Cancer Letters 418 (2018) 230–238

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Original Articles

Extratumoral PD-1 blockade does not perpetuate obesity-associated inflammation in esophageal adenocarcinoma

Karen C. Galvin [a, 1], Melissa J. Conroy [a, 1], Suzanne L. Doyle [a], Margaret R. Dunne [a], Ronan Fahey [b], Emma Foley [a], Katie E. O'Sullivan [a], Derek G. Doherty [c], Justin G. Geoghegan [d], Narayanasamy Ravi [e], Cliona O'Farrelly [b], John V. Reynolds [a, e], Joanne Lysaght [a, *]

[a] Trinity Translational Medicine Institute, Department of Surgery, Trinity College Dublin, Ireland
[b] Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland
[c] Department of Immunology, Trinity College Dublin, Ireland
[d] Liver Transplant Unit, St. Vincent's University Hospital, Dublin, Ireland
[e] National Esophageal and Gastric Center, St. James's Hospital, Dublin, Ireland

ARTICLE INFO

Article history:
Received 10 November 2017
Received in revised form
8 January 2018
Accepted 9 January 2018

Keywords:
Cancer
PD-1
Immunotherapy
Inflammation
T cells
Esophageal adenocarcinoma

ABSTRACT

Checkpoint inhibitors, such as anti-PD-1 (Programmed death-1), are transforming cancer treatment for inoperable or advanced disease. As the incidence of obesity-associated malignancies, including esophageal adenocarcinoma (EAC) continues to increase and treatment with checkpoint inhibitors are being FDA approved for a broader range of cancers, it is important to assess how anti-PD-1 treatment might exacerbate pre-existing inflammatory processes at other sites.

Outside the EAC tumor, the omentum and liver were found to be enriched with substantial populations of PD-1 expressing T cells. Treatment of omental and hepatic T cells with anti-PD-1 (clone EH12.2H7) did not enhance inflammatory cytokine expression or proliferation, but transiently increased CD107a expression by $CD8^+$ T cells. Importantly, PD-1-expressing T cells are significantly lower in EAC tumor post neoadjuvant chemoradiotherapy, suggesting that combination with specific conventional treatments may severely impair the efficacy of anti-PD-1 immunotherapy.

This study provides evidence that systemically administered anti-PD-1 treatment is unlikely to exacerbate pre-existing T cell-mediated inflammation outside the tumor in obesity-associated cancers, such as EAC. Furthermore, our data suggests that studies are required to identify the negative impact of concomitant therapies on PD-1 expression in order to boost overall response rates.

1. Introduction

PD-1 (CD279) is a member of the CD28 superfamily, with a key role in establishing and maintaining immune tolerance by inhibiting the proliferation and function of T cells in the periphery during acute inflammatory responses [1]. However, PD-1 has also been identified as a key negative regulator of anti-tumor T cell responses, and targeting this pathway has become a major focus of cancer immunotherapy research [2]. With the recent FDA approval of immunotherapeutics targeting the PD-1 pathway for recurrent and metastatic gastric and gastroesophageal junction adenocarcinoma, and previous approvals for melanoma, non-small cell lung cancer, Hodgkins lymphoma, bladder cancer, renal cell carcinoma, head and neck cancer and urothelial cancer, most clinical and scientific research has naturally focused on tumor responses. However, it is important also to determine whether PD-1 and PD-L1 inhibition impacts on systemic inflammation, particularly in the context of cancer arising in the background of inflammation and obesity. In particular, it is important to address whether these therapies result in pro-inflammatory processes at sites such as the liver, and to elucidate the clinical consequence.

In humans, we and others have established that activated T cells

* Corresponding author. Cancer Immunology and Immunotherapy Group, Department of Surgery, Trinity Translational Medicine Institute, St. James's Hospital, Dublin 8, Ireland.
E-mail address: jlysaght@tcd.ie (J. Lysaght).
[1] Dr. M. Conroy and Dr. K. Galvin contributed equally to this manuscript.

https://doi.org/10.1016/j.canlet.2018.01.039
0304-3835/

and macrophages are key mediators of adipose tissue and hepatic inflammation, and that inflammatory factors such as TNF-α, IL-1β, IL-6 and IFN-γ contribute to both local and systemic pathological inflammation [3–6]. To date, no study has addressed the off-target effects of systemically administered anti-PD-1 therapy on T cell mediated inflammation in cancer patients, particularly at sites of dysregulated inflammation, such as the omentum and liver [10]. Therefore, this study ascertained if blocking the PD-1 pathway would exacerbate T cell mediated inflammation within the omentum and liver of patients with an exemplar model of inflammatory driven and obesity-associated cancer, esophageal adenocarcinoma (EAC). If the recent FDA approval of immunotherapies, such as Keytruda is to extend from gastric and gastroesophageal junction adenocarcinoma to EAC and additional inflammatory-driven or obesity-associated cancers, assessing the efficacy solely based on changes within the tumor is not sufficient for long-term tumor control, drug tolerance and clinical outcomes. The effects of such systemic immunotherapies on other tissue compartments must be carefully assessed to maximize drug efficacy, safety and side-effect management.

2. Materials and methods

2.1. Subjects

Forty five consecutive consenting patients with esophageal adenocarcinoma, attending the National Esophageal and Gastric Center at St. James Hospital, Dublin from 2011 to 2016 were enrolled in this study. The patient cohort was similar in age and ethnicity, and 34 had received neo-adjuvant chemo-radiotherapy. The patient group included 39 males and 6 females, representative of the male predominance in esophageal adenocarcinoma, with an average age of 64.4 years. The mean BMI at time of surgery was 26.5 and CT-defined visceral fat area was 146 cm [2] (Table 1). EAC tumors were obtained from consenting patients either prior to and post neoadjuvant treatment. Control blood and omentum were taken from a group of age matched non-cancer control patients attending St. James's Hospital, Dublin for laparoscopic cholecystectomy. Healthy liver was obtained prior to transplantation from the Liver Transplant Unit, St. Vincent's University Hospital, Dublin. All cancer patients were evaluated by a dietician. Body mass index, waist circumference and anthropometric variables were measured as described previously [3]. Visceral fat area was assessed by computer tomography as previously described, with more than 160 cm [2] and 80 cm [2] defining visceral obesity in males and females, respectively [7].

Table 1
Patient demographics table.

Age (years) (range)	64.4 (46–89)
Sex ratio (M:F)	39:6
Esophageal Adenocarcinoma patients	45
Tumor stage[a]	
T1	5
T2	2
T3	27
T4	3
Nodal status[a]	
Positive	19
Negative	18
Mean BMI (kg/m^2) (range)	26.5 (18.4–36.8)
Underweight (BMI<19.9)	6.25%
Normal weight (BMI 20–24.9)	33.3%
Overweight (BMI 25–29.9)	29.2%
Obese (BMI > 30)	27.1%
Mean waist circumference (cm) (range)	99.3 (40.3–191)
Centrally obese by waist circumference[b]	47.9%
Mean VFA (cm^2) (range)	146.4 (4.65–353.05)
Viscerally obese by VFA[c]	48.3%
Received Neoadjuvant CRT[d]	75.5%

[a] Eight subjects tumor stage and nodal status could not be determined. Two subjects BMI could not be determined.
[b] Obese waist circumference ≥94 cm for men and ≥80 cm for women (Alberti et al. 2006).
[c] Obese visceral fat area (VFA) > 160 cm^2 for men and >80 cm^2 for women (Doyle et al. 2013). Determined for 31 patients.
[d] Chemo-radiotherapy (CRT). 6.25% of patients had chemotherapy only.

The work was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Patients provided informed consent for sample acquisition and data analysis and the study received ethical approval from the St James's Hospital Ethics Review Board and St. Vincent's University Hospital Ethics Committee. Patient samples were pseudonymised to protect the privacy rights of the patients.

2.2. Sample preparation

Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation using Ficoll-Paque™ Plus (GE Healthcare, Uppsala, Sweden). Omental samples (10 g from each patient) and liver samples (<0.1 g from each patient) were digested enzymatically to obtain stromal vascular fraction (SVF) and intrahepatic immune cells respectively, as previously described [3,8]. Tumor tissue biopsies were digested in 125U/ml collagenase type IV in HBSS containing 4% FBS for 30 min on a shaking incubator at 37 °C at 180 RPM before being passed through a 70 μm polypropylene filter (Filcon; BD Bioscience, San Jose, California, USA) to discard debris. Cells were washed twice with HBSS and centrifuged at 1300 RPM for 3 min. Serum was isolated from peripheral blood by collecting and centrifuging whole blood in SerumClotActivator tubes (Greiner) at 3000 RPM for 10 min at 4 °C. Adipose conditioned media (ACM) and Liver conditioned media (LCM) was prepared as previously described [8].

2.3. Antibodies and flow cytometry

Freshly-isolated PBMC, SVF and liver were stained with monoclonal antibodies (mAbs) specific for human surface markers (CD3, CD4, CD8, PD-1), obtained from BD Biosciences (Oxford, UK). For intracellular cytokine staining, cells were stimulated with 10 ng/ml of phorbal myristate acetate and 1 μg/ml of ionomycin (PMA/I) for 1 h, followed by the addition of 10 μg/ml of brefeldin A for a further 3 h. Cells were stained with mAbs specific for human surface markers (CD3, CD8) BD Biosciences (Oxford, UK) for 30 min. As human CD4 cannot be reliably detected following treatment with PMA, $CD4^+$ T cells were represented by $CD3^+CD8^-$ cells, the vast majority of this population is made up of $CD4^+$ T cells, however it will also contain minor populations of innate lymphocytes. Cells were fixed and permeabilized, then stained with mAbs specific for the cytokines IFN-γ and TNF-α, obtained from BD Biosciences (Oxford, UK). Cells were acquired using a CyAn ADP flow cytometer (Beckman Coulter) and analysed with FlowJo software (TreeStar Inc.).

2.4. Assessing degranulation by CD107a expression

PBMC, SVF and intrahepatic immune cells were added to a 24 well plate at densities of 1×10^6 cells per ml, stimulated with 10 ng/ml of phorbol 12-myristate 13-acetate and 1 μg/ml of ionomycin (PMA/I). PE-conjugated CD107a (BD Biosciences) was added to the cells. After 1 h, 10 μg/ml of Brefeldin A was added to each of the wells. The cells were incubated in a CO_2 incubator at 37 °C for a