Interleukin 15-stimulated natural killer cells can kill both pancreatic cancer and stellate cells

J. VAN AUDENAERDE (1), J. DE WAELE (1), E. MARCQ (1), E. LION (2), J. VAN DEN BERGH (2), R. JESENOFSKY (3), A. MASAMUNE (4), G. ROEYEN (5), P. PAUWELS (6), F. LARDON (1), M. PEETERS (7), E. SMITS (1) / [1] University of Antwerp, Antwerp, Belgium, Center for Oncological Research, [2] University of Antwerp, Antwerp, Belgium, Laboratory of Experimental Hematology, [3] University of Heidelberg, Heidelberg, Germany, Department of Medicine II, [4] Tohoku University Graduate School of Medicine, Sendai, Japan, Division of Gastroenterology, [5] Antwerp University Hospital, Edegem, Belgium, Department of Hepatobiliary, Endocrine and Transplantation Surgery, [6] Antwerp University Hospital, Edegem, Belgium, Department of Pathology, [7] Antwerp University Hospital, Edegem, Belgium, Department of Oncology

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related death in Western countries with a 5-year survival rate below 5%. Within the tumour microenvironment, a strong desmoplastic reaction occurs that is orchestrated by activated pancreatic stellate cells (PSC), resulting in a functional and mechanical shield. Tackling this stromal shield is needed to overcome treatment resistance.

Aim: Since conventional therapies have limited effects, we investigated innate immunotherapy for PDAC, more specifically the possibility to kill pancreatic cancer cells (PCC) as well as PSC with interleukin (IL-)15 activated natural killer (NK) cells.

Methods: Peripheral blood NK cells were purified from healthy controls and stimulated overnight with recombinant human IL-15. The effect of IL-15 stimulation on the surface expression of several NK cell receptors was evaluated. NK cell-mediated cytotoxicity against three PCC lines and three PSC lines was measured using a 4h flow cytometric annexin V/propidium iodide assay. The impact of cell-to-cell contact and soluble mechanisms on NK cell-mediated killing were assessed using a transwell system and neutralizing antibodies, respectively. Subsequently, the cytotoxic potential of NK cells towards PSC, both isolated from PDAC patients, was tested in an autologous setting. Finally, we charted the expression of several NK cell ligands on PSC using flow cytometry.

Results: IL-15-activated NK cells have the capacity to significantly kill PCC and PSC cell lines (9-35% and 20-50%, respectively), as compared to resting peripheral blood NK cells, in a contact-dependent manner. NK cell-mediated killing was confirmed in the autologous setting in 4 out of 5 patients. IL-15 induces significant upregulation of TIM-3 and NKG2D. Our experiments investigating the mechanism of IL-15-activated NK cell cytotoxicity point towards involvement of these two receptors. Furthermore, we observed expression of PD-L1, PD-L2, MICA/B, ULBPs and Galectin-9 on PSC of PDAC patients.

Conclusions: We are the first to demonstrate that both human PCC and PSC can be killed in vitro by IL-15-stimulated NK cells. Our results in the autologous setting stresses the therapeutic potential of IL-15 in the treatment of pancreatic cancer and reveals possible future targets to tackle remaining PSC.

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Characterization of the immune microenvironment and relation to preoperative treatment of synchronous resection of primary tumor and liver colorectal cancer metastases.

M. VAN DEN EYNDE (1), B. MLECNIK (2), G. BINDEA (3), J. MACHIELS (4), A. JOURET-MOURIN (5), P. BALDIN (6), A. KARTHEUSER (7), D. LEONARD (8), C. REMUE (7), J. GIGOT (8), C. HUBERT (9), Y. HUMBLET (4), N. HAICHEUR (10), F. MARLIOT (10), F. PAGÈS (10), J. GALON (3) / [1] Cliniques Universitaires Saint Luc, Woluwe-Saint-Lambert, Belgium, Digestive Oncology, [2] Centre de Recherche des Cordeliers, Paris, France, Integrative cancer Immunology, [3] Centre de Recherche des Cordeliers, Paris, France, Integrative cancer immunology, [4] Cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, Oncology, [5] Cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, Pathology, [6] Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, Pathology, [7] Cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, Digestive Surgery, [8] cliniques universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, Digestive Surgery, [9] Cliniques Universitaires Saint-Luc, Woluwe-Saint-Lambert, Belgium, Digestive Surgery, [10] Hôpital Européen Georges Pompidou, Paris, France, Immunology

Introduction: Previous reports suggest that the adaptive Th1 immune response observed in resected primary colorectal tumor (PCT) and liver colorectal metastases (LCM) is a recognized prognostic factor and is often associated with a tumor response to preoperative treatment.

Aim: We investigated, in a subgroup of patients synchronously resected for PCT and LCM, if this immune microenvironment could be different in PCT compared to LCM and the relation to the preoperative treatment administered

Methods: From a cohort of 161 patients undergoing curative LCM resection, 29 patients with synchronous resection of LCM (n = 102) and PCT after different preoperative treatment were analyzed. The density of immune cells (CD3, CD8, CD45R0, CD20, FoxP3) was quantified with a dedicated image analysis software on whole-slide imaging in the core (CT) and invasive margin (IM) of all synchronous LCM and PCT. Comparisons were made using the Wilcoxon-Mann-Whitney test. A CD3/CD8 Immunoscore (I) was calculated and ranged from 0 (I0), when low densities of both cell types are found in the CT and IM of the tumor, to 4 (I4) when high densities for both markers are found in both regions. Distribution of (I) was analyzed and compared using the Fisher's exact test.

Results: Global analysis of immune cell density in LCM and corresponding PCT showed no significant correlation. Compared to PCT, LCM were more frequently associated with a high T-cells infiltration in CT and IM ($p < 0.001$). Conversely, high CD20 and FoxP3 density were higher in the CT of PCT ($p < 0.005$). PCT had low proportion of I3-4 (13,8%) compared to the least (41,4%, p=0,04), the mean of all (62,1%, p=0,0003) and the most infiltrated LCM per patient (65,5%, p=0,0001). A significant difference for immune microenvironment was observed in LCM and PCT after preoperative treatment. Anti-EGFR treatment was significantly associated with an increase of T-cells in CT of LCM but not in PCT ($p < 0.001$).

Conclusions: PCT had different immune microenvironment and was significantly associated with a worse immunoscore compared to the synchronously resected LCM. Preoperative treatment had different impact on synchronous PCT and LCM immune microenvironment. Anti-EGFR treatment increases T cells densities in the CT of the LCM but not in the PCT, possibly suggesting a specific treatment effect in this tumor region.

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