Abstract

Cancer-associated fibroblasts (CAFs) play a significant role in tumor progression within the tumor microenvironment. Previously, we used near-infrared photoimmunotherapy (NIR-PIT), a next-generation cancer cell-targeted phototherapy, to establish CAF-targeted NIR-PIT. In this study, we investigated whether dual-targeted NIR-PIT, targeting cancer cells and CAFs, could be a therapeutic strategy.

A total of 132 cases of esophageal cancer were analyzed for epidermal growth factor receptor (EGFR), human epidermal growth factor 2 (HER2), and fibroblast activation protein (FAP) expression using immunohistochemistry. Human esophageal cancer cells and CAFs were co-cultured and treated with single- or dual-targeted NIR-PIT *in vitro*. These cells were co-inoculated into BALB/c-*nu/nu* mice and the tumors were treated with single-targeted NIR-PIT or dual-targeted NIR-PIT *in vivo*.

Survival analysis showed FAP- or EGFR-high patients had worse survival than patients with low expression of FAP or EGFR (log-rank, $p < 0.001$ and $p = 0.074$, respectively), while no difference was observed in HER2 status. *In vitro,* dual (EGFR/FAP)-targeted NIR-PIT induced specific therapeutic effects in cancer cells and CAFs along with suppressing tumor growth *in vivo*, whereas single-targeted NIR-PIT did not show any significance. Moreover, these experiments demonstrated that dual-targeted NIR-PIT

could treat cancer cells and CAFs simultaneously with a single NIR light irradiation. We demonstrated the relationship between EGFR/FAP expression and prognosis of patients with esophageal cancer and the stronger therapeutic effect of dual-targeted NIR-PIT than single-targeted NIR-PIT in experimental models. Thus, dual-targeted NIR-PIT might be a promising therapeutic strategy for cancer treatment.

Keywords: Cancer-associated fibroblasts, fibroblast activation protein, near-infrared photoimmunotherapy, esophageal cancer, target therapy