COMPARISON OF VIRAL VECTOR TRANSDUCTION METHODS FOR TUMOR-INFILTRATING LYMPHOCYTES

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Background Tumor-infiltrating lymphocytes (TIL) therapy has been developed and its efficacy has been proven mainly for malignant melanoma. TILs are composed of various T cell clones with T cell receptors (TCRs) that can target tumor antigens. However, TILs are highly differentiated and exhausted. To improve the efficacy of TIL therapy, engineering various genes that are associated with immune activation and persistence is necessary. We tried to find methods to increase the gene transfer efficiency using lentiviral vectors.

Methods TILs were cultured in 2 steps; initial expansion and rapid expansion (REP). For initial expansion, tumor tissue from colorectal cancer patients was minced into fragments (1-2 mm) and cultured in a 24-well plate with IL-2 for 2 weeks. Gene transfer was conducted with initially expanded TILs with a lentiviral vector with Zsgreen. IL-2 and CD3/28 beads were used and compared for stimulation. Transduction efficiency was compared between protamine and retronectin during viral transduction. We compared three conditions with protamine according to the timing of viral transduction during REP (before, in the middle of, and after). We analyzed the fluorescence expression level of Zsgreen using a flow cytometer.

Results On post-transduction day 6, transduction efficiency for IL-2 stimulation was 4.3%, while CD3/28 beads achieved 13%. The doses of CD3/28 beads were compared and set as 1:1. Transduction efficiency was higher for protamine (17%) than retronectin (1.4%). For the timing of viral transduction, transduction in the middle of REP (17%) and before REP (16.8%) showed higher transduction efficiency than transduction after REP (5.6%).

Conclusions We showed different lentiviral transduction efficiency for TILs according to culture methods. Further studies with larger sample size and optimization of the culture protocol are necessary for better application of TIL therapy.