European Journal of Cancer 86 (2017) 15–27

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

Determination of poor prognostic immune features of tumour microenvironment in non-smoking patients with lung adenocarcinoma

Tomonari Kinoshita [a,b], Chie Kudo-Saito [c], Reiko Muramatsu [a], Tomonobu Fujita [a], Miyuki Saito [a], Haruna Nagumo [a], Toshiharu Sakurai [a], Shinobu Noji [a], Emi Takahata [a], Tomonori Yaguchi [a], Nobuo Tsukamoto [a], Yuichiro Hayashi [d], Kaoru Kaseda [b], Ikuo Kamiyama [b], Takashi Ohtsuka [b], Kenji Tomizawa [e], Masaki Shimoji [e], Tetsuya Mitsudomi [e], Hisao Asamura [b], Yutaka Kawakami [a,*]

[a] *Division of Cellular Signaling, Institute for Advanced Medical Research, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 1608582, Japan*
[b] *Division of General Thoracic Surgery, Department of Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 1608582, Japan*
[c] *Division of Molecular & Cellular Medicine, Group for Translational Research, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo, Tokyo 1040045, Japan*
[d] *Department of Pathology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 1608582, Japan*
[e] *Division of Thoracic Surgery, Department of Surgery, Kinki University Faculty of Medicine, 377-2, Ohnohigashi, Osaka-Sayama, Osaka 5898511, Japan*

Received 8 June 2017; received in revised form 9 August 2017; accepted 23 August 2017

KEYWORDS
Non-small cell lung cancer;
Adenocarcinoma;
Smoking history;
Tumour microenvironment;

Abstract We have previously demonstrated that the prognostic significance of tumour-infiltrating $CD8^+$ T cells significantly differs according to histological type and patient smoking habits in non-small cell lung cancer (NSCLC). This work suggested that infiltrating $CD8^+$ T cells may not be activated sufficiently in the immunosuppressive microenvironment in non-smokers with adenocarcinoma. To understand the immunogenic microenvironment in NSCLC, we characterised immune cells comprehensively by performing an immunohistochemical evaluation using an alternative counting method and multicolour staining method (n = 234), and assessed immune-related gene expression by using genetic analytical

* *Corresponding author*: Division of Cellular Signaling, Institute for Advanced Medical Research, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail address: yutakawa@keio.jp (Y. Kawakami).

http://dx.doi.org/10.1016/j.ejca.2017.08.026

Immunosuppression

approaches (n = 58). We found that high infiltration of activated $CD8^+$ T cells expressing interferon gamma (IFN-γ) and granzyme was correlated with postoperative survival in patients with non-adenocarcinoma. On the contrary, $CD8^+$ T-cell accumulation was identified as a worse prognostic factor in patients with adenocarcinoma, particularly in non-smokers. Infiltrating $CD8^+$ T cells were significantly less activated in this microenvironment with high expression of various immunoregulation genes. Potentially immunoregulatory $CD8^+$ $FOXP3^+$ T cells and immunodysfunctional $CD8^+$ $GATA3^+$ T cells were increased in adenocarcinoma of non-smokers. $CD4^+$ $FOXP3^+$ regulatory T cells expressing chemokine receptor-4 (CCR4)- and chemokine ligand (CCL17)-expressing $CD163^+$ M2-like macrophages also accumulated correlatively and significantly in adenocarcinoma of non-smokers. These characteristic immune cells may promote tumour progression possibly by creating an immunosuppressive microenvironment in non-smoking patients with lung adenocarcinoma. Our findings may be helpful for refining the current strategy of personalised immunotherapy including immune-checkpoint blockade therapy for NSCLC.

1. Introduction

Recent clinical trials have demonstrated the efficacy of immune checkpoint inhibitors in advanced non-small-cell lung cancer (NSCLC) [1–4]. However, not all patients respond to these immunotherapies. Significant clinical responses, including durable long-term responses after programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) blockade therapy, have been reported in patients with advanced squamous cell non-small-cell lung carcinoma (SQ) [3]. However, this treatment did not prolong survival in some patients with non-SQ, mainly those with adenocarcinoma (AD). Crossing of survival curves of patients treated with immunotherapy or chemotherapy implies the presence of distinctive immunoresistant populations [4]. Although some immunoresistance factors such as total mutation burden and strong driver mutation have been clinically reported [3–6], the cellular and molecular mechanisms involved in the responsiveness or ineffectiveness of these immunotherapeutics remain unclear.

An increase in tumour-infiltrating lymphocytes (TILs) has been demonstrated to be a good prognostic marker in NSCLC [7]. However, the use of $CD8^+$ T cells as a prognostic marker in NSCLC patients remains controversial because an increase in $CD8^+$ T cells has been associated with both better [8,9] and worse [10,11] prognoses. Our previous study showed that the prognostic implication of TILs significantly differs according to histological type and smoking habit in NSCLC patients, which suggests that infiltrating $CD8^+$ T cells may be inactivated by a possible immunosuppressive microenvironment in non-smokers with AD [12].

In this study, as an extension of our previous work, we performed further detailed cellular and molecular analyses on the tumour microenvironment of completely resected NSCLC (particularly adenocarcinoma) by using tile-counting and multicolour methods as well as gene expression analyses to assess immunogenic features in detail.

2. Materials and methods

2.1. Patients

Two hundred and thirty-four patients with localised NSCLC who underwent complete tumour resection during the period from 2000 to 2011 at Keio University Hospital were enrolled in the histological study. Fifty-eight patients treated from 2012to 2014 at Kinki University Hospital were enrolled in the DNA microarray study (Table 1). The need to obtain written informed consent from each patient was waived by the institutional review boards of Keio University in March 2011 and of Kinki University in May 2016.

2.2. Immunohistochemical analysis and tile-counting method

Immunostaining with antibodies was performed automatically, as described in our previous article [12]. As distribution of tumour- infiltrating immune cells are heterogeneous, we applied the tile-counting method. In this method, the numbers of TILs except for forkhead box P3 $(FOXP3)^+$ cells in 1-mm^2 tiles were automatically counted, and the mean number of the three tiles showing the highest infiltration was used in the analyses. The density of $FOXP3^+$ cells was measured by the mean density of whole tumour areas after subtraction for those stained with anti-FOXP3 antibody (Ab) and isotype matched control Ab for accurate evaluation of nuclear-stained $FOXP3^+$ cells (Supplementary Fig. 1) [13–15].

2.3. Multiple immunofluorescence staining

We used anti-CD4 (Ventana), anti-CD8 (DAKO), anti-FOXP3 (Abcam), anti-CD163 (Novocastra), anti-CCR4 (BD Pharmingen), anti-CCL17 (Abcam), anti-IL-13 (Abcam), and anti-GATA3 (BIOCARE). The signal intensity of horseradish peroxidase was amplified by