Can the assessment of lymphocyte exhaustion serve as a prognostic predictor after lung cancer surgery?

Kenji Morimoto, Yoshie Morimoto, Junji Uchino

Department of Pulmonary Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan
Correspondence to: Dr. Junji Uchino. Department of Pulmonary Medicine, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Hirokoji-agaru, kawaramachi-dori, Kamigyo-ku, Kyoto 602-0857, Japan. Email: uchino@koto.kpu-m.ac.jp.

T-cell receptors (TCRs) and costimulatory receptors—both activating signals—are directed for differentiation of T-cells in the direction of through to effector cells. Notably, both TCRs and costimulatory receptors can be suppressed by different signals. Costimulatory receptors coordinate the induction of antigen-specific signals from TCRs, including a group of inhibitory immune-checkpoint molecules that comprise cytotoxic T-cell antigen-4 (CTLA-4), programmed cell death 1 (PD-1), lymphocyte activation gene 3 (LAG3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) (1).

TIM-3 belongs to a family of type I membrane proteins and suppresses IFN-γ production in Th1 cells (2). It is commonly expressed in exhausted T-cells with other checkpoint receptors (3). However, the mechanisms by which TCRs and other factors regulate TIM-3 expression are not well characterized. Equivalently, a majority of tumor-infiltrating lymphocytes (TILs) in tumors express TIM-3, but it is unclear what roles in the microenvironment of tumor or which tumor antigens are involved in the elevation of TIM-3 in effector T-cells and Treg cells. TIM-3 elevation, as well as the elevation of other checkpoint receptors, is correlated with CD8^{+} T-cell exhaustion. About melanoma, elevation of both PD-1 and TIM-3 is a good indicator of non-reactive CD8^{+} T-cell populations (4). It has also been shown that TIM-3 is expressed on tumor antigen-specific T-cells in the peripheral blood of patients with various tumors, and

several recent reports have demonstrated that TIM-3 is also expressed on tumor cells themselves (5-7). It was suggested that the expression of TIM-3 on tumor cells might directly promote tumor metastasis and result in tumor advance by other mechanisms. These mechanisms include direct suppression of CD4^{+} T-cell function and suppression of IL-6 to STAT3 signaling. Against this background, TIM-3 has been suggested to be a predictive marker for poor survival outcomes in a wide variety of malignancies, including prostatic and renal cell carcinomas, as well as in colorectal and gastric cancers (8).

In this study, they evaluated the expression of TIM-3 in surgically resected lung cancer specimens. This study included 139 patients who had not received any preoperative treatment. More than 10% of the TILs and more than 5% of the tumor cells were positive for TIM3. Results showed that 7.9% (11 cases) of TILs and 6.5% (9 cases) of tumor cells were TIM3 positive, and that there was no correlational statistics between TIM-3 expression and clinical characteristics. Notably, they evaluated the co-expression patterns of TIM-3 with PD-1 and PD-L1 in TILs. Previously, the co-expression of PD-1(+) TIM-3(+) was considered to be indicative of exhausted T-cells (9), which correlated with poor prognostic factors in ovarian cancers (10).

Although they did not analyze prognostic factors for co-expression, high TIM-3 levels in TILs were associated with significantly shorter recurrence-free survival (RFS), overall survival (OS), and poorer prognosis. The lack of correlation

of OS with TIM-3 expression in tumor cells may support the hypothesis that TIM-3 is more functional for TILs than cancer cells. An investigation of the expression and prognosis of PD-1 and TIM-3 or CD8 in TILs in esophageal cancer discovered that TIM-3 expression correlated with poor survival , in agreement with the current findings, and that the co-expression of PD-1 and TIM-3 indicated a worse prognosis than TIM-3 expression alone (11).

As a retrospective study, there are inherent limitations to our research. For example, there is no uniformity in the characteristics of patients who are positive and negative for TIM-3 and have differences with respect to driver mutation and performance status, etc., which may affect the differences in terms of survival. Further, the overall TIM-3 positivity rate was low in both tumor cells and TILs, which may undermine the credibility of our data. However, the same conclusions regarding correlations between the TIM-3 expression and RFS and OS were noted by Su *et al.*, suggesting the reproducibility of the results (12). Besides, the present study found stronger TIM-3 expression and prognostic correlates in early-stage I-II cases. Strong expression of TIM-3 in TILs, even in early-stage cases, leads to lymphocyte exhaustion and may have attenuated immune responses to minimal residual disease. Nevertheless, because of the limited number of patients who tested positive for TIM-3 and due to limited information regarding the postoperative chemotherapy, this study should be validated via a prospective clinical study.

The production of inflammatory cytokines such as IFN-γ, TNF-α, and IL-2 is strongly suppressed, and innate immune function is reduced in TILs that are positive for both TIM-3 and PD-1, which are considered poor prognostic factors, compared with TILs that are negative for TIM-3 and positive for PD-1 or negative for both TIM-3 and PD-1 (13). TILs positive for TIM-3 and PD-1 are the most exhausted populations of T-cells, while TILs negative for TIM-3 and positive for PD-1 are a mixed population of exhausted and effector T-cells (14). Su *et al.* reported that patients positive for both TIM-3 and PD-1 had the highest RFS and the shortest OS, whereas patients negative for both TIM-3 and PD-1 had the highest RFS and a longer OS; the RFS and OS of patients positive for either, but not both TIM-3 and PD-1, were between those that for patients positive or negative for both. Strong correlations between expression of TIM-3 and PD-1 in TILs have been shown, and TILs showing TIM-3 positivity are also often positive for PD-1, which may be more prone to poor prognoses due

to strong exhaustion of lymphocytes (12). In our study, we observed a weak positive association between TILs and the expression of PD-L1 in tumor cells (correlation coefficient =0.255, P=0.002). Although the association between PD-L1 status and RFS and OS is not clearly understood, positive PD-L1 may be a poor prognostic factor in postoperative cases, and evaluating PD-L1 expression postoperatively may help predict the risk of recurrence.

TIM-3 interacts with several ligands, including Galectin-9, high mobility group box 1 (HMGB1), phosphatidylserine, and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (15). Among these, HMGB1 plays a key role in the expression of transcription factors, such as p53 and NF-κB. HMGB1 is released from activated dendritic cells and macrophages or from necrosed cells (including cancerous cells) into the extracellular space where it induces innate immunity by serving as a damage-associated molecular pattern (DAMP) (16). TIM-3 competitively inhibits the binding of HMGB1 and DAMPs to suppress innate immunity. Although some anticancer drugs induce highly immunogenic cell-death and exhibit antitumor potential by the mechanisms mentioned above, TIM-3 expression has been reported to suppress innate immunity and suppress the action of anticancer drugs (17). Combinatorial treatment with anti-TIM-3 antibodies and anticancer drugs may disrupt the function of TIM-3 and can enhance innate immunity and antitumor efficacy (17). In the background of high TIM-3 expression, the strategy of combining anti-TIM-3 antibodies with cisplatin-based postoperative adjuvant chemotherapy can possibly prolong the life span or can even prevent postoperative relapse. Clinical trials investigating anti-TIM-3 antibodies are currently underway, and it seems likely that anti-TIM-3 antibodies will be approved soon (18,19). In addition, clinical trials (NCT03680508) investigating the combination of anti-TIM-3 and anti-PD-1/PD-L1 antibodies in liver cancer are currently underway, and if the results are encouraging, we expect that these strategies would get extended to other cancers as well.

Additionally, it has also been suggested that PD-1 antibodies can predict lymphocyte exhaustion. The most recent report examined 247 metabolites in plasma samples from lung cancer patients treated with PD-1 antibodies (before and after treatment), and reported that accumulation of a four-item combination comprising the following: enterobacteria-derived metabolite (hippuric acid), energy metabolism-related metabolite (butyrylcarnitine), and reactive oxygen-related metabolites (cystine, GSSG)

in the plasma for up to 4 weeks after PD-1 antibody administration was able to better determine the efficacy of PD-1 antibodies (20). Further testing of T-cells revealed that a total of four combinations, comprising mitochondrial activation (PPARγ coactivator 1 expression and ROS) and CD8$^+$PD-1^{hi} and CD4$^+$ T-cell frequencies up to 2 weeks after treatment, can efficiently determine the efficacy of PD-1 inhibitory antibodies. In addition, we also confirmed that there was a strong correlation between plasma metabolites and the activation and energy metabolism status of T-cells. Due to these strong correlations, the four above-mentioned T-cell markers were finally chosen as the most sensitive biomarkers among all the metabolites and T-cell markers studied. These results indicate that the activation and metabolic status of immune cells can be assessed using blood samples, and that the efficacy of PD-1 inhibitory antibodies can potentially be predicted. Surgical resection specimens, such as those used in the present study, can be used to assess lymphocytic exhaustion in TILs, whereas the prediction of therapeutic efficacy and prognosis using blood samples, as previously described, may be beneficial for predicting the prognosis of patients treated with, for instance, radiotherapy.

Acknowledgments

Footnote

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