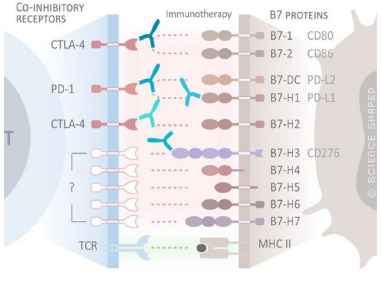
B7-H4 immune checkpoint protein as mediator of resistance to targeted therapy in renal cancer cells

Maite Emaldi and Caroline E. Nunes-Xavier
Biomarkers in Cancer Department, Biocruces Bizkaia Health Research Institute, Barakaldo, Spain

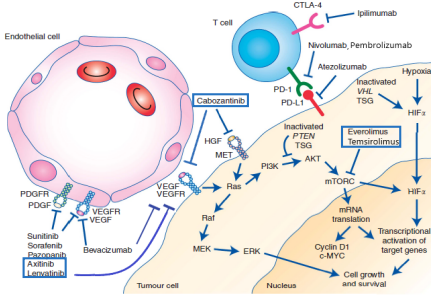
INTRODUCTION

B7 family of immunoregulatory proteins bind to co-signalling receptors of the PD-1/CTLA-4 family in T cells and induce co-inhibitory signals for their inactivation (**Figure 1**). Several B7 family members are overexpressed in various tumour types, including renal cell carcinoma (RCC), correlating with cancer progression and poor prognosis. B7 proteins can cause pro-tumorigenic effects related to metastatic capacity and resistance to anti-cancer drugs by mechanisms which go beyond their immunoregulatory role. Radiotherapy and chemotherapy have been considered ineffective for RCC treatment, but combination of immunotherapy and targeted therapies like VEGFR and mTOR inhibitors (**Figure 2**) has been shown to increase the response rate of RCC patients. Nevertheless, many patients finally develop resistance to immunotherapy and targeted therapy.



Modified figure from Flem-Karlsen et al., 2020

***Figure 1.* B7 proteins and co-inhibitory receptors.** B7 transmembrane proteins are shown on tumour cells with their inhibitory co-receptors on T cells. Unknown receptors are marked with a question mark and antibody molecules are shown for B7 members targeted by immunotherapy.



Modified figure from Greef & Eisen, 2016

***Figure 2.* Mechanism of action of immunotherapy and targeted therapies for RCC.** Axitinib, Lenvatinib and Cabozantinib are shown to visualise the inhibition carried out on tyrosine kinase receptors in endothelial and tumour cells. Everolimus and Temsirolimus are shown as part of the mTOR signalling pathway inhibition in tumour cells.

HYPOTHESIS & OBJECTIVES

B7-family of immune checkpoint proteins could be involved in the resistance to targeted therapy in renal cancer cells due to their association with poor prognosis in RCC patients. To test this hypothesis, the following objectives were proposed:

- To analyse the global gene expression profile of B7 family members in renal cancer cells upon treatment with different targeted therapies.
- To test by functional *in vitro* experiments the effect of B7-expression on the sensitivity to targeted therapies in renal cancer cells.

MATERIALS & METHODS

The workflow below indicated was followed to analyse the effect of B7 proteins on the sensitivity to targeted therapy in RCC cells by silencing experiments.

Targeted therapy treatment and cell viability analysis → B7 gene expression analysis (RNA isolation, reverse transcription, RT-qPCR) → B7-H4 silencing by transient transfection → Targeted therapy treatment and cell proliferation analysis

REFERENCES

- Flem-Karlsen, K., Fodstad, Ø., & Nunes-Xavier, C. (2020). B7-H3 Immune Checkpoint Protein in Human Cancer. *Current Medicinal Chemistry*, 27(24), 4062-4086.
- Greef, B., & Eisen, T. (2016). Medical treatment of renal cancer: new horizons. *British Journal Of Cancer*, 115(5), 505-516.

RESULTS

Caki-1, 786-O and A-498 RCC cell lines were treated with targeted therapies. Treatment resulted in significant decrease of cell viability (**Figure 3**). Gene expression analysis by quantitative PCR revealed differential expression patterns of the B7-family members in human renal cancer cell lines upon targeted therapy. B7-H4 gene expression was upregulated after treatment with various targeted therapies in Caki-1 and 786-O renal cancer cells (**Figure 4**). Consequently, expression of B7-H4 was knocked down using small interfering RNA (siRNA). Knock down of B7-H4 decreased cell viability of renal cancer cells and increased their sensitivity to various targeted drugs (**Figure 5**).

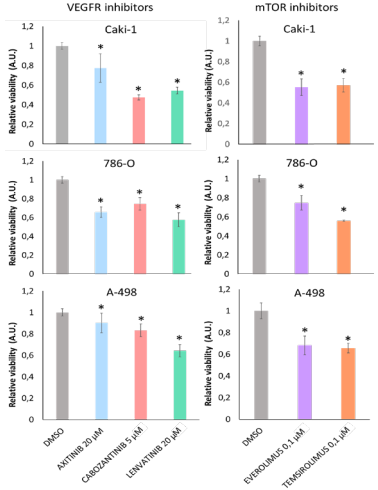


***Figure 3.* Cell viability of renal cancer cells upon targeted therapy treatments.** Viability was measured by crystal violet 72 h after treatment with drug or vehicle (DMSO), as indicated. Data are shown as relative viability normalised to untreated cells in arbitrary units (A.U.) ± S.D. Statistically significant results ($p < 0.05$) are marked with *.

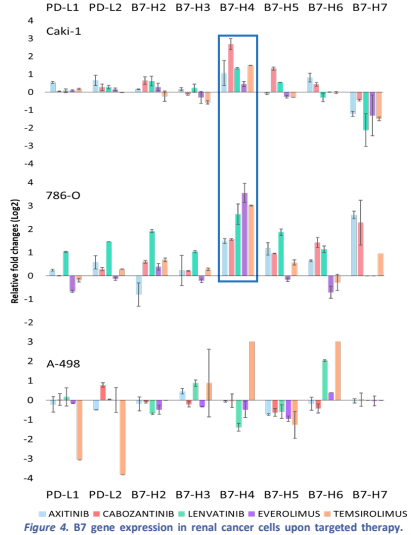


***Figure 4.* B7 gene expression in renal cancer cells upon targeted therapy.** Relative fold changes of B7-family members were measured by RT-qPCR 24 h after treatment with drugs, and are represented in Log2 scale.

CONCLUSIONS

- B7-H4 mRNA expression is increased upon treatment with targeted therapy in renal cell carcinoma cell lines.
- Knocking down B7-H4 increases sensitivity to VEGFR inhibitors and mTOR inhibitors.
- Targeting B7-H4 immune checkpoint protein could be beneficial to increase treatment efficacy in renal cell carcinoma cases resistant to current therapies.

ACKNOWLEDGEMENTS

UNIFOR
Stiftelsen til fremme av forskning innen nyresykdommer/The Foundation for the Promotion of Research in Kidney Diseases

MINISTERIO DE CIENCIA E INNOVACIÓN
Instituto de Salud Carlos III
biocruces bizkaia

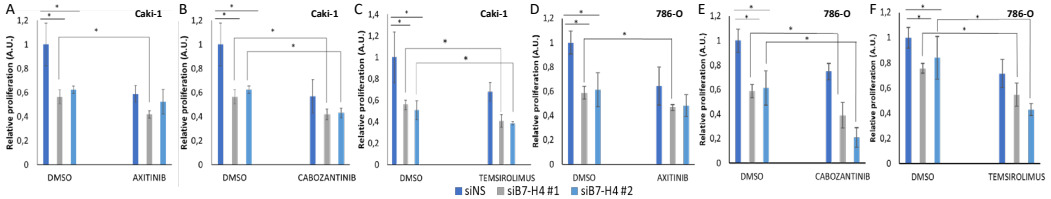


***Figure 5.* Cell proliferation of Caki-1 (A, B, C) and 786-O cells (E, F, G) after B7-H4 silencing and targeted therapy treatment.** Proliferation was measured by MTS after 72 h of treatment with Axitinib (**A, D**), Cabozantinib (**B, E**), Temsirolimus (**C, F**) or vehicle (DMSO), as indicated. Data are shown as relative viability normalised to untreated cells in arbitrary units (A.U.) ± S.D. Statistically significant results ($p < 0.05$) are marked with *.