Abstract

Objectives: No immunotherapeutic protocol has yet been established in never-smoking patients with lung cancer harboring driver oncogenic mutations, such as epidermal growth factor receptor *(EGFR)* mutations. The immunostimulatory effect of Ad-REIC, a genetically engineered adenovirus vector expressing a tumor suppressor gene, reduced expression in immortalized cells *(REIC)*, has been investigated in clinical trials for various solid tumors. However, the immunostimulatory effect of the Ad-REIC in *EGFR*-mutant lung cancer with a non-inflamed tumor microenvironment (TME) has not been explored.

Materials and methods: We used a syngeneic mouse model developed by transplanting *Egfr*-mutant lung cancer cells into single or double flanks of C57BL/6J mice. Ad-SGE-REIC, a 2nd-generation vector with an enhancer sequence, was injected only into the tumors from one flank, and its antitumor effects were assessed. Tumor-infiltrating cells were evaluated using immunohistochemistry or flow cytometry. The synergistic effects of Ad-SGE-REIC and PD-1 blockade were also examined.

Results: Injection of Ad-SGE-REIC into one side of the tumor induced not only a local antitumor effect but also a bystander abscopal effect in the non-injected tumor, located on the other flank. The number of PD-1^{+}CD8^{+} T cells increased in both injected and non-injected tumors. PD-1 blockade augmented the local and abscopal antitumor effects of Ad-SGE-REIC by increasing the number of CD8^{+} T cells in the TME of *Egfr*-mutant tumors. Depletion of CD8^{+} cells reverted the antitumor effect,

suggesting they contribute to antitumor immunity.

Conclusion: Ad-SGE-REIC induced systemic antitumor immunity by modifying the TME status from non-inflamed to inflamed, with infiltration of $CD8^+$ T cells. Additionally, in *Egfr*-mutant lung cancer, this effect was enhanced by PD-1 blockade. These findings pave the way to establish a novel combined immunotherapy strategy with Ad-SGE-REIC and anti-PD-1 antibody for lung cancer with a non-inflamed TME.

Keywords: *EGFR* mutation, non-small cell lung cancer, antitumor immunity, non-inflamed tumor, Ad-SGE-REIC, gene therapy, PD-1