Investigating the role of antioxidant enzyme sulfiredoxin in the chemoresistance of prostate cancer cell lines

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Introduction

Prostate cancer (PCa) is the second most prevalente cancer in men in Brazil and worldwide. The advanced stage with worse prognosis of PCa are resistant to chemical castration, and the most used treatment is exposure to chemotherapeutics, like docetaxel. However, even this commom therapy presentes a significant rate of resistance in tumor cells. Recent studies suggest that the action of antioxidante enzyme Sulfiredorin, which is overexpressed in metastatic PCa, helps in tumor chemoresistance. This work aims to evaluate the chemosensitivity of non-tumor cells (PNT-2) and advanced PCa cells (DU145 and PC3) after silencing the mRNA to Sulfiredoxin (SRXN1), in order to evaluate its potential to be used as an adjuvant treatment with docetaxel in the clinic.

Materials and Methods

Four prostate cell lines were used, each one representing a distinct phase of PCa progression. PNT-2 is an epithelial and non-tumor cell; LNCaP is an androgen-sensitive tumor cell; DU145 and PC-3 are androgen-insensitive cells that represents the most advanced stages and castration-resistant PCa. All prostate cells were exposed to crescent doses of docetaxel (1 up to 25nM) to the construction of a dose-response curve. After 24, 48 and 72h of exposition to the drug, cell viability was evaluated by MTT assay. The knockdown was carried out by siRNA against SRXN1 (25pmol) with the objective of standardizing the method and verifying if there is a real decrease in the expression of the target mRNA. RT-qPCR were performed before and after 24, 48 and 72h of silencing.

Results

The dose-response curve demonstrate that there is an important difference in the chemoresistance of prostate cell lines. For PNT-2, the exposition (72h) to small doses of docetaxel (6nM) were able to reduce cell viability in approximately 60.8% (figure 1.A). As for LNCaP, at the same time of exposure, 8nM of docetaxel was needed to obtain a decay of about 61.3% (figure 1.B). In the case of DU145, the same 8nM (72h) would achieve a decreasing of 56.4% in cell viability (figure 1.C). Analyses of PC-3 cells demonstrated that even the dose of 25nM (72h) reached a decay rate of only 35.4% (figure 1.D) . These results ensure that higher doses and longer exposure time are necessary to treat more advanced stages of PCa.

Results

Besides, after Sulfiredoxin knockdown, we observed a significant reduction in the mRNA level to SRXN1 in all prostate cell lines. PNT-2 presented a reduction of 59%; DU145 of 75%; PC-3 had a decreasing of 80%, and LNCaP of 86% (Figure 2), reinforcing that Sulfiredoxin inhibitors can be efficient as adjuvant treatment.

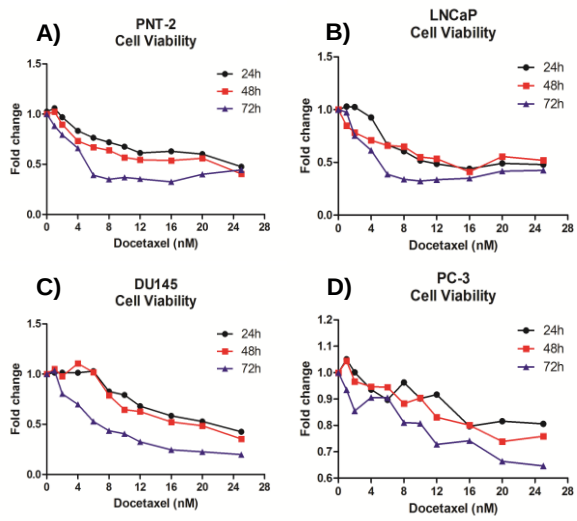

Figure 1 - Cell viability graph (MTT assay) of (A) PNT-2, (B) LNCaP, (C) DU145, and (D) PC3 lines treated with docetaxel (1 up to 25 nM) at times of 24 (black), 48 (red) and 72 (blue) hours. Values are presented as mean in fold change, with the number 1 representing 100% viable cells at time and dose 0.

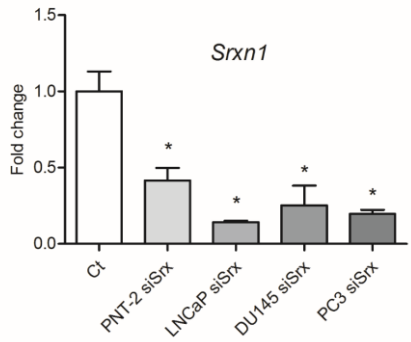

Figure 2- Graph of *Srxn1* gene expression after silencing with siRNA (siSrx) during 48 h in prostate lines: PNT-2, LNCaP, DU145 and PC3. Values are presented as mean and ±S.D in fold change normalized with *ACTB* expression. *P<0.05.

Conclusion

Therefore, considering recent results that demonstrate the importance of the antioxidant enzyme Sulfiredoxin in PCa, as well as the low number of new researches for post-docetaxel treatment strategies, we highlight the importance of investigating the SXRN1 silencing as an adjuvant treatment for PCa. These results can be used by the precision medical clinic in the future, helping to increase the survival of PCa patients in advanced stages.

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