Polymeric micelles containing anti-survivin siRNA and paclitaxel in combination: A promising strategy to reverse the drug resistance in the treatment of ovarian cancer

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ABSTRACT SUMMARY

Ovarian cancer is the second most common gynecological malignancy worldwide. Its current therapy is based on cytoreductive surgery followed by platinum and taxane-based combination chemotherapy. Frequently, it is complicated by multidrug resistance (MDR). The discovery that survivin, a small anti-apoptotic protein is involved in chemoresistance, opens a new scenario to overcome MDR in cancer¹, since siRNA could be used to inhibit the expression of survivin in cancer cells. The down-regulation of survivin by siRNA sensitizes cancer cells to chemotherapeutic agents, such as paclitaxel² (PXL).

With this in mind, we have developed easy-to-prepare polymeric micelles (PM) co-loaded with PXL and anti-survivin siRNA, so-called survivin siRNA/PXL PM. The activity of the nanopreparations was evaluated on a PXL-resistant ovarian cancer cell line, SKOV3-tr, by tumor cells growth inhibition, down-regulation of survivin expression, and chemosensitization of the treated tumor cells to PXL. We found a significant sensitization of resistant SKOV3-tr cells to PXL after the treatment with PM co-loaded with siRNA and PXL³ (Figure 1).

To confirm the encouraging in vitro results, the antitumor efficiency of the developed multifunctional PM was evaluated in an animal model of SKOV3-tr and demonstrated that survivin siRNA/PXL-co-loaded PM elicited a significant inhibition of the tumor growth of a human xenograft model of SKOV3-tr in mice.

INTRODUCTION

Survivin, a 16.5 kDa protein, is a member of the inhibitor of apoptosis protein (IAP) family. Different authors have studied the suppression of survivin by using siRNA⁴, followed by sensitization of resistant cancer cells to chemotherapeutic agents. Unfortunately, the clinical use of siRNA is still hampered by poor biological stability and low intracellular uptake in vivo. To address this problem, we reversibly modified the survivin siRNA with a phosphothioethanol (PE) portion via the reducible disulfide bond, obtained the so-called survivin siRNA-S-S-PE conjugate⁵. Then, we prepared multifunctional PEG₂₀₀₀-PE-based PM containing the resulting survivin siRNA-S-S-PE conjugate and PXL in combination. Finally, the in vitro and in vivo activity of such combination was evaluated in SKOV3-tr cell lines and in nu/nu mice SKOV3-tr xenografts.

EXPERIMENTAL METHODS

The survivin siRNA-S-S-PE conjugate was synthesized as reported by Salzano G. et al⁶. The PM containing survivin siRNA-S-S-PE and PXL were prepared by hydration of a thin polymeric film. Briefly, an organic solution of PXL in methanol (1 mg/mL) was added to the PEG₂₀₀₀-PE solution in chloroform (20 mg/mL). Organic solvents were removed by N₂, and the film was further dried under vacuum. Then, the polymeric film was hydrated with 1 mL of survivin siRNA-S-S-PE in PBS at pH 7.4. The resulting dispersion was gently vortexed to form mixed micelles, so-called survivin siRNA/PXL PM, PEG₂₀₀₀-PE-based plain PM, or PM containing survivin siRNA-S-S-PE or scrambled siRNA-S-S-PE alone or in combination with PXL were prepared similarly. The PM were characterized in terms of mean diameters and size distribution, siRNA and PXL encapsulation efficiency. In vitro down-regulation of survivin, chemosensitization studies and cytotoxicity of survivin siRNA/PXL PM were evaluated in SKOV3-tr cancer cell lines. Down-regulation of survivin protein levels and cell viability were investigated by ELISA and Cell Titer Blue assay, respectively. Finally, the anti-tumor activity of survivin siRNA/PXL PM and the down-regulation of survivin in vivo were evaluated in mice with SKOV3-tr xenografts.

RESULTS AND DISCUSSION

Survivin siRNA/PXL PM are characterized by a very narrow size distribution with a mean diameter of about 25 nm and PI< 0.2 (Table 1). HPLC analysis of non-encapsulated siRNA and PXL showed an encapsulation efficiency of about 50% and 70%, respectively.

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<th>Table 1. Physical characteristics of survivin siRNA/PXL PM.</th>
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In SKOV3-tr cells, the treatment with survivin siRNA/PXL PM induced a strongly enhanced antiproliferative effect compared to free PXL (Figure 1A). A significant down-regulation of survivin protein levels in these cells (of about 30%) was also observed (Figure 1B). No significant effects were observed after the treatment with the same concentrations of PM containing scrambled siRNA and with free survivin siRNA-S-S-PE conjugate.
The studies of the microtubules organization confirmed the key role of the survivin down-regulation in the sensitization of SKOV3-tr to PXL treatment. As shown in the Figure 1C, the treatment with PXL yielded significant changes in microtubules morphology only when cells where pre-treated with survivin siRNA PM. In particular, SKOV3-tr cells displayed a significant lack of microtubule organization reflective of cell death (Fig. 1C, image D).

Figure 1. (A) Effect of survivin siRNA/PXL PM on SKOV3-tr cells. The cell viability was measured after 72 hrs by the Cell Titer Blue assay. Data = mean ± SD (n =3). ***p < 0.001 values were obtained by comparing each treatment to survivin siRNA/PXL PM treated cells. (B) Down-regulation of survivin protein levels in SKOV3-tr, evaluated by ELISA assay. (C) Effect of PXL on microtubule stabilization after survivin down-regulation in SKOV3-tr cells. Cells were stained for β-tubulin (green). The nuclei (blue) were stained with DAPI. Untreated cells (A); cells treated with free PXL for 24h (B); cells treated with survivin siRNA PM for 72 h (C); cells treated survivin siRNA PM for 48 h followed by treatment with PXL for 24 h (D).

Then, we evaluated the effect of the combination therapy in vivo. A significant reduction of the tumor growth following the administration of survivin siRNA/PXL PM was observed in mice with SKOV3-tr xenografts (Figure 2A). Post-mortem tumor weights in mice treated with this preparation, was also significantly reduced compared to all control groups (Figure 2B). In the same model, the administration of PXL, both in Cremophore solution and in PM (scrambled siRNA/PXL PM group), was not effective. No significant bodyweight loss was observed during the study confirming low non-specific toxicity of the treatments (data not shown).

Figure 2. SKOV3-tr tumor growth in mice. A. In vivo antitumor activity of survivin siRNA/PXL PM in SKOV3-tr xenografts. Survivin siRNA/PXL PM were administered at a concentration of survivin siRNA and PXL of 1 and 10 mg/kg, respectively, once per week for 5 consecutive weeks. Relative tumor volume values (tumor volume in mm³ on day ‘n’ (Vn) / tumor volume at the start of the treatment (Vo) versus time in days) are reported. B. Post-mortem tumor weights. On day 30, tumors were excised, weighed, and plotted.

Finally, to provide the evidence that the inhibition of the tumor growth mediated by survivin siRNA/PXL PM treatment was due to its ability to down-regulate survivin in vivo, transcriptional mRNA levels of the survivin gene were evaluated by RT-PCR in tumor tissues. As shown in Figure 3, survivin mRNA was strongly down-regulated by survivin siRNA/PXL PM treatment. Compared to saline treated group and to scrambled siRNA/PXL PM group, the inhibitory rate of survivin mRNA levels was of about 90% after treatment with survivin siRNA/PXL PM.

Figure 3. Real Time-PCR of survivin mRNA expression in tumor tissues. Values were calculated based on standard curves generated for each gene. Normalization of samples was determined by dividing copies of survivin transcripts by copies of β-actin. *p values ≤ 0.05 were considered statistically significant and were obtained by comparing each treatment to survivin siRNA/PXL PM treated cells.

CONCLUSION

A new micellar nanopreparation was developed for the co-delivery of the anti-survivin siRNA as siRNA-S-S-PE conjugate and PXL for the treatment of ovarian cancer. The developed system allows to easily co-encapsulate chemotherapeutic drugs and siRNA for multifunctional therapy of MDR tumors. The developed system is able to efficiently down-regulate survivin, an antiapoptotic protein, in vivo. The use of the combination of survivin siRNA and PXL in the same PM allowed achieving a potent anti-tumor effect in an animal model of SKOV3-tr. In the same model, tumor growth was unaffected when using Taxol. These results suggest the future pre-clinical development of survivin siRNA/PXL PM for the treatment of the aggressive human cancer.

REFERENCES