European Urology

European Urology 47 (2005) 703–709

Inhibition of bcl-2 Enhances the Efficacy of Chemotherapy in Renal Cell Carcinoma

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Accepted 26 November 2004

Available online 14 December 2004

Abstract

Objectives: Renal cell cancer (RCC) is highly resistant to chemotherapy. Increased expression of the antiapoptotic gene bcl-2 in tumors is known to be associated with poor responses to systemic treatment of cancer. Down-regulation of bcl-2 expression using antisense oligonucleotides (asON) has been shown to increase chemosensitivity in clinical phase I–III studies with various cancers. However, no studies on the efficacy of this approach in RCC have been reported so far. This study aimed to evaluate whether bcl-2 asON could enhance efficacy of chemotherapy in human RCC.

Material and Methods: Expression of bcl-2 mRNA and protein was analyzed in different RCC cell lines by RT-PCR and Western blot. Cells with high or low bcl-2 mRNA and protein expression were treated with different concentrations of bcl-2 asON in combination with cisplatin. AsON-induced down-regulation of bcl-2 mRNA and protein was documented by RT-PCR and Western blot. Treatment effects on cell viability were analyzed by colorimetric tetrazolium (MTT) assay. Immunohistochemical staining of M30-positive cells was performed for quantification of apoptotic cells.

Results: Transfection of high bcl-2 expressing cells with bcl-2 asON alone induced no reduction of cell viability at a concentration range from 100–1000 nM. In combination therapy, pretreatment with asON significantly enhanced MTT reduction after cisplatin treatment. IC_{50} concentrations of cisplatin were 1 μg/ml with and 2.7 μg/ml without prior incubation. The marked reduction of cell viability correlated with an 8-fold increase of apoptotic cells after combination treatment. Only a minor increase of cisplatin effectivity was noted after asON preincubation of cells with lower bcl-2 expression.

Conclusions: The combination of cisplatin and bcl-2 antisense ON exerts significantly greater effects on cell viability and apoptosis than either agent used alone on human RCC cells. These data indicate that inhibition of bcl-2 expression may be an attractive therapeutic strategy in RCC tumors with high bcl-2 expression.

Keywords: bcl-2; Antisense; Renal cell cancer; Molecular therapy

1. Introduction

Renal cell carcinoma accounts for approximately 2% of adult cancers with over 30,000 new cases per year in the United States and an associated 12,000 deaths [1]. Almost half of the patients with RCC die within 5 years of diagnosis and 5-year survival for those with metastatic disease is less than 10% [2].

Despite numerous studies with different treatment modalities, advanced RCC remains highly resistant to systemic therapy. Between 1990 and 1998, 33 chemotherapeutic agents were studied in 51 phase II trials comprising 1347 RCC patients [2]. No chemotherapy

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doi:10.1016/j.eururo.2004.11.013

with objective response rates higher than 15% has been established. Various reasons, including inherent chemoresistance, pharmacological mechanism of chemotherapeutic action, and the increased expression of antiapoptotic genes such as bcl-2 have been related to the ineffectivity of conventional chemotherapy in RCC [3].

Members of the bcl-2 family of proteins are important regulators of programmed cell death pathways with individual members that can suppress (e.g. bcl-2, bcl-xl) or promote (e.g. Bax, Bad) apoptosis [4,5]. The localization of bcl-2 and bcl-xl to cellular membranes, particularly in mitochondria, inhibits the release from mitochondria of substances involved in either signaling or execution of apoptosis, such as cytochrome c, procaspase 3, and apoptosis-inducing factor [5]. The role and function bcl-2 members has been reviewed in detail [6]. Increased expression of the anti-apoptotic proteins bcl-2 and bcl-xl is involved in the development and progression of many tumors [7]. Elevated levels of anti-apoptotic proteins have been demonstrated in virtually every type of cancer. Specifically bcl-2 is overexpressed in more than half of all human cancers. bcl-2 expression is frequently upregulated in RCC and significantly higher in RCC than in normal renal tissue [8,9]. Although correlation of immunohistochemical bcl-2 staining with RCC patient survival has been reported [10] this parameter does not seem to be a statistical significant prognosticator of RCC patient outcome [9]. However, high levels of bcl-2 protein in RCC tumors have been correlated with low incidence of tumor cell apoptosis. Thus, down-regulation of bcl-2 may be a promising approach in RCC treatment.

Antisense oligonucleotides (asON) are chemically modified stretches of single-stranded DNA that are complementary to mRNA regions of a target gene. AsON effectively inhibit target gene expression by specifically hybridizing with complementary mRNA regions of the target gene and formation of RNA/DNA duplexes, thereby inhibiting gene expression primarily by activation of ribonuclease (RNase) H, which subsequently cleaves the target mRNA. Additional mechanisms by which these duplexes release down-regulation of gene expression have been described in detail [11,12]. Generally, antisense inhibitors offer the opportunity to modulate dysregulated gene expression in cancer on an early molecular level. Phosphorothioate ON which are the most commonly used chemical modification today are stabilized to resist nuclease degradation by substitution of one of the nonbridging oxygen atoms at each phosphorus by a sulfur atom.

bcl-2-specific asON have shown broad anti-cancer activities in pre-clinical models and are currently in several phase III trials [13]. Antisense bcl-2 strategy has particularly been shown to strongly increase antitumoral effects of different chemotherapeutical agents [3].

Despite poor chemosensitivity and high bcl-2 positivity in RCC, efficacy of combined bcl-2 antisense treatment plus chemotherapy has not yet been evaluated in a human renal cell cancer model. Since ON highly accumulate in the kidney [14] high local concentrations may be expected to affect target gene expression, rendering this organ a promising site for therapeutic strategies with antisense compounds.

In the present study we intended to evaluate the effects of asON-mediated suppression of bcl-2 in combination with cisplatin chemotherapy. Cisplatin was chosen since it is one of the most important anticancer drugs and application in RCC has been reported. Recently promising results using combination chemotherapy including cisplatin have been described [15].

2. Materials and methods

2.1. Cell lines

Human SK-RC 35, SK-RC 47 and SK-RC 58 renal cell cancer cell lines were originally established from primary nephrectomy specimens of RCC patients [16]. RCC cell lines were cultured in RPMI-1640 (Invitrogen, Karlsruhe, Germany) containing 1% sodium pyruvate, 10% fetal calf serum (FCS), 1% L-glutamin, 10 U/ml penicillin and streptomycin (BIOCHROM, Berlin, Germany). Cells were maintained at 37 °C in a 5% CO_2, humidified incubator.

2.2. Oligonucleotide synthesis and sequence

Oligonucleotides used in these studies were phosphorothioate oligodeoxyribonucleotides. Antisense and mismatch ON have the same sequence as previously described [17]. Mismatch (MM) ON have mismatches in 2 positions. The antisense construct is complementary to the first six codons of the bcl-2 mRNA. ON were purified by high-performance liquid chromatography (HPLC). Synthesis and purification of ON was performed by EUROGENTEC (Seraing, Belgium).

The used sequences were as follows:

AS: 5′-TCT CCC AGC GTG CGC CAT-3′
MM: 5′-TCT CCC AGC ATG TGC CAT-3′

2.3. Treatment of cells with antisense oligonucleotides

For RT-PCR and Western blot experiments cells were cultured and treated in 25 ccm cell culture flasks (Nunc, Wiesbaden, Germany). 5×10^5 cells were seeded and treated one day later with ON at the indicated concentrations in the presence of a cationic lipid (Tfx_{20}, PROMEGA, Mannheim, Germany), in an ON/Tfx ratio of 1:4 in 2.5 ml serum-free medium. After 2 hours transfection time