HETEROGENEITY AND CHANGES OF T-CELL SUBSET PROPORTION DURING THE EXPANSION OF TUMOR-INFILTRATING LYMPHOCYTES IN NON-SMALL CELL LUNG CANCER

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Background Adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TILs) has emerged as an additional treatment option for non-small cell lung cancer (NSCLC). However, the absence of predictive factors for therapeutic efficacy and successful expansion and lack of knowledge of changes in TILs characteristics during expansion are hurdles to the clinical introduction of TIL therapy. Herein, we report the characteristics of TILs and their changes during manufacturing.

Methods 103 cases of NSCLC operational specimens were prospectively collected at the single institute. In the initial expansion (IE) step, lung cancer tissues were cut into 1 to 2-mm diameter fragments and expanded within 2 hours of surgery. In the rapid expansion (REP) step, post-IE TILs were cultured with allogeneic peripheral blood mononuclear cells, interleukin-2, and human anti-CD3 antibody. The memory subsets and PD-1 expression status of TILs were estimated via flow cytometry (CD3, CD4, CD8, CD45, CD45RA, CCR7, and PD-1). IFN-γ ELISA assay with TILs (4.0×10^5) and autologous cancer cells (1.0×10^5) was evaluated. We investigated clinicopathologic characteristics including the level of stromal TIL (sTIL) and tertiary lymphoid structures (TLS) in hematoxylin and eosin stained slides.

Results The median IE cells per fragment were 2.5×10^5 (range 0.02 ~ 30.8×10^5), and the success rate of IE is 81.6% (84 of 103 cases, cut-off value of 0.8×10^5). The sTIL and IE cells per fragment showed positive correlation (Pearson’s r=0.215, p= 0.029). Increased TLS grades were associated with IE cells per fragment (Jonchheere-Terpstra test, p=0.008). Effector memory type was a major subset in both IE (n=39; CD8+, mean 72.4%; CD4+, mean 86.1%) and REP (n=29; CD8+, mean 85.6%; CD4+, mean 91.6%). Mean PD-1+ cells were 30.4% in IE (n=25) and 12.6% in REP (n=20). 75% (12 of 16) of the REP TIL cases showed a more than 2-fold increase of IFN-γ secretion against autologous cancer cells. Cases with less than 2-fold increased IFN-γ secretion showed higher PD-1+ cell proportion (n=2, 47.3%) in REP than cases with above 2-fold increase (n=6, 2.0%). Cases with higher IFN-γ fold change showed a decrease of PD-1+ cell proportion during IE to REP (n=3).

Conclusions We successfully manufactured expanded TILs from NSCLC specimens for TIL therapy. The TLS and sTIL may be potential markers for the prediction of successful TIL expansion in NSCLC. Effector memory cells were the main subset of therapeutic TIL products. PD-1 expression was decreased during REP and it was associated with higher IFN-γ secretion against autologous cancer cells.