

No significant difference in the expression of PD-L1, CD80 and MHC class I between iFib and iMSC. iFib and iMSC were cultured with 50 ng/ml IFN- γ for 24hr. Their expression of PD-L1 and CD80 was evaluated by flow cytometry (upper and middle). iFib and iMSC were culture with or without 50 ng/ml IFN- γ . After 24 hr, the expression of MHC class I (H-2K^d or H-2K^b) was evaluated by flow cytometry (lower).



The antitumor effect of Allo-iMSC/CCL19 therapy in RENCA tumor model. BALB/c mice were s.c. injected with 5×10^5 RENCA cells. Either syn-iMSC, allo-iMSC, syn-iMSC/CCL19 or allo-iMSC/CCL19 were injected i.t. on days 10 and 13 after tumor inoculation. Tumor sizes in individual mice (left) and mean \pm S.D. (right) are shown.



Generation of an OVA-expressing RENCA cell line. (A) The vector map of pWZLneo-cOVA. The retroviral vector containing OVA cDNA, pWZLneo-OVA, was constructed. The pBlueRIP-OVA vector including OVA cDNA was purchased from Addgene and then digested with HindIII and ligated to HindIII-digested pKO Scrambler V907 (V907-OVA). Next, V907-OVA was digested with BgIII and EcoRI, then ligated to BamHI and EcoRI-digested pWZLneo vector. pWZLneo-cOVA vector was transfected into Plat-E packaging cells (Cell Biolabs). 48 hrs after transfection, supernatant was collected to infect to RENCA Luc ZsGreen. Transfectants were cultured in medium with G418 (600 µg/ml). **(B)** G418-resistant clones were picked up and then OVA mRNA expression was checked by genomic PCR method.



Relative mRNA expression in tumor tissues after the local cell therapy. CT26-bearing mice were injected i.t. with allo-iMSC or allo-iMSC/CCL19 on day 10 after tumor inoculation. Two days after cell therapy, tumor tissues were harvested and mRNAs were isolated. Quantitative RT-PCR were performed to detect mRNA expression of *Ccl19*, *Ccr7*, *p35*, *p40*, *Itgax*, *Batf3*, *Ifn-* γ and *Gzmb*. mRNA expression of β -actin was evaluated as an internal control and normalized.



Allo-iMSC/CCL19 therapy showed no effect on the percentages of tumor-specific CD8⁺ T cells in draining lymph nodes. CT26-bearing mice were treated with PBS, Syn-iMSC, Allo-iMSC, Syn-iMSC/CCL19 or Allo-iMSC/CCL19 10 days after tumor inoculation. Draining lymph nodes were harvested on 12 days and analyzed by flow cytometry. Representative dot plots of AH1 (MuLV gp70 Tetramer)-tetramer⁺ CD8⁺ cells, gated on CD45⁺ CD3⁺ cells (left) and mean \pm S.D. (right) in draining lymph nodes are shown.



FTY720 treatment decreased T cells in tumor sites. CT26-bearing mice were injected i.p. with FTY720 (2 mg/kg). Spleen or tumors were harvested and CD45⁺ CD3⁺ cells were examined by flow cytometry. Propidium iodide (PI) were used to distinguish live and dead cells. Representative dot plots and the percentages of CD45⁺ CD3⁺ cells are shown.



Asialo GM1 is expressed on NK and NKTcells but not on T cells. CT26 tumor were harvested and analyzed by flow cytometry. Propidium iodide (PI) were used to distinguish live and dead cells. Histogram and percentage of asialo GM1⁺ cells among NK (NKp46⁺ CD3⁻), NKT (NKp46^{middle} CD3^{middle}) and CD8T (NKp46⁻ CD3⁺ CD8⁺) cells are shown.