Combination Treatment of Human Breast Cancer Cells with Possible RNAi Enhancer and siRNA Targeting Polo-Like Kinase 1

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Purpose
RNAs are easily degraded by RNase, and their ability to penetrate the cell membrane is poor. Therefore, the establishment of an efficient system for delivering RNAs into target cells is required. We previously developed polycation liposomes (PCLs) for the delivery of small interfering RNA (siRNA) to tumors. In the present study, PCLs were used to deliver siRNA targeting polo-like kinase 1 (siPlk1) into cancer cells. In addition, verteporfin, an approved drug for the treatment of age-related macular degeneration, was also used to treat cancer cells. Verteporfin has recently been shown to suppress tumor cell proliferation through autophagy inhibition. It has also recently been reported that inhibition of autophagy leads to the stabilization of Argonaute2 (Ago2), which is the effector component of RNA-induced silencing complexes. In this study, we have investigated for the first time a combination cancer therapy using siPlk1 and verteporfin. We hypothesized that verteporfin acts as an autophagy inhibitor and an RNA interference (RNAi) enhancer, and that the treatment with verteporfin enhances the anticancer effects of siPlk1.

Methods
The expression of light chain 3 (LC3), a marker of autophagy, in MDA-MB-231 human breast cancer cells was examined by Western blotting to evaluate the inhibitory effect of verteporfin on the autophagy of the cells. MDA-MB-231 cells, which are known to have the mutation of k-ras genes, were used since autophagy is highly observed in cancer cells with k-ras mutation. The inhibitory effect of PCLs carrying siPlk1 (siPlk1-PCLs) on the growth of MDA-MB-231 cells was evaluated by WST-8 assay. Knockdown of Plk1 protein in MDA-MB-231 cells by combination treatment with siPlk1 and verteporfin was evaluated by Western blotting. PCLs carrying both siPlk1 and verteporfin (siPlk1-V-PCLs) were prepared and used to evaluate their combined effect on cell growth.

Results
The result of Western blotting showed that the expression of LC3 in MDA-MB-231 cells was markedly suppressed by verteporfin treatment, indicating that the autophagy of the cells was inhibited. On the other hand, the expression of Ago2 in the cells was markedly increased by verteporfin treatment. Cell growth was significantly inhibited by the treatment with siPlk1-PCLs compared with PCLs carrying control siRNA. The combination use of siPlk1 and verteporfin showed significant Plk1-knockdown effect compared to siPlk1 treatment alone. Furthermore, the siPlk1-V-PCL treatment significantly inhibited cell growth compared with siPlk-PCL or verteporfin treatment. It may be possible to obtain additive/synergistic anticancer effects by combination of verteporfin and siPlk1.

Conclusion
As verteporfin alone suppressed the growth of MDA-MB-231 cells, this suppression may have occurred through autophagy inhibition. It is likely that the autophagy inhibition followed by increased Ago2 expression in the MDA-MB-231 cells caused by verteporfin treatment resulted in the enhancement of the RNAi effect of siPlk1. Although further investigation is required to clarify the precise mechanism of this combination effect, the findings of the present study suggest that combination therapy using siPlk1 and verteporfin has strong potential as a treatment for cancer.