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***In vitro* and *in vivo* activity of sphingosine kinase 1 LNA oligonucleotides in gastric cancer**

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Background

Gastric cancer is still a major health problem, although declining in incidence. The prognosis at advanced stage of disease with the current chemotherapeutic treatment strategies remains poor. Therefore, novel treatment strategies and molecular targets for gastric cancer therapy are desperately needed. Sphingosine kinase (SphK) 1 represents a promising novel target for anti-cancer therapy. However, the most commonly used small-molecule inhibitors of SphK are unspecific inhibitors of SphK1. Here we investigated the effect of SphK1 downregulation by locked nucleic acid antisense oligonucleotides (LNA-ASO) in gastric cancer *in vitro* and *in vivo*.

Methods

Two gastric cancer cell lines (Mkn28 and N87) were assessed for cell viability and cell death by electronic cell counting and FACS analysis, respectively. SphK1 target modulation of mRNA was measured by RT-PCR. For combination therapy, SphK1 LNA-ASO was combined with doxorubicin and analyzed for cell viability and cell death. For *in vivo* studies athymic nude mice were inoculated with Mkn28 cells bilaterally and treated thrice weekly i.p. with SphK1 LNA.

Results

LNA-ASO targeting SphK1 reduced SphK1 mRNA levels in a dose-dependent manner (1.9–4.9-fold) whereas the control LNA-ASO did not reduce SphK1 mRNA levels at all. Transfection with 12.5 nM SphK1 LNA-ASO resulted in growth inhibition of Mkn28 gastric cancer cells (up to 57%) beginning from 24h after transfection. Notably, the combination of SphK1 LNA-ASO with doxorubicin resulted in significant chemosensitizing anti-proliferative activity. The anti-tumor activity coincided with an increase in the number of apoptotic cells both in the monotherapy and in the combination group as determined by FACS analysis. However, *in vivo* SphK1 therapy does not show anti-tumor activity. In line with this finding we did not observe target downregulation *in vivo*.

Conclusions

SphK1 target downregulation induces apoptosis and reduces cell counts of gastric cancer cells indicating a role for SphK1 as a functionally relevant molecular target in gastric cancer cells. Moreover, downregulation of SphK1 sensitizes gastric cancer cells to doxorubicin. Although SphK1 ASO treatment did not result in tumor growth inhibition *in vivo*, our data show that SphK1 is a functionally relevant molecular target in gastric cancer cells.