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Soluble immune checkpoint receptor landscape in liver metastases of neuroendocrine neoplasms: a new perspective for immunotherapy

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Background: Patients with neuroendocrine liver metastases (LM-NEN) have poor survival rates and limited therapeutic options. Accumulating evidence suggests that the immune landscape plays an important role in NEN progression and metastases, particularly in LM-NENs that display an immune infiltrating profile rich in immune effector cells - specifically T-cells, which are well known to be central to tumour control. Checkpoint Receptors (CRs) on these immune effector cells serve as pivotal regulators of immune function, best known for their involvement in suppressing anti-tumour immunity. CR-blockade with Nivolumab (anti-Programmed Death-1 (anti-PD-1)) has obtained FDA approval in multiple cancers. Due to this success, several studies have investigated the efficacy of anti-PD-1 treatment for NENs. However, results suggest that only a limited number of NEN patients will benefit from anti-PD-1 therapy, as the tumours do not display significant positivity for the PD-1 ligand (PD-L1). Recent evidence has described an extensive network of soluble (cell-free) CRs beyond PD-1 that act in concert to not only inhibit but also stimulate anti-tumour immunity. The function of these inhibitory and stimulatory soluble CRs in NENs is unknown and unexplored. We hypothesise that soluble CRs can modulate tumour immunity in LM-NENs and could possibly serve as biomarkers of response for immunotherapeutic strategies. Aims of this study were (a) to delineate the role of soluble CRs in NENs, and (b) to investigate their contribution to the tumour microenvironment using a novel human immunocompetent organotypic tissue slice model for LM-NENs established in our lab.

Methods: Plasma samples from LM-NEN (n=27), primary liver cancer (n=11), colorectal liver metastases (n=11) and healthy controls (n=15) were analysed for 15 soluble CRs including sLag3 and sCD28 by multiplex Luminex assay. Precision cut tumour slices (PCTS) were prepared and cultured for up to 15 days. Tissue slice viability was confirmed by measuring ATP, cleaved cytokeratin 18 (CK18) and Lactate dehydrogenase (LDH) release. Proliferative capacity (Ki67) and neuroendocrine differentiation (Chromogranin A) were assessed by Immunofluorescence. The expression of genes relevant to innate and adaptive immune responses, including checkpoint receptors, were quantified by RT-PCR in tumour tissue and tumour-free liver tissue. LM-NEN PCTS culture supernatants were quantified for production of soluble CRs by Luminex assay.

Results: LM-NEN was associated with significantly higher plasma levels of 10 stimulatory and inhibitory soluble CRs compared to healthy controls and other tumours. The sCR secretome was liver specific and was not dependent based on anatomical primary site of the original tumour. PCTS from LM-NENs were successfully established and maintained in culture for up to 15 days with stable histological, differentiation and proliferative properties compared to the original tumour. PCTS produced soluble CRs and had tumour-bound expression of CRs beyond PD-1, with similar patterns as observed in the

plasma. Immune-specific gene signatures were readily detectible in LM-NEN PCTS, confirming immune infiltration in the tumour microenvironment, and this was also confirmed by immunohistochemistry.

Conclusions and future perspectives: Patients with LM-NENs exhibit a distinct immune checkpoint signature compared to healthy controls. Both stimulatory and inhibitory soluble checkpoint markers were detected in the systemic circulation and PCTS supernatants of LM-NENs, revealing a novel role for soluble CRs beyond the PD-1 pathway in the immunopathogenesis of LM-NEN. This was successfully modelled in our LM-NEN PCTS model, enabling new opportunities to perform pre-clinical testing of novel immunotherapeutics such as CR receptor inhibitors.

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