Immunosuppressive Microenvironment in Neuroendocrine Neoplasms

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INTRODUCTION

Proliferation rate and histological characteristics define clinical diagnoses and therapy approaches in neuroendocrine neoplasms. In neuroendocrine tumors (NET) and neuroendocrine carcinomas (NEC) low response rates to anti-PD1 monotherapies have been reported, that emphasize a need for development of new innovative treatment strategies. [1,2]

OBJECTIVES

We aimed to investigate the tumormicroenvironment of neuroendocrine tumors to assess whether checkpoint inhibitor pathways like PD-1/PD-L1 are involved in immune escape and whether additional immune escape mechanisms need to be targeted to enable a functional active anti-tumor T cell response in NET and NEC.

MATERIALS & METHODS

Tissue samples of 77 patients diagnosed with neuroendocrine neoplasias (48 gastroenteropancreatic NET and 29 NEC of different localizations) were used in this study.

To characterize the tumor immune landscape in these NET and NEC, we analyzed the expression of immune cells through immunohistochemistry and mRNA immunoprofiling by NanoString® technology and digital spatial profiling via high resolution immunofluorescence imaging coupled with multiplex RNA expression technology.

RESULTS

Lower Intratumoral CD3+ T Cell Infiltration in NET Compared to NEC

Evaluating whole slides NEC showed a significant higher intratumoral CD3+ T cell infiltration than NET (data not shown). There was no significant difference in stromal CD3+ T cell infiltration. Expression of checkpoint molecules like PD1, PD-L1 and TIM3 on immune cells was low or nearly absent.

mRNA immune profiling n NET and NEC

Genes associated with T / Nk cell function like GZMA, GZMK and PRF1 and antigen presentation (PSMB7, HLA-DMB) were lower expressed in NET G3 / NEC and NET G1/ G2 compared to healthy tissue. However, genes known to be involved in attraction of myeloid cells, cancer sustenance /progression like TNFRSF11B, IL8, CXCL2, CXCL16, SPP1, IL1RAP, MIF and VEGFA showed higher expression levels in NET G3 / NEC and NET G1 / G2 compared to healthy tissue. Interestingly, in NET and NEC we also observed also higher expression of CD209/DC-SIGN, a marker of DCs and TAMs compared to healthy ileal tissue.

Low expression of IFNγ inducible genes in NET and NEC without spatial heterogeneity

Two microarray slides consisting of 12 NEC and 18 NET G3 were available for Digital Spatial RNA Profiling. Regions of interest (ROI) were selected based on Ki67 positivity and immunofluorescence imaging of CD45, CD68 and synaptophysin expresion to scan tumor regions. (Fig 1)

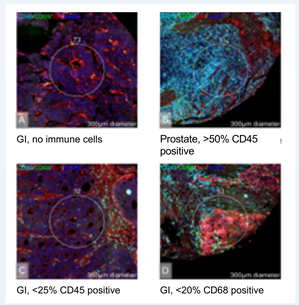

Figure 1: fluorescent labeling aided in characterizing tumor vs immune cells

RESULTS

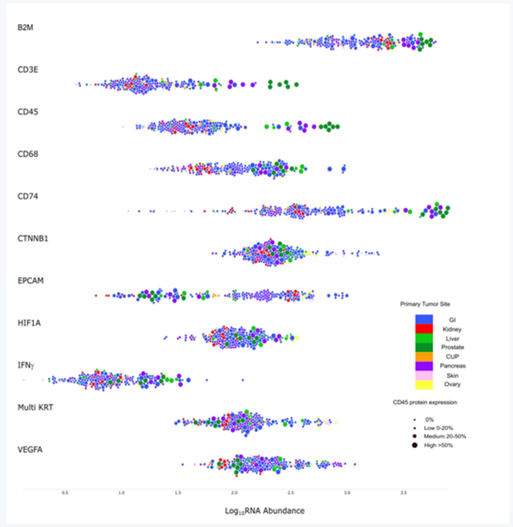

Figure 2: Swarm blot to stratify the RNA abvundance of high and low expressed genes relative to the scoring of ROIs with high CD45 positive cells

Digital spatial profiling showed low abundance of CD3E and IFNγ plus *IFNγ* signature genes in both immune and tumor cells.

In contrast, genes with immunosuppressive functions like *VEGFA*, *HIF1α*, *CD47*, *CD74,* and *CD44*, as well as signaling pathway associated molecules like *AKT*, *PTEN*, *STATs,* and *CTNNB1* coding for PI3K and WNT signaling, were highly expressed in both tumor cell and immune cell enriched regions. (Figures 2,3)

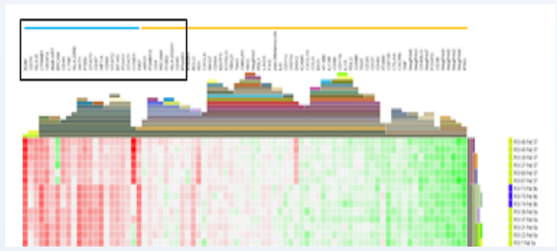

Figure 3: RNA abundance heatmeap of genes related to the immune response in NET and NEC

CONCLUSIONS

Both, NET and NEC lack signs of an activation of the adaptive immune system.

They rather display a gene signature associated with infiltration of myeloid cells including high expression of several immunosuppressive genes known to be expressed by tumor cells or stromal cells. The lack of expression of chemokines which attract T cells seems to be a reason for the lack of intratumoral T cell infiltration.

We have identified several immunosuppressive molecules, which are already targetable by approved drugs like bevacizumab directed against VEGF or for which drugs are in development. Besides STAT3 inhibitors, these include inhibitors of CD47, a ‘don’t eat me’ signal that inhibits macrophage phagocytosis for immune evasion [3] or the blockade of MIF-CD74 signaling on myeloid cells to decrease the expression of immunosuppressive factors from myeloid cells and to increase the capacity of DCs to activate CTL. [4]

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