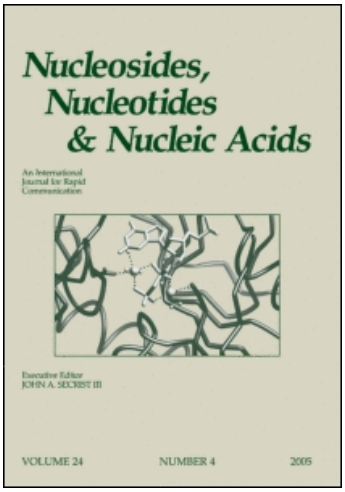

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Survivin mRNA Antagonists Using Locked Nucleic Acid, Potential for Molecular Cancer Therapy

Niels Fisker[a]; Majken Westergaard[a]; Henrik Frydenlund Hansen[a]; Jens Bo Hansen[a]
[a] Santaris Pharma A/S, Hoersholm, Denmark

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SURVIVIN mRNA ANTAGONISTS USING LOCKED NUCLEIC ACID, POTENTIAL FOR MOLECULAR CANCER THERAPY

Niels Fisker, Majken Westergaard, Henrik Frydenlund Hansen, and Jens Bo Hansen □ *Santaris Pharma A/S, Hoersholm, Denmark*

□ *We have investigated the effects of different locked nucleic acid modified antisense mRNA antagonists against Survivin in a prostate cancer model. These mRNA antagonists were found to be potent inhibitors of Survivin expression at low nanomolar concentrations. Additionally there was a pronounced synergistic effect when combining the mRNA antagonists against Survivin with the chemotherapeutic Taxol. This effect was demonstrated at concentrations of antagonists far lower than any previously demonstrated, indicating the high potential of locked nucleic acid for therapeutic use. Further characterisations* in vivo *are ongoing.*

Keywords Locked nucleic acid; survivin; mRNA; LNA; antisense

INTRODUCTION

Survivin is known to be selectively expressed in most cancers and an elevated expression of Survivin is often associated with poor prognosis for the patients.[1] In addition, several studies show that Survivin down-regulation sensitises cancer cells to radiation and chemotherapy, which makes it a prominent molecular target for cancer therapy using mRNA antagonists.[2,3]

We have identified different mRNA antagonists against Survivin. These antagonists are a class of antisense oligonucleotides modified with locked nucleic acid (LNA). The LNA mRNA antagonists used in this study were designed as fully phosphorothioated 16 nucleotide long gapmers flanked with LNA. We and others have previously shown that LNA enhance the potency of single stranded mRNA antagonists.[4,5] Currently, a clinical phase I/II study in patients with Chronic Lymphocytic Leukaemia has commenced with an LNA modified mRNA antagonist targeting Bcl-2 (SPC2996).

We have studied the Survivin mRNA and protein levels following in vitro transfection of mRNA antagonists by quantitative PCR (qPCR) and ELISA,

Address correspondence to Niels Fisker, Santaris Pharma A/S, Boege Allé 3, DK-2970 Hoersholm, Denmark. E-mail: NFN@santaris.com

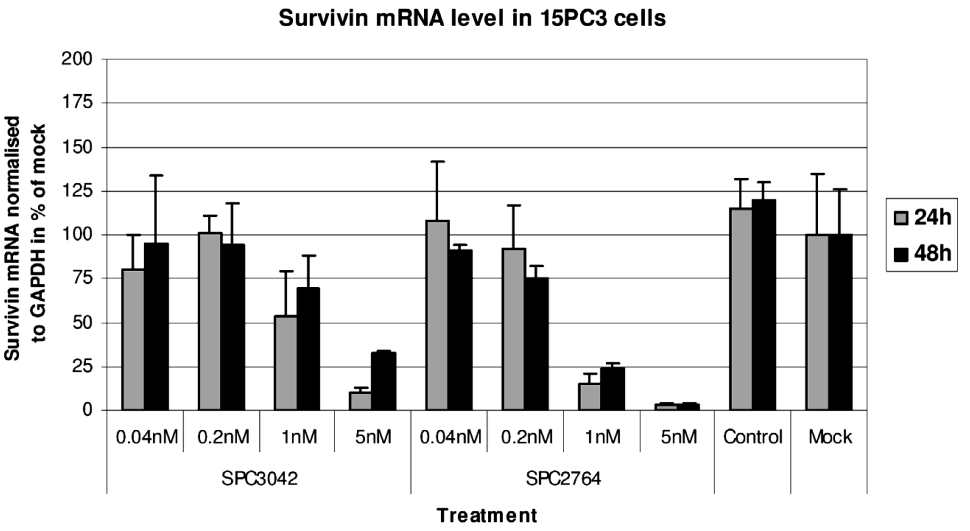

FIGURE 1 Survivin mRNA levels in 15PC3 cells upon treatment with LNA mRNA antagonists analyzed by qPCR at 24 and 48 hours after transfection. Error bars indicate the standard deviation from three individual experiments. The control used is a scrambled LNA mRNA antagonist at 5 nM concentration. Mock resembles untreated cells.

respectively. Survivin mRNA levels were normalised to GAPDH mRNA and Survivin protein levels to total protein. The effect on cell division were studied by cell cycle and apoptosis induction assays. All experiments were carried out using the human prostate cancer model cell line 15PC3. The Survivin mRNA antagonists SPC3042 and SPC2764 were combined with Taxol, which is known to stabilize microtubules, in order to elucidate possible synergistic effects with this chemotherapeutic.

RESULTS

The results showed that the SPC3042 and SPC2764 were potent inhibitors of Survivin mRNA with estimated IC_{50} values of approximately 1 nM at 24 hours after transfection (Figure 1). The Survivin protein levels correlated to the mRNA levels (data not shown).

Results from nuclear staining followed by FACS showed that both LNA mRNA antagonists had an effect on the cell cycle leading to an arrest of cells in the G2/M phase (Figure 2). This effect was most prominent for SPC2764 resulting in an increased accumulation of cells in late phases of the cell cycle and cellular polyploidy. This result was confirmed as a downstream effect of Survivin modulation by similar experiments with a non-modified siRNA against Survivin (data not shown).

Results obtained by an apoptosis dependent caspases 3/7 assay showed that SPC3042 had a pronounced effect on cell viability by inducing cellular

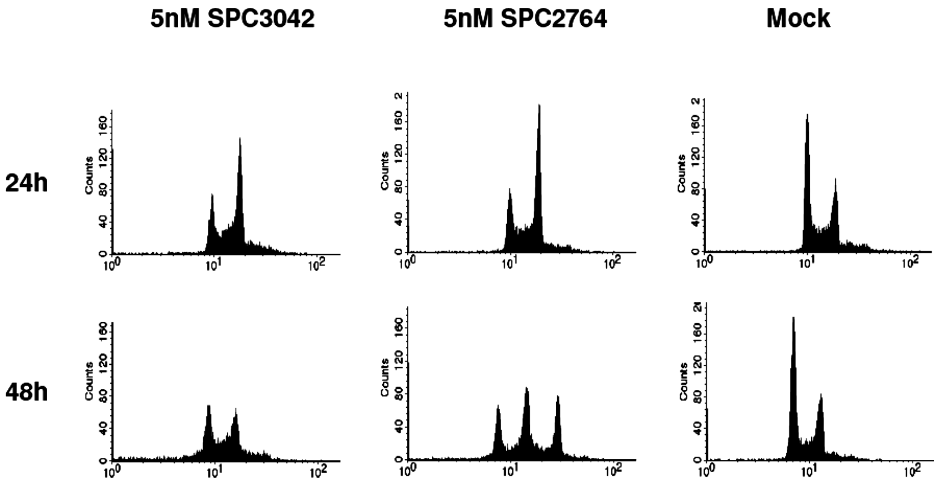

FIGURE 2 Accumulation of cells within different phases of the cell cycle measured by nuclear staining and flow cytometry. From left to right the first peak indicates cells within the G1 phase then cells in G2/M phase and thereafter polyploid cells.

apoptosis (Figure 3). This effect could be further induced by treatment in combination with 10 nM Taxol. On the contrary, SPC2764 did not elicit a strong apoptotic induction which might explain the accumulation of polyploid cells from this treatment compared to SPC3042.

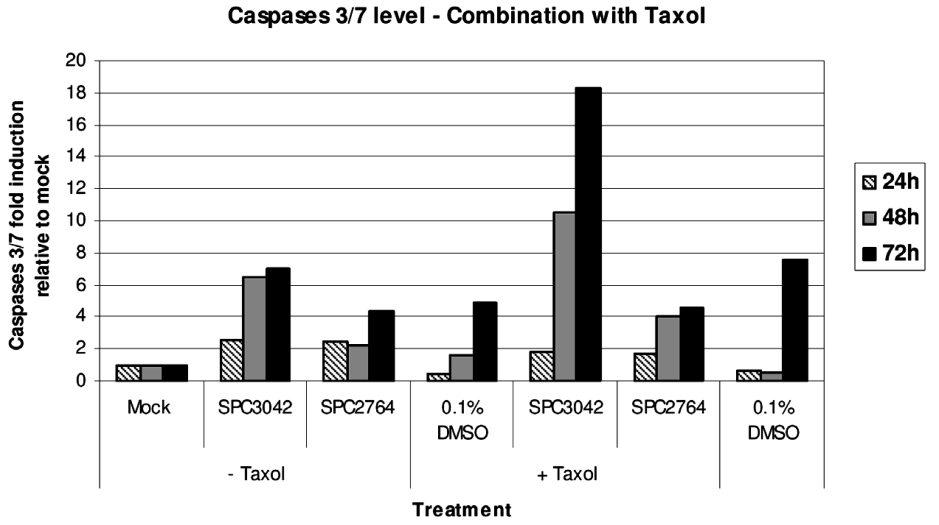

FIGURE 3 Apoptosis induction measured by the relative increase in caspases 3/7 levels in 15PC3 cells at 24, 48, and 72 hours after transfection. Cells were treated with 10 nM SPC3042 and SPC2764 with or without 10 nM Taxol in 0.1% DMSO. Mock resembles untreated cells.

CONCLUSION

We have demonstrated the inhibitory potential of LNA modified mRNA antagonists targeting Survivin. LNA is known to enhance the therapeutic potential of oligonucleotides by increasing the stability, affinity and reduce toxicity compared to first generation antisense compounds.

The LNA mRNA antagonists used in this study were shown to inhibit Survivin mRNA with IC_{50} values of approximately 1 nM. As a result of Survivin knockdown, cancer cells are forced to arrest in the G2/M phase of the cell cycle and/or apoptosis. In addition, SPC3042 sensitizes cancer cells to treatment with the chemotherapeutic Taxol which in combination leads to increased apoptosis. Further characterisations in vivo on LNA mRNA antagonists against Survivin are currently ongoing.

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