B7-H3 immune checkpoint protein expression is associated with immune exhaustion, overall survival and metastasis in clear cell renal cell carcinoma

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BACKGROUND

Immune checkpoint inhibitors in combination with targeted therapy is improving the response rates of advanced or metastatic renal cancer patients. However, many treated patients either do not respond or develop resistance to therapy, making novel immune checkpoint-based immunotherapies of potential clinical benefit for specific groups of patients.

METHODS

B7-H3 mRNA and protein expression has been evaluated in human renal cancer cell lines by quantitative PCR and immunoblot, and *in vitro* functional assays were performed upon B7-H3 knockdown. B7-H3 protein immunostaining has been performed by immunohistochemistry on tissue microarray samples from two distinct renal cancer cohorts, and clinical correlations have been explored. The immune exhaustion gene expression profile has been obtained by nanoString technology from a panel of renal cancer whole tissue sections.

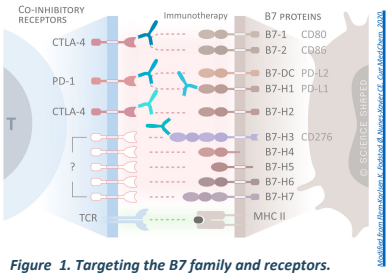


Figure 1. Targeting the B7 family and receptors. *The B7 proteins are shown on tumor cells with the co-inhibitory receptors they interact with on T cells. Unknown receptors are indicated with a question mark. Proteins targeted by immunotherapy are shown bound to an antibody molecule. TCR: T cell receptor.*

RESULTS – PART I

B7-H3 is highly expressed in renal cancer cells, and knocking down its expression resulted in lower cell viability.

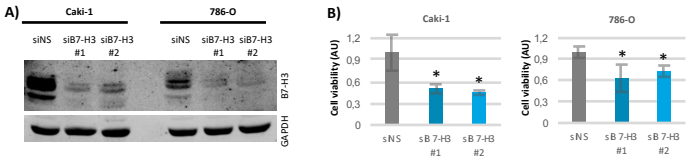


Figure 2. A) *Immunoblot of cell lysates from Caki-1 and 786-O human renal cancer cells upon B7-H3 knockdown using two different siRNAs.* ***B)*** *Cell viability was measured by MTS assay after 72 h post transfection of siRNAs.* P-values < 0.05.*

RESULTS – PART II

In a first cohort of 129 renal cancer specimens, including 96 clear cell renal cell carcinomas (CCRCC), 17 chromophobe renal cell carcinomas (ChRCC), and 16 papillary renal cell carcinomas (PRCC), we explored the correlations between B7-H3 and pathological parameters. B7-H3 was highly expressed in tumor cells from 40% of samples, which correlated with poor outcome and overall survival (OS). In a second cohort of 52 metastatic CCRCC (mCCRCC) patients, approximately half of primary tumors expressed B7-H3 at the primary site, whereas 30% of the metastatic tumors were positive for B7-H3 expression. B7-H3 expression in primary tumors correlated with tumor necrosis, sarcomatoid transformation, disease-free survival, and synchronous metastasis, while B7-H3 expression in metastasis was correlated to metastases to the lymph nodes.

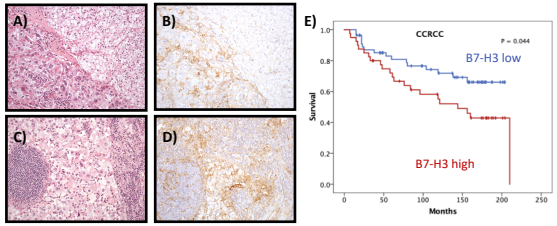
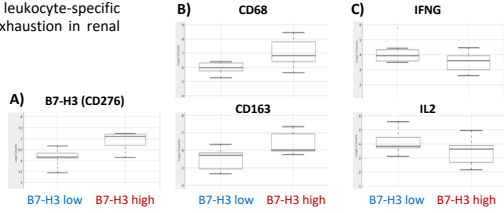


Figure 3. *Immunohistochemical staining of B7-H3.* ***A) B)*** *CCRCC primary tumor.* ***C) D)*** *matching lymph node metastases.* ***A) C)*** *hematoxylin/eosin;* ***B) D)*** *B7-H3 immunostaining.* ***E)*** *Immunohistochemical staining of B7-H3 from CCRCC specimen correlates with patient overall survival.*

RESULTS – PART III

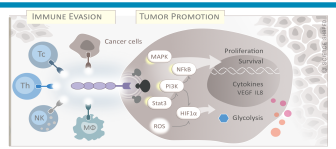
Molecular analysis revealed correlation between B7-H3 expression and leukocyte-specific gene expression profiles and signaling pathways implicated in immune exhaustion in renal cancer samples.

Figure 4. *Nanostring gene expression analysis.* ***A)*** *Good correlation was observed between B7-H3 (CD276) mRNA expression from whole tissue sections and the matching B7-H3 protein expression (categorized as B7-H3 low and B7-H3 high) from TMAs in Figure 3.* ***B)*** *Gene expression of macrophage markers CD68 and CD163 is increased in B7-H3 high tumors.* ***C)*** *Interferon gamma (IFNG) and interleukin 2 (IL2) expression levels, related to immune exhaustion, are lower in B7-H3 high tumors.*



CONCLUSIONS

Together, our findings suggest a role for B7-H3 in renal cancer immune exhaustion, and highlight B7-H3 as an actionable novel immune checkpoint protein in advanced and metastatic renal cancer.



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