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Chemosensitization of bladder cancer cells by survivin-directed antisense oligodeoxynucleotides and siRNA

Susanne Fuessel[a,*], Jana Herrmann[a], Shuangli Ning[a], Matthias Kotzsch[b], Kai Kraemer[a], Uta Schmidt[a], Oliver W. Hakenberg[a], Manfred P. Wirth[a], Axel Meye[a]

[a]*Department of Urology, Technical University Dresden, Fetscherstr. 74, D-01307 Dresden, Germany*
[b]*Institute of Pathology, Technical University Dresden, Fetscherstr. 74, D-01307 Dresden, Germany*

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Abstract

Survivin is known to be overexpressed in numerous tumor types including human bladder cancer and to cause resistance to radiation and chemotherapy. Therefore, we tested the antisense oligodeoxynucleotide AS-SVV286 and the small interfering RNA si-SVV284 to down-regulate survivin in the BCa cell lines EJ28 and 5637 thereby acting as sensitizers for chemotherapy. Pretreatment with these inhibitors followed by chemotherapy caused an enhanced decrease in cell viability. The observed reduction in cell counts associated with increased rates of apoptosis paralleled the degree of reduction of survivin expression that was achieved more efficiently by the siRNA than by the AS-ODN. Nevertheless, both therapy approaches in combination with all tested chemotherapeutics provoked a remarkable inhibition of viability and may serve as suitable additive tools for chemosensitization of bladder cancer cells.

Keywords: Antisense oligodeoxnucleotides; Bladder cancer; Chemosensitization; siRNA; Survivin

1. Introduction

Survivin, a member of the inhibitor of apoptosis protein (IAP) family, is one of the most prominent cancer-associated genes analyzed extensively during recent years. Apart from fetal tissues, selective expression of survivin was observed in numerous tumor types. It is often associated with enhanced recurrence risk and poor prognosis. Furthermore, the up-regulation of survivin and other anti-apoptotic proteins such as bcl-2 is associated with the resistance of tumors to apoptosis-inducing therapies [1,2].

Survivin occupies an outstanding position among the IAP family due to its complex mode of anti-apoptotic action and its additional function in the control of cell division. Several studies revealed survivin as an indispensable factor in the concerted action with different cell cycle regulators assisting completion of cytokinesis [3–8]. Depletion of survivin by dominant negative mutants [3,9], antisense cDNA [9–11], antisense oligodeoxynucleotides

* Corresponding author. Tel.: +49 351 458 4544; fax: +49 351 458 5771.
E-mail address: susanne.fuessel@uniklinikum-dresden.de (S. Fuessel).

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(AS-ODN) [3,12,13] or small interfering RNA (siRNA) constructs [4,5,8] caused incomplete cytokinesis and re-entry into mitosis followed by the appearance of multinucleated cells.

Inhibition approaches using AS-ODN [3,12–18] or siRNA constructs [19–23] were performed with the intention to impair tumor cell growth and to sensitize cells to apoptosis-inducing agents. Reduction of tumor cell proliferation, cell colony formation and tumorigenicity caused by a remarkable increase in apoptosis and by cell cycle alterations were observed in most of these studies exposing survivin to be an excellent target of specific anti-tumor treatments.

In bladder cancer (BCa)—with $>60{,}000$ estimated new cases and $>12{,}000$ estimated new deaths in the US per year one of the most common cancers [24]—a dependence of survivin mRNA and protein expression on tumor grade and stage was shown for bladder tissue samples and urine specimens [25–28]. The successful down-regulation of survivin which is overexpressed in numerous BCa cell lines by AS-ODN and siRNA was reported recently [16,21]. BCa cell proliferation was inhibited selectively by such nucleic acid based inhibitors that were reported to be taken up efficiently in vitro and ex vivo by urothelial cells [29].

For superficial high-grade BCa, transurethral resection accompanied by the intravesical instillation of the immunotherapeutic Bacillus Calmette-Guérin and/or chemotherapeutics (CT) such as mitomycin C (MMC) suffers from unsatisfactory treatment efficacy [30]. The failure of locally or systemically applied CT such as cisplatin (CDDP), gemcitabine (GEM) or MMC as a major problem in the treatment of BCa is frequently caused by the up-regulation of anti-apoptotic genes [31]. High recurrence rates of 50–70% and the progression of high-risk primary tumors to muscle-invasive stages despite repeated interventions emphasize the need for more specific therapeutic approaches. Such adjuvant strategies are expected to reduce the hazard of recurrence and progression selectively by increasing the susceptibility of tumor cells to CT.

Therefore, the aim of this study was to sensitize BCa cells by a specific pretreatment with one AS-ODN or one siRNA construct directed at the same survivin mRNA motif to different commonly used CT (MMC, CDDP, GEM) in order to increase their cytotoxic efficacy.

2. Material and methods

2.1. AS-ODN and siRNA constructs

A previous systematic in vitro evaluation of different survivin-directed AS-ODN (Synthesized by Invitrogen, Karlsruhe, Germany) revealed the construct AS-SVV286 as highly efficient [16]. Furthermore, the siRNA construct si-SVV284 (Synthesized by Eurogentec, Seraing, Belgium) was described by several groups [19,21,23,32] to attenuate survivin expression effectively and persistently in different tumor cell lines. The siRNA GL3 (MWG Biotech, Ebersberg, Germany) which was targeted at the luciferase mRNA and the nonsense (NS) ODN NS-K1 [12] served as controls for siRNA and antisense treatments [16,21], respectively.

2.2. Cell culture and treatment

The cells lines EJ28 and 5637 (ATCC; Manassas, USA) were selected as model systems of human BCa for this study since they are characterized by a remarkable overexpression of survivin both at the transcript and the protein levels [16]. In both cell types, survivin could be downregulated most efficiently by survivin-directed AS-ODN and siRNA constructs [16,21]. Moreover, this targeted survivin inhibition was associated with an obvious reduction in viability and proliferation [16,21]. The cells were cultivated without antibiotics under standard conditions (37 °C, humidified atmosphere containing 5% CO_2) as recommended by the providers.

Three days after seeding cells into 96-well or 6-well plates, they were transfected with a mixture of ODN or siRNA (each 250 nM) and Lipofectin (ratio 1:3 w/w), both diluted with serum-free OptiMEM according to the manufacturer's instructions (Invitrogen). After the transfection (4 h, 37 °C), the cells were washed with PBS and incubated in culture medium for 24 h. Thereafter, the cells were incubated with the chemotherapeutic agents CDDP or GEM (both for 24 h) or MMC (for 2 h) followed by a washing with PBS and further cultivation as described recently [33]. In viability assays in 96-well plates, two different concentrations of each CT (CT1 and CT2, as specified in Fig. 1) chosen from pre-tests were applied. In all other experiment series doses of