Testosterone-repressed Prostate Message-2 Is an Antiapoptotic Gene Involved in Progression to Androgen Independence in Prostate Cancer

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ABSTRACT

Although initially reported as an androgen-repressed gene in the rat prostate, the functional role of testosterone-repressed prostate message-2 (TRPM-2) in apoptosis remains undefined. Inhibition of castration-induced apoptosis by calcium channel blocker treatment in androgen-dependent Shionogi tumors resulted in the prevention of TRPM-2 gene up-regulation, suggesting that TRPM-2 is not directly androgen-repressed, but is regulated by apoptotic stimuli. The overexpression of the TRPM-2 gene in human androgen-dependent LNCaP prostate cancer cells by stable transfection rendered them highly resistant to androgen ablation in vivo. We then tested the efficacy of antisense TRPM-2 oligodeoxynucleotide (ODN) therapy in the Shionogi tumor model and demonstrated that the systemic administration of antisense TRPM-2 ODNs in mice bearing Shionogi tumors after castration resulted in a more rapid onset of apoptosis and time to complete regression, as well as a significant delay of emergence of androgen-independent recurrent tumors compared to control ODN treatment. Collectively, these findings illustrate that TRPM-2 is an antiapoptotic rather than an androgen-repressed gene that confers resistance to androgen ablation and thereby helps accelerate the progression to androgen independence.

INTRODUCTION

No therapy exists that is superior to androgen ablation in patients with advanced prostate cancer. Approximately 80% of patients achieve symptomatic and/or objective response after androgen ablation; however, the progression to androgen independence ultimately occurs and remains the main obstacle to improving the survival and quality of life in this disease (1). Traditionally, new nonhormonal therapies have been evaluated in patients with hormone refractory disease. When used in this end-stage setting, no nonhormonal agent has improved survival (2). Because androgen resistance develops, in part, from the up-regulation of antiapoptotic genes after androgen withdrawal, the identification and targeting of genes involved in apoptosis and AI (3) progression may lead to the development of novel therapies capable of delaying hormone refractory recurrences.

TRPM-2, also known as clusterin or sulfated glycoprotein-2, was first isolated from ram rete testes fluid (3), and it is associated with a wide variety of physiological and pathological processes, including tissue remodeling, lipid transport, reproduction, complement regulation, and apoptotic cell death (4). TRPM-2 has been regarded as a marker for cell death because its expression is up-regulated in various normal and malignant tissues undergoing apoptosis (5–9). Recent studies provide conflicting data on the association between enhanced TRPM-2 expression and apoptotic activity (10–12). Although it was initially named as an androgen-repressed gene up-regulated in regressing prostate tissues after androgen ablation (13), the functional role of TRPM-2 in castration-induced apoptosis remains undefined. In prostate cancer, TRPM-2 expression is associated with apoptotic cell death and AI recurrences. The introduction of TRPM-2 cDNA into LNCaP prostate cancer cells increases the resistance to apoptosis induced by tumor necrosis factor α treatment (14). The increased expression of TRPM-2 in prostate cancer correlates with the increasing Gleason score (15). However, the functional significance of TRPM-2 expression during AI progression has not been demonstrated.

Controlled study of the complex molecular processes associated with AI progression in prostate cancer has proved difficult because it cannot be replicated in vitro, and few animal models exist that reproducibly mimic the clinical course of the disease in men. The Shionogi tumor model is an AD mouse mammary carcinoma xenograft that is particularly useful in studying mechanism of castration-induced apoptosis and AI progression. In this model, AD tumors in male mice undergo complete regression after castration but recur as rapidly growing AI tumors after 1 month (16). The highly reproducible regression and recurrence pattern provides a reliable end point to test the efficacy of agents targeting castration-induced apoptosis and their effects on time to AI progression. Of the available human prostate cancer cell lines, only the LNCaP cell line is AD, PSA-secreting, and immortalized in vitro. As in human prostate cancer, serum PSA levels in the LNCaP tumor model are initially regulated by androgen and directly proportional to tumor volume, with loss of androgen-regulated PSA gene expression after castration as a surrogate endpoint of AI progression (17).

In the present study, we report for the first time that the TRPM-2 gene is an apoptosis-associated gene rather than an androgen-repressed gene. We then evaluated the effects of TRPM-2 overexpression on time to AI progression in the LNCaP tumor model. Finally, we tested whether adjuvant use of antisense TRPM-2 ODNs enhances castration-induced apoptosis and delays AI progression in the AD Shionogi tumor model.

MATERIALS AND METHODS

Shionogi Tumor Growth. The Toronto subline of the transplantable SC-115 AD mouse mammary carcinoma (18) was used in this study. For in vitro experiments, Shionogi tumor cells were cultured in DMEM (Life Technologies, Inc., Gaithersburg, MD) supplemented with 5% heat-inactivated FCS. For in vivo study, adult male DDS strain mice received s.c. injections of 5 × 106 tumor cells. When Shionogi tumors became 1–2 cm in diameter, usually 2–3 weeks after injection, castration was performed through an abdominal incision under methoxyflurane anesthesia. Details of the maintenance of mice, tumor stock, and operative procedures are described in a previous publication (19). Mice were maintained in accordance with institutional accredited guidelines.

CCB Treatment. One day before the castration, male DDS mice bearing the Shionogi tumor were randomly selected for treatment with CCBs (n = 5) versus no treatment as the control (n = 5). Beginning 1 day before castration, 588-μg nifedipine and 100-μg norvasc were administered p.o. three times daily to each mouse in the CCB group for 14 days.

Tumor volume was measured twice weekly and calculated by the formula length × width × depth × 0.5236 (20).
TUNEL Staining. A modified TUNEL technique (21) was used to detect apoptotic cells in Shionogi tumors using the ApopTag In Situ Apoptosis Detection System (Oncor Inc., Gaithersburg, MD) according to the manufacturer’s protocol. The number of positively stained cells per high power field in five random fields was counted and averaged.

Expression Plasmid and Transfection of LNCaP Cells. LNCaP cells were kindly provided by Dr. Leland W. K. Chung (University of Virginia, Charlottesville, VA) and maintained in RPMI 1640 (Life Technologies, Inc.) supplemented with 5% heat-inactivated FCS. pRC-CMV expression vector containing the 1.6-kb CDNA fragment encoding human TRPM-2 was kindly provided by Dr. Martin Tenniswood (W. Alton Jones Cell Science Center, Lake Placid, NY). The expression vector was transfected into LNCaP cells by the liposome-mediated gene transfer method (22). Briefly, 2 × 10^6 LNCaP cells were plated in 6-cm plates. The next day, 5 μg of purified TRPM-2 cloned pRC-CMV or pRC-CMV alone (as a control) was added to LNCaP cells after a preincubation for 30 min with 5 μg of lipofectamine reagent and 3 ml of serum-free OPTI-MEM (Life Technologies, Inc.). Drug selection in 300 μg/ml of Geneticin (Sigma Chemical Co., St. Louis, MO) was begun 3 days after the transfection. Colonies were harvested 2 weeks after drug selection using cloning cylinders, and cell lines were expanded for in vivo injection.

Assessment of in Vivo LNCaP Tumor Growth and Determination of Serum PSA Levels. One million cells of each of the LNCaP sublines were inoculated s.c. with 0.1 ml of Matrigel (Becton Dickinson Labware, Lincoln Park, NJ) in the flank region of 6- to 8-week-old male athymic nude mice (BALB/c strain; Charles River Laboratory, Montreal, Canada). Each experimental group consisted of six mice. Mice were castrated via a scrotal approach when tumors reached 100 and 200 mm^3 in volume. Tumor volume was measured once weekly and calculated as described above. Blood samples were obtained with tail vein incisions of mice once weekly. Serum PSA levels were determined by an enzymatic immunoassay kit with a lower limit of sensitivity of 0.2 μg/liter (Abbott IMX, Montreal, Canada) according to the manufacturer’s protocol. Data points were reported as mean values ± SD.

Antisense TRPM-2 ODNs. Phosphorothioate ODNs used in this study were obtained from Nucleic Acid-Protein Service Unit, University of British Columbia (Vancouver, Canada). The sequences of antisense TRPM-2 ODNs corresponding to the mouse TRPM-2 translation initiation site were 5’-GCA-CAGCGAGGAGGAATCTTCAT-3’. Two base TRPM-2 mismatch ODNs (5’-GCA-CAGCGAGGAGGATATTTCAT-3’) were used as the control.

Treatment of Cells with ODNs. Lipofectin, a cationic lipid (Life Technologies, Inc.) was used to increase the ODN uptake of cells. Shionogi cells were treated with various concentrations of ODNs after a preincubation for 20 min with 4 μg/ml lipofectin in serum-free OPTI-MEM (Life Technologies, Inc.). After 4 h, the medium containing ODNs and lipofectin was replaced with the standard culture medium described above.

Northern Blot Analysis. Total RNA was isolated from Shionogi tumor tissues and cultured Shionogi tumor cells by the acid-guanidum thiocyanate-phenol-chloroform method. Poly(A)^+ mRNA was then purified from total RNA using oligo(deoxythymidylate) cellulose. The electrophoresis, hybridization, and washing conditions were carried out as previously reported (17). Mouse TRPM-2 and G3PDH cDNA probes were generated by reverse transcription-PCR from total RNA of mouse brain using primers 5’-AAT-GAGCTCCCAAGAATGTCTGAC-3’ (sense) and 5’-AAA-GAGGCGTGTC-TATGTAGCCAGATGAT-3’ (antisense) for TRPM-2, and 5’-ATGTTGAGG-GTCGGTGGTGAACGATG-3’ (sense) and 5’-AAATGTTGCTAGATG-GACCTT-3’ (antisense) for G3PDH. The density of bands for TRPM-2 was normalized against that of G3PDH by densitometric analysis.

Western Blot Analysis. Samples containing equal amounts of protein (15 μg) from lysates of the cultured Shionogi cells and Shionogi tumors were electrophoresed on an SDS-polyacrylamide gel and transferred to a nitrocellulose filter. The filters were blocked in PBS containing 5% nonfat milk powder at 4°C overnight and then incubated for 1 h with a 1:400-diluted C-18, an antimonu TRPM-2 goat polyconal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA); 1:1000-diluted MAB065, an antirat TRPM-2 monoclonal antibody (CHEMICON INTERNATIONAL INC., Tumecula, California); or 1:600-diluted C2–10, an antihuman PARP mouse monoclonal antibody that reacts with mouse PARP (PARMINGEN, Mississauga, Canada). The filters were then incubated for 30 min with horseradish peroxidase-conjugated goat or antimonu IgG antibody (Amersham Life Science, Arlington Heights, IL), and specific proteins were detected using an enhanced chemiluminescence Western blotting analysis system (Amersham Life Science).

In Vivo ODN Treatment. Male DD/S mice bearing the Shionogi tumor were castrated and randomly selected for treatment with antisense TRPM-2 versus mismatch control ODNs. Each experimental group consisted of seven mice. Beginning the day of castration, each mouse received injections i.p. of 12.5 mg/kg of antisense TRPM-2 or mismatch control once daily for 15 days. The tumor volume was measured as described above. Data points were reported as mean tumor volumes ± SD.

RESULTS

TRPM-2 mRNA Expression Increases after Castration in the Shionogi Tumor Model. Northern blot analyses were used to characterize changes in TRPM-2 mRNA expression in AD intact tumors before castration, regressing tumors 4 and 7 days after castration, and AI recurrent tumors 28 days after castration. As shown in Fig. 1, compared to AD intact tumors before castration, TRPM-2 mRNA expression is up-regulated ~14-fold and 10-fold 4 and 7 days after castration, respectively and is maintained at 8-fold higher levels in AI tumors. The pattern of TRPM-2 up-regulation in the Shionogi tumor model after castration is similar to that in the rat ventral prostate (5) and human prostate cancer xenografts (9).

Inhibition of Castration-induced Apoptosis and TRPM-2 Up-regulation in Shionogi Tumors by CCBs. To determine whether changes of TRPM-2 expression after castration result from up-regulation of an androgen-repressed gene or are directly associated with apoptosis, mice bearing Shionogi tumors were treated beginning 1 day before castration with oral CCBs (nifedipine and norvasc) three times per day for 14 days. After castration, Shionogi tumors in mice treated with CCBs continue to grow with a doubling time of 8 days, compared to complete regression of Shionogi tumors in control mice (data not shown). As reported in our recent study (23), Shionogi tumor regression after castration was associated with a 10-fold increase in the number of apoptotic bodies detected by TUNEL staining, between 3 and 5 days postcastration. By 4 days postcastration, numerous apoptotic bodies (~20/high power field) in untreated control tumors were observed, but not in tumors treated with CCBs (Fig. 2, A and B). Furthermore, TRPM-2 up-regulation in Shionogi tumors after castration was significantly inhibited by CCB treatment. TRPM-2 mRNA levels in tumors treated with CCBs were 90%, 86%, and 77% lower than controls at 4, 7, and 10 days after castration, respectively (Fig. 2, C and D).

Overexpression of TRPM-2 Accelerates Time to AI Progression in the LNCaP Tumor Model. To determine the effects of TRPM-2 overexpression on time to AI progression after androgen ablation, LNCaP cells were transfected with TRPM-2 cDNA ex-
pression vector pRC-CMV/TRPM-2 or the pRC-CMV vector alone as a control. As shown in Fig. 3A, abundant levels of TRPM-2 mRNA were detected in four independent TRPM-2-transfected clones (LNCaP/T1 to LNCaP/T4), whereas the parental LNCaP (LNCaP/P) and the control vector-transfected cell line (LNCaP/C) did not express detectable TRPM-2 mRNA levels. LNCaP/P, LNCaP/C, LNCaP/T1, and LNCaP/T2 were selected for in vivo study, with $1 \times 10^8$ of each cell line inoculated s.c. in intact male nude mice. After castration, LNCaP/P and LNCaP/C tumor growth was inhibited for 4 weeks, after which LNCaP/P and LNCaP/C tumor volumes increased 2.5- and 2.3-fold, respectively by 9 weeks after castration. In contrast, LNCaP/T1 and LNCaP/T2 tumor volumes continued to grow after castration, increasing 4.5- and 4.7-fold, respectively by 9 weeks after castration (Fig. 3B). Serum PSA in mice bearing LNCaP/P and LNCaP/C tumors decreased by 64% and 71%, respectively by 1 week after castration and increased from 4 to 9 weeks after castration by 2.1- and 2.3-fold, respectively. In comparison, serum PSA in mice bearing LNCaP/T1 and LNCaP/T2 tumors decreased by 40% and 54%, respectively before increasing to 3.9- and 4.1-fold, respectively by 9 weeks (Fig. 3C) beginning 2 weeks after castration.

Antisense ODN-mediated Inhibition of TRPM-2 Expression in Shionogi Tumor Cells. Antisense TRPM-2 ODNs corresponding to the mouse TRPM-2 translation initiation site and mismatch control ODNs containing two base changes were used in this study. The effect of treatment with antisense TRPM-2 ODNs on TRPM-2 mRNA expression in Shionogi tumor cells was evaluated by Northern blot analysis. As shown in Fig. 4, daily treatment of Shionogi tumor cells with antisense TRPM-2 ODNs (50, 100, 500 or 1000 nM) for 2 days reduced TRPM-2 mRNA levels by 2, 15, 81, or 98%, respectively. In contrast, TRPM-2 mRNA levels were unchanged by the two-base mismatch control ODN treatment (Fig. 4C).

To determine whether decreases in TRPM-2 mRNA levels induced by antisense ODNs were accompanied by corresponding decreases in protein levels, Western blot analysis was used to measure changes in TRPM-2 protein levels in Shionogi tumor cells after daily treatment with antisense TRPM-2 ODNs for 4 consecutive days. Substantial inhibition of both unprocessed (60-kDa) and mature (40-kDa) forms of TRPM-2 protein were observed with 1000-nM antisense TRPM-2 ODNs, but not with mismatch control ODN treatment (Fig. 4C).

Inhibition of AI Progression in the Shionogi Tumor Model by Antisense TRPM-2 ODN Treatment. Male mice bearing Shionogi tumors 1–2 cm in diameter were castrated and randomly selected for...
treatment with antisense TRPM-2 versus mismatch control ODNs. Mean pretreatment tumor volume was similar in both groups. Beginning the day of castration, 12.5 mg/kg of ODNs were administered once daily by i.p. injection for 15 days. As shown in Fig. 5, Shionogi tumors regressed faster, and complete regression occurred earlier in mice treated with the antisense TRPM-2 ODN compared to those treated with mismatch control ODNs. Furthermore, antisense TRPM-2 ODN treatment significantly delayed the recurrence of AI tumors compared to mismatch control ODN treatment. After an observation period of 50 days postcastration, the mean tumor volume in the mismatch-treated control group was six times that of the antisense-treated group. AI tumors recurred in five of seven mice after a median of 46 days in the antisense TRPM-2 ODN treatment group, whereas AI tumors recurred in all mice after a median of 35 days in the mismatch ODN treatment group. No side effects, including weight loss, gait disturbances, or anorexia were observed with antisense TRPM-2 or mismatch ODN treatment over the 50-day observation period.

Fig. 3. Effect of TRPM-2 overexpression on LNCaP tumor growth and serum PSA in nude mice after castration. a, total RNA was extracted from PC3 (positive control for the screening of TRPM-2 mRNA expression), LNCaP/P, LNCaP/C, and four clones of TRPM-2 transfectants (LNCaP/T1 to LNCaP/T4) and analyzed for TRPM-2 and G3PDH levels by Northern blotting. b, male nude mice received injections s.c. of each LNCaP subline, and after castration, tumor volume was measured once weekly. Each point represents the mean tumor volume in each experimental group containing six mice with SD. c, blood samples for the measurement of serum PSA levels were obtained with the tail vein of the mice after castration once weekly. Serum PSA levels were determined by an enzymatic immunoassay kit according to the manufacturer’s control (Abbott IMX, Montreal, Canada). Each point represents the mean PSA level in each experimental group containing six mice with SD.

Fig. 4. Sequence-specific inhibition of TRPM-2 expression by the antisense TRPM-2 ODN in Shionogi tumor cells. a, Shionogi tumor cells were treated daily with various concentrations of the antisense TRPM-2 ODN or a two-base TRPM-2 mismatch ODN as a control for 2 days. poly(A)1 RNA was extracted from culture cells, and TRPM-2 and G3PDH levels were analyzed by Northern blotting. No Tx, untreated cells. b, quantitative analysis of TRPM-2 mRNA levels after normalization to G3PDH mRNA levels in Shionogi tumor cells after treatment with various concentrations of antisense TRPM-2 or mismatch control ODNs was performed by using laser densitometer. Each point represents the mean of triplicate analyses with SD. c, Shionogi tumor cells were treated with various concentrations of antisense TRPM-2 or mismatch control ODN once daily for 4 days, protein was extracted from culture cells, and TRPM-2 and β-tubulin protein levels were analyzed by Western blotting. Unprocessed form of TRPM-2, size = 60 kDa; mature form of TRPM-2, size = 40 kDa. No Tx, untreated cells.
We then examined the effects of in vivo ODN treatment on TRPM-2 mRNA expression in Shionogi tumors by Northern blotting. In this experiment, beginning the day of castration, each of three tumor-bearing mice were administered 12.5 mg/kg of antisense TRPM-2 or mismatch control ODNs i.p. once daily, and tumor tissues were harvested for RNA extraction 3 days after castration. Antisense TRPM-2 ODN treatment resulted in a 70% reduction in TRPM-2 mRNA levels in Shionogi tumors compared to mismatch control ODN-treated tumors (Fig. 6).

To determine whether more rapid regression of antisense ODN-treated tumors resulted from an earlier onset of castration-induced apoptosis, Western blotting of tumor tissues was used to evaluate the cleavage of the PARP protein, a substrate of the caspase activated during the final process of apoptotic execution (24). Proteins were extracted 3 days postcastration from each of three Shionogi tumors in mice administered antisense TRPM-2 or mismatch control ODNs under the same treatment schedule described above. The 116-kDa intact form of PARP was observed in both antisense TRPM-2 ODN-treated and mismatch control ODN-treated Shionogi tumors, whereas the 85-kDa PARP cleavage fragment was clearly detectable only in antisense TRPM-2 ODN-treated Shionogi tumors (Fig. 7).

Changes in TRPM-2 mRNA levels in various normal mouse organs after antisense ODN treatment were also evaluated. The Shionogi tumors, spleen, kidney, brain, and prostate were harvested 3 days postcastration for RNA extraction from mice administered antisense TRPM-2 or mismatch control ODNs under the same treatment schedule described above. Antisense TRPM-2 had no effect on TRPM-2 expression levels in the spleen, kidney, and brain, whereas TRPM-2 mRNA levels in the AD Shionogi tumor and mouse prostate tissues were reduced after antisense TRPM-2 ODN treatment compared to mismatch control ODN treatment (Fig. 8).

**DISCUSSION**

TRPM-2 was initially considered an androgen-repressed gene associated with the regressing rat prostate gland after castration and regarded as a marker and putative mediator of apoptosis (4, 5). In more recent studies, however, TRPM-2 up-regulation was dissociated from apoptosis (10–12, 14, 15, 25). For example, TRPM-2 expression was not associated with increased apoptotic activity in sex-hormone-
induced prostatic dysplasia in the Noble rat (10). Similarly, TRPM-2 expression was enhanced in the absence of increased apoptotic activity and reduced androgen stimulation in rat prostate cancer and ventral prostate tissues (25). The overexpression of TRPM-2 in LNCaP prostate cancer cells enhances the resistance to apoptosis induced by tumor necrosis factor α (14). Collectively, these conflicting data do not clearly define the functional role of TRPM-2 associated with apoptosis induced by various types of stimuli.

TRPM-2 mRNA is highly up-regulated in Shionogi tumors after castration and in AI recurrent tumors. These changes in TRPM-2 are similar to previously reported findings in the rat prostate (5) and the human prostate cancer xenograft (9). To differentiate between androgen-repressed versus apoptosis-associated up-regulation, CCBs were administered before castration to prevent castration-induced apoptosis because an early event in apoptotic cascade involves increases in the intracellular-free calcium concentration, with subsequent endonuclease activation (26, 27). Castration-induced apoptosis and up-regulation of TRPM-2 was prevented by CCB treatment, thereby supporting TRPM-2 as an apoptosis-related rather than androgen-repressed gene. To determine whether TRPM-2 up-regulation after androgen ablation helps mediate the progression to androgen independence, we generated TRPM-2 overexpressing LNCaP prostate cancer cell lines. Although tumor take rates and growth in intact male mice were similar between parental and overexpressing TRPM-2 tumors, after castration, tumor growth and serum PSA increased severalfold faster in TRPM-2-transfected tumors. These findings provide the first clear evidence that increased TRPM-2 results in the acquisition of androgen resistance and accelerates time to AI progression.

The up-regulation of TRPM-2 in Shionogi tumors after castration and the acquisition of androgen resistance with TRPM-2 overexpression suggests that preventing TRPM-2 up-regulation precipitated by androgen ablation may enhance castration-induced apoptosis and delay the AI progression of prostate cancer. Antisense ODN, a chemically modified stretch of single-stranded DNA that is complementary to mRNA regions of a target gene and thereby effectively inhibits gene expression by forming RNA/DNA duplexes (28), offers one strategy to specifically target TRPM-2 gene expression. Phosphorothioate ODNs are water soluble, stable agents manufactured to resist nuclease digestion. After parenteral administration, phosphorothioate ODNs become associated with high capacity, low affinity serum binding proteins (29). In this study, the phosphorothioate antisense TRPM-2 ODN corresponding to the mouse TRPM-2 translation initiation site reduced TRPM-2 expression levels in a sequence-specific and dose-dependent manner. In vivo administration of antisense TRPM-2 ODN accelerated castration-induced tumor regression and delayed the time to AI progression compared to that of mismatch control ODNs. Consistent with the in vitro data, in vivo treatment with the antisense TRPM-2 ODN also reduced TRPM-2 mRNA levels. The earlier detection of PARP cleavage fragments in Shionogi tumors after antisense TRPM-2 ODN treatment suggests that the inhibition of TRPM-2 up-regulation after castration results in an earlier induction of castration-induced apoptosis.

Because TRPM-2 plays a critical role in some normal organs, including the brain, kidney, spleen, and prostate (4, 30, 31), the effects of antisense TRPM-2 ODNs on TRPM-2 expression levels in these organs were also examined. Although antisense ODNs significantly reduced TRPM-2 levels in the AD prostate and tumor tissues undergoing castration-induced apoptosis, TRPM-2 expression was not altered by antisense ODNs in the other organs examined. We reported similar differential decreases in Bcl-2 levels after treatment with mouse antisense Bcl-2 ODNs in Shionogi tumors (32). We speculate that tissues undergoing apoptosis may be more sensitive to antisense TRPM-2 ODN treatment relative to intact organs because of the preferential uptake of ODNs in these tissues for reasons of biodistribution or increased membrane permeability. However, reduced target mRNA levels in mouse tissues have been reported by Monia et al. (33) after i.v. administration of antisense C-ras ODNs, although no significant toxicity was observed. A phase I dose-escalation trial using antisense Bcl-2 ODNs in nine patients with lymphoma reported tumor responses with no significant toxicity (34). These findings suggest that therapeutic doses of systemic phosphorothioate antisense ODNs targeted against important cellular proteins do not cause significant toxicity in normal tissues.

Integration and appropriate timing of combination therapies, based on biological mechanism of progression and castration-induced changes in gene expression, may provide means to delay AI progression in a major way (32, 35). New nonhormonal therapies for prostate cancer have traditionally been evaluated in patients with hormone refractory disease, and when used in this end-stage setting, none has demonstrated improved survival (2). A more rational strategy to delay emergence of the AI phenotype would initiate treatment earlier to enhance castration-induced apoptosis by targeting the adaptive changes in gene expression precipitated by androgen withdrawal. The present study provides evidence for a functional role of TRPM-2 in androgen resistance and AI progression, and it demonstrates that reduction of TRPM-2 gene expression by antisense TRPM-2 ODNs can delay progression to androgen independence.

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