

**Title:**

**Promising gene therapy using an adenovirus vector carrying *REIC/Dkk-3* gene for the treatment of biliary cancer**

**Short title: Ad-REIC gene therapy for biliary cancer**

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## **Abstract**

**Background:** We previously demonstrated that the reduced expression in immortalized cells (*REIC*)/dickkopf-3 (*Dkk-3*) gene was downregulated in various malignant tumors, and that an adenovirus vector carrying the *REIC/Dkk-3* gene, termed Ad-REIC induced cancer-selective apoptosis in pancreatic cancer and hepatocellular carcinoma.

**Objective:** In this study, we examined the therapeutic effects of Ad-REIC in biliary cancer using a second-generation Ad-REIC (Ad-SGE-REIC).

**Methods:** Human biliary cancer cell lines (G-415, TFK-1) were used in this study. The cell viability and apoptotic effect of Ad-SGE-REIC were assessed *in vitro* using an MTT assay and Hoechst staining. The anti-tumor effect *in vivo* was assessed in a mouse xenograft model. We also assessed the therapeutic effects of Ad-SGE-REIC therapy with cisplatin. Cell signaling was assessed by Western blotting.

**Results:** Ad-SGE-REIC reduced cell viability, and induced apoptosis in biliary cancer cell lines via the activation of the c-Jun N-terminal kinase pathway. Ad-SGE-REIC also inhibited tumor growth in a mouse xenograft model. This effect was further enhanced in combination with cisplatin.

**Conclusions:** Ad-SGE-REIC induced apoptosis and inhibited tumor growth in biliary cancer cells. *REIC/Dkk-3* gene therapy using Ad-SGE-REIC is an attractive therapeutic tool for biliary cancer.

**Key words:** REIC/Dkk-3, gene therapy, apoptosis, biliary cancer, chemotherapy, cisplatin

## **Abbreviations:**

CDDP, cisplatin; REIC, Reduced expression in immortalized cell; Dkk-3, dickkopf 3; ER, endoplasmic reticulum; FBS, fetal bovine serum;

MOI, multiplicities of infection;

MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenylformazan;

PBS, phosphate-buffered saline; SAPK, stress-activated protein kinase; JNK, c-Jun N-terminal kinase;

PBMC, peripheral blood mononuclear cells; PD-1, programmed cell death-1; SE, standard error.