Prostate Cancer, Radiotherapy, Hypoxia and Immune Response

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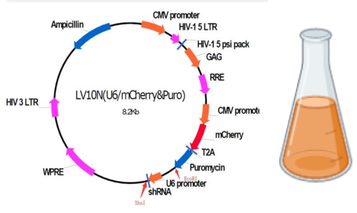
Radiation therapy is the primary therapy for treating cancer patients, and especially prostate cancer. In parallel, radiotherapy promotes the activation of immune responses and expression of immunological molecules that have the potential to affect T-cell infiltration. This dual role of radiation in the course of therapy offers a background for immunological interference and combination of radiation with immune checkpoint inhibitors and cytokines that are already available in clinical practice. Recently, studies have shown that the Hypoxia inducible factor HIF-1α regulates the expression of PD-L1 in cancer cells, contributing to the immune escape of cancer cells. Hypoxia and HIF1α also define resistance of cancer cells to radiotherapy . We examined the role of hypoxia and of HIF1α in defining expression of PD-L1 in prostate cancer PC cell lines and the eventual role of HIF1α targeting in PC immunotherapy.

Experimental Protocols

Permanent silencing of HIF1α genes in prostate cancer cell lines 22Rv1, DU145, PC3

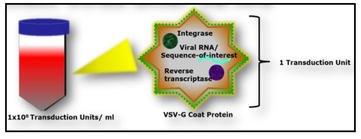
Preparation and transformation of DH5-alpha bacteria, extraction of DNA plasmid from transformed bacterial colony and confirmation that the extracted plasmids are the desirable ones

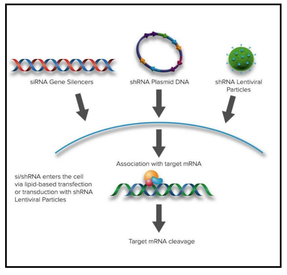


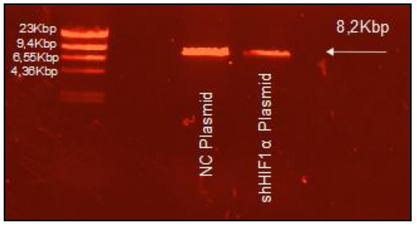
Producing lentivirus in HEK293 cells using a 2nd Generation Lentiviral System

Co-transfection

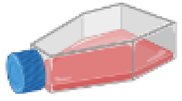
Viral collection after 48-hours







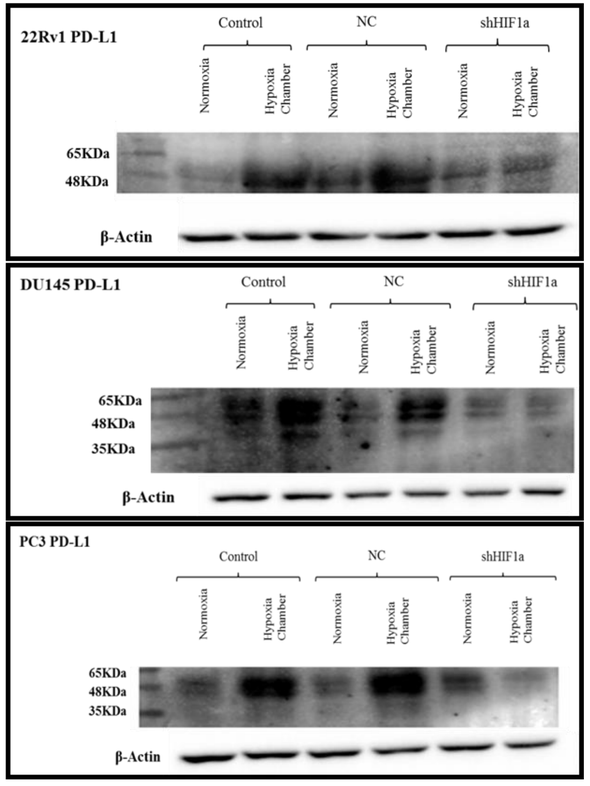
The shRNA becomes stably integrated into the host cell genome leading to the permanent knockdown



Infection of Target cells (22Rv1, DU145, PC3)

Induction of Hypoxic conditions via Hypoxia Chamber

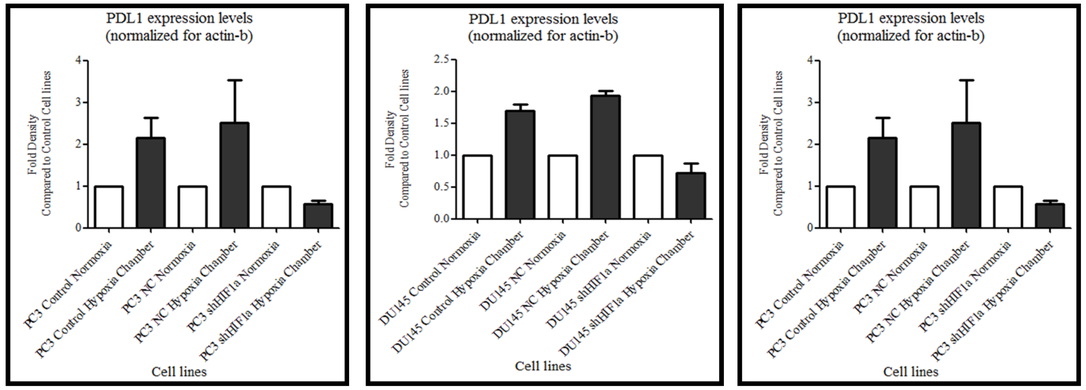
Results



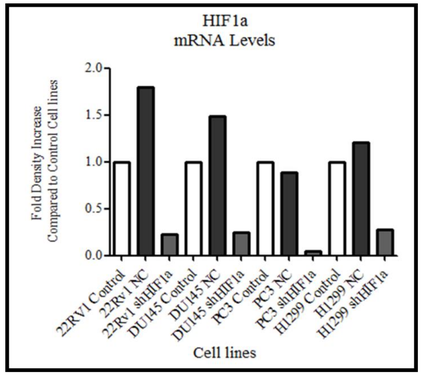
(A)

Western blot images (A) and band densitometry (B) showing the expression pattern of PD-L1 in the 22Rv1 (androgen dependent), and DU145 and PC3 (androgen independent) prostate cancer cell lines, under normoxia and hypoxia. The response is examined in parental cell lines and cell lines with stably suppressed HIF1α gene (shHIF1α). PD-L1 is up-regulated under hypoxic conditions, a phenomenon that is abrogated after silencing of HIF1α gene.

(B)



(C)



RT-PCR analysis of the mRNA expression of the HIF1α gene (normoxic conditions), of the 22Rv1, DU145, PC3 shHIF1α cell lines in comparison with the control cell lines, showing effective suppression of the HIF1α mRNA levels in sh-cell lines .

Conclusions

- Despite the development of targeted therapies, there is no significant progress in prostate cancer (PC) that promises better results and improved prognosis.
- Hypoxic conditions lead to increased levels of PD-L1 gene expression in PC cell lines, promoting immune escape of cancer cells.
- PD-L1 up-regulation under hypoxic conditions is mediated by HIF1α, and its silencing suppressed the response of PC-cell lines to hypoxia.
- Suppression of HIF1α emerges as an eventual target to enhance immune response and hypoxia-mediated radioresistane.

References

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