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# Cationic liposome-mediated delivery of reovirus enhances the tumor cell-killing efficiencies of reovirus in reovirus-resistant tumor cells

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ABSTRACT

Reovirus induces tumor cell death efficiently and specifically, and thus is currently undergoing clinical testing as an anticancer agent. In the intracellular trafficking of reovirus, proteolytic disassembly of reovirus capsid-proteins and subsequent penetration of viral particles into the cytosol are crucial steps. Cathepsins B and L are largely responsible for the proteolytic disassembly of reovirus. Reovirus efficiently lyses tumor cells exhibiting relatively high activities of cathepsins B and L, while tumor cells with low activities of cathepsins B and L are often refractory to reovirus, probably due to inefficient endo/lysosomal escape. In this study, in order to enhance the tumor cell-killing efficiencies of reovirus by promoting endo/lysosomal escape, especially in reovirus-resistant tumor cells, reovirus was complexed with a cationic liposome transfection reagent. Reovirus alone and reovirus-cationic liposome complex (reoplex) exhibited similar levels of tumor cell-killing efficiencies in reovirus-susceptible tumor cells, while reoplex mediated more than 30% higher levels of tumor cell-killing activities in reovirus-resistant tumor cells than reovirus alone. Reoplex-mediated tumor cell death was efficiently induced in the tumor cells pretreated with cathepsin inhibitors. The mRNA levels of interferon (IFN)-β and apoptotic genes were significantly elevated following reoplex treatment. These results suggest that cationic liposomes efficiently promoted delivery of reovirus to the cytosol, leading to induction of apoptosis.


## 1. Introduction

Reovirus, which is a non-enveloped double-stranded RNA virus, has been actively studied as a novel and promising anticancer agent because of its efficient oncolytic activity. Clinical trials, including phase III trials, using reovirus against various types of tumors are underway internationally (Galanis et al., 2012; Karapanagiotou et al., 2012; Kicielinski et al., 2014). Reovirus preferentially infects and kills tumor cells rather than normal cells. Ras-activated tumor cells are efficiently killed by reovirus, possibly because efficient translation of viral proteins occurs in Ras-activated tumor cells due to the inactivation of double-stranded RNA-dependent kinase (PKR), which permits efficient production of the progeny virus (Coffey et al., 1998; Norman et al., 2004). On the other hand, several reports, including ours, have demonstrated that the Ras-activation status does not appear to be correlated with the level of reovirus-mediated tumor cell killing (Hwang et al., 2013; Song et al., 2009; Terasawa et al., 2015; Twigger et al., 2012).

The cellular activities of cathepsins B and L, which are cysteine proteases mainly located in the endo/lysosomes, are also a crucial determinant for reovirus-mediated tumor cell killing (Alain et al., 2007; Kim et al., 2007; Terasawa et al., 2015). Cathepsins B and L mediate degradation of the virus outer capsid proteins in the endo/lysosomes, yielding so-called infectious subviral particles (ISVP) (Baer et al., 1999; Ebert et al., 2002). ISVP efficiently escape from endo/lysosomes into the cytosol following further structural changes of ISVP, leading to efficient tumor cell killing. The activity levels of cathepsins B and L are often up-regulated in tumor cells

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(Coulibaly et al., 1999; Vasiljeva et al., 2006). Reovirus efficiently kills tumor cells with high activities of cathepsins B and L, while tumor cells showing low activities of cathepsins B and L are often refractory to reovirus, probably because proteolytic disassembly of the outer capsid proteins of reovirus and subsequent penetration from endo/lysosomes to the cytosol does not efficiently occur in tumor cells showing low activities of cathepsins B and L. Reovirus-resistant tumor cells are efficiently killed by ISVP (Terasawa et al., 2015), which are produced by incubation of reovirus particles with chymotrypsin, although ISVP show low thermostability (Middleton et al., 2002). These findings led us to hypothesize that reovirus-resistant tumor cells would be efficiently killed, if reovirus efficiently escapes from endo/lysosomes into the cytosol, even though the activity levels of cathepsins B and L in tumor cells are low.

In this study, in order to achieve efficient reovirus-mediated killing, especially in reovirus-resistant tumor cells, complexes of reovirus and a cationic lipid-based transfection reagent (reoplex) were formed and were added to reovirus-resistant tumor cells as well as reovirus-susceptible tumor cells. It is well-known that a cationic lipid-based transfection reagent promotes efficient escape of plasmid DNA and nucleic acid-based agents, including small interfering RNAs (siRNAs), from endo/lysosomes to the cytosol (Cardarelli et al., 2012; Sakurai et al., 2000). Reoplex efficiently

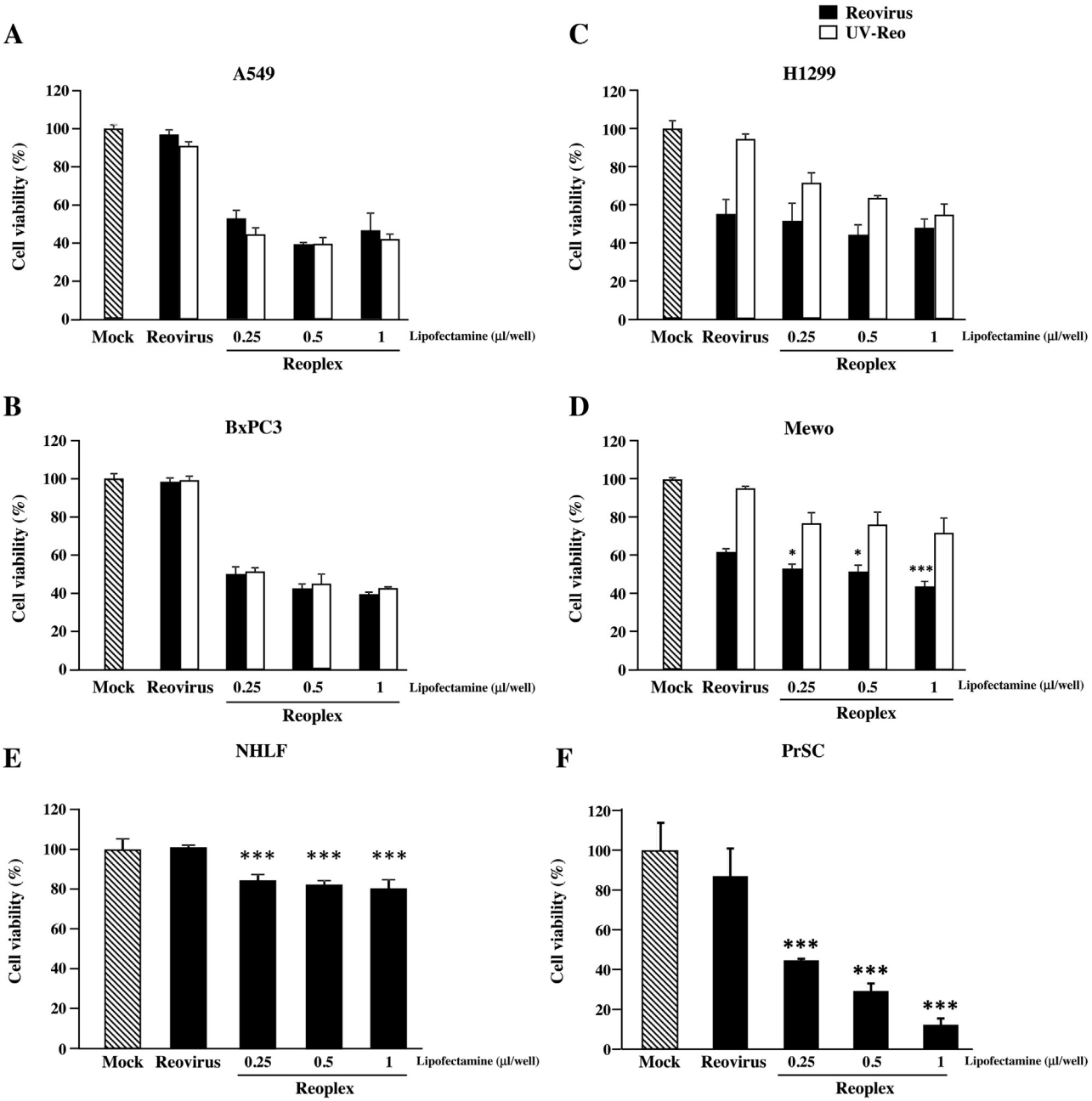


**Fig. 1.** Tumor cell viabilities following treatment with reoplex. A549 (A), BxPC-3 (B), H1299 (C), and Mewo cells (D) were incubated with reovirus, reoplex, and UV-reoplex at doses equivalent to an MOI of 10. Following a 6-h incubation, the medium was replaced with fresh medium, and then incubated for an additional 42 h. UV-reoplex contained UV-inactivated reovirus. Open and closed bars indicate the data using live reovirus and UV-inactivated reovirus, respectively. NHLF (E) and PrSC (F) were treated with reovirus and reoplex as described above. The mock group received no treatment. Cell viabilities were determined 48-h after incubation by an alamarBlue assay. Cell viability of the mock group was normalized to 100%. The data are presented as the mean ± S.D. (n = 4). *$p < 0.05$, ***$p < 0.0001$, compared with reovirus.