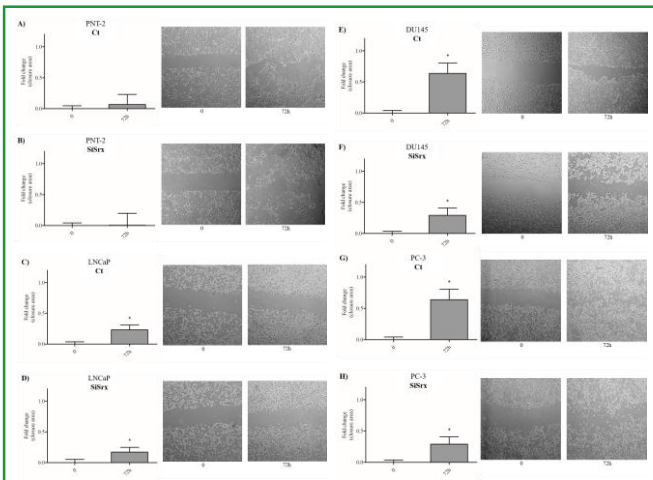

Figure 2. Graphs and representative images presenting the wound healing assay of PNT-2 (A, B), LNCaP (C,D), DU145 (E,F) and PC-3 cells (G,H) without (Control, Ct) and with knockdown to Srx (SiSrx) after 72h of exposition to siRNA-mediated silencing. Graphs data are expressed as fold change related to total coverage area at time 0, which was considered as 1. ∗Denotes statistical significance with $p < 0.0001$.

Besides, Srx silencing leaded to differential regulation of oxidative stress-related genes in prostate cells, and alterations in proliferation/apoptosis genes. After exposition to siSrx, we observed an upregulation of PRXD1, PRXD4, and BCL2 in PNT-2 cells (Figure 3); DJ1 and MKI67 levels decreased in LNCaP, and AP1 and BAX increased (Figure 4); DU145 had a downregulation just in MKI67 (Figure 5), and the effects in PC-3 were an increasing in AP1, PRXD1, PRXD4 and MKI67, as well as a decreasing in DJ1 (Figure 6).

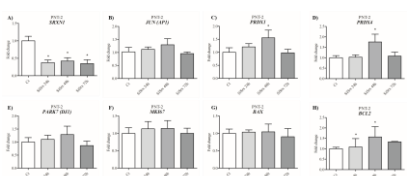

Figure 3. Graphs representing mRNA levels of different genes from PNT-2 cells before (Control, Ct) and after Srx silencing (SiSrx) during 24, 48, and 72 h. Data are expressed as fold change normalized with *ACTB* expression. * means significant difference with p< 0.05.

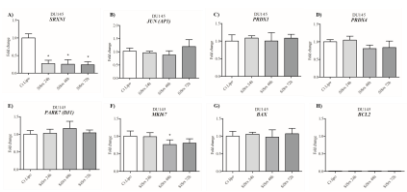

Figure 4. Graphs representing mRNA levels of different genes from LNCaP cells before (Control, Ct) and after Srx silencing (SiSrx) during 24, 48, and 72 h. Data are expressed as fold change normalized with *ACTB* expression. * means significant difference with p< 0.05.

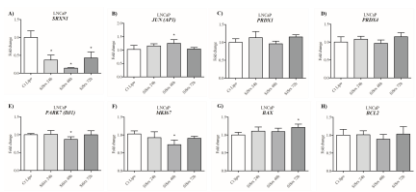

Figure 5. Graphs representing mRNA levels of different genes from PC-3 cells before (Control, Ct) and after Srx silencing (SiSrx) during 24, 48, and 72 h. Data are expressed as fold change normalized with *ACTB* expression. * means significant difference with p< 0.05.

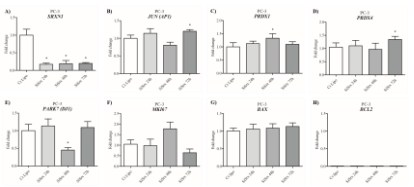

Figure 6. Graphs representing mRNA levels of different genes from DU145 cells before (Control, Ct) and after Srx silencing (SiSrx) during 24, 48, and 72 h. Data are expressed as fold change normalized with *ACTB* expression. * means significant difference with p< 0.05.

Conclusion

Our results reinforce that the antioxidant enzyme Srx plays a role in PCa progression, being important to the viability, migration, and survival of castration-resistant cells. Inside the precision medicine, the understanding of Srx regulation may contribute to the development of new inhibitors and more specific adjuvant treatments, especially for patients with alteration in this enzyme that present a worse prognosis.

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