

Expression of TMPSS22, Androgen Receptor and its Co-Activators are Increased Following Suppression of Bcl-2 by Antisense Oligonucleotides: But the TMPSS22 Fusion Protein Partner ERG is Not Detectable in this Compensatory Response.

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Abstract

Employing both *in vivo* and *in vitro* prostate cancer models, antisense oligonucleotides (oligos) have targeted and suppressed regulatory proteins associated with tumor growth, proliferation and apoptosis. In efforts to identify compensatory changes in the expression of non-targeted genes this study evaluated mono- and bispecific oligos capable of targeting and equally suppressing the expression of bcl-2 (an apoptosis inhibitor). Previous studies have shown that oligo treated LNCaP cells compensate for diminished bcl-2 by suppressing caspase-3 (an apoptosis promoter) while enhancing the expression of AKT-1 (another apoptosis inhibitor). In addition, we found enhanced expression of non-apoptosis related proteins including the androgen receptor (AR), its p300 and IL-6 co-activators, (but not 5-alpha reductase), polymerase transcription mediator MED-12 and growth regulating signal transducer STAT-3. These enhancements culminated in increased expression of the proliferation marker KI-67 and mitosis checkpoint regulator cyclin D1. This suggests that therapeutic approaches to restore apoptosis through suppression of bcl-2 lead to altered protein expression of non-targeted genes not only involving apoptosis, but also androgen sensitivity and transcription, resulting in increased proliferation, altered regulation of mitosis, and a pattern of gene expression associated with more aggressive tumors.

To further evaluate adaptive compensatory mechanisms related to androgen sensitivity we now evaluate the level of expressed TMPSS22 and ERG, and their contribution to tumor aggressiveness. TMPSS22 is associated with prostatic carcinogenesis and its expression is enhanced by the over expression of ERG. Fusion proteins formed from TMPSS22 and ERG is found in 40-80% of human prostate tumors, and ERG over expression is thought to disrupt AR signaling contributing to the development of androgen independent tumors. Results indicate significant enhancement of TMPSS22 along with AR and its co-activators p300 and IL-6, following treatment, while ERG expression was either suppressed or not detectable.

Introduction

Gene therapy is in theory specific, but encounters difficulties when applied. Although suitable targets are found in many pathways, and while tumors express altered patterns of gene activity, the actual transcription of most growth regulating genes is not much different from normal. Resistance develops because the biochemical pathways involved are complex, frequently redundant and regulated to varying degrees by combinations of both stimulatory and inhibitory factors; particularly in the process of apoptosis. We previously reported that non-targeted genes are directly affected by, what was believed to be; specific therapy and that some are susceptible to altered expression to an extent having the potential to reverse the effects of the originally intended treatment. This process of compensation [1] may drive tumors to increased aggression, and in this prostate model (an androgen sensitive LNCaP line) tumor cells appear to become increasingly more sensitive (than usual) to androgen. Just as bacteria and viruses mutate to evade antibiotic and antiviral agents, tumor cells are under similar selective pressure to evade therapy, whether chemically induced (chemotherapy) or that based on suppression of gene translation (including antisense oligonucleotide [oligo] mediated gene therapy). Studies of this kind are increasingly relevant since oligo based therapy is entering the clinical environment, and is particularly directed against apoptosis regulating proteins like bcl-2 and clusterin. In the current state of the art, unintended compensatory mechanisms and their consequences are poorly understood. These compensatory mechanisms involve many aspects of cell growth (and death) including not only apoptosis involving both mitochondrial and signal transducing pathways, but androgen sensitivity and transcription patterns, resulting in increased proliferation, altered mitosis, and a pattern of gene expression associated with more aggressive tumors. Such a broad range of unintended changes in gene expression could contribute to the emergence and selection of more aggressive cells and compromise treatment efficacy.

Gene therapy has been clinically employed for the treatment of human prostate tumors and together with radio- [2, 3] or chemotherapy [4] antisense oligos have been administered against inhibitors of apoptosis (bcl-2 and clusterin) in attempts to increase this (often tumor suppressed and desirable) activity. In addition to oligo mediated approaches, the apoptotic process is also being targeted by Genentech using a bioavailable small molecule (venetoclax [GDC-0199/ABT-199]) which is currently in Phase I and II clinical trials for the treatment of a variety of tumor types.

In previous studies we found that LNCaP cells treated with antisense oligos directed against bcl-2 (administered in a nanoparticle suspension of lipofectin) compensated by suppressing caspase-3 [1] (an apoptosis promoter), and enhancing AKT-1 (an apoptosis inhibitor) [5], androgen receptor [6] (AR), and AR co-activators p300

[7] and IL-6 expression [8]. In addition, PD-1, its ligand PD-L1 (immune checkpoint blockage markers) and fas-ligand, which activate apoptosis through signal transduction, were also enhanced [9], as were suppressor protein p53 [10], oncogene v-myc [11], polymerase transcription mediator MED-12 [12] and signal transducer STAT-3 [5, 12]. These changed patterns of expression culminated in greater expression of KI-67, a marker exclusively associated with proliferation, expressed throughout all phases of cell division (G₁, S, G₂, and mitosis) but absent while cells rest (G₀), and whose expression is considered a prognostic indicator for some tumors (breast). Cyclin D1 expression is similarly increased but not to the large extent of KI-67 [13]. This suggests that (at least in this LNCaP model), therapeutic approaches to restore apoptosis (including the use of antisense oligos to suppress bcl-2) can lead to altered expression of non-targeted genes and regulatory changes not only involving apoptosis, but also androgen sensitivity, (suppressor/oncogene) transcriptional activity and immune responsiveness. As noted, we demonstrated that cell surface proteins which regulate immune checkpoint blockade (PD-1 and PD-L1 and fas-ligand) and activate apoptosis via signal transduction were also enhanced [9], presumable leading to further inhibition of T-cell activity. Compensatory effects identified with these proteins are important since the PD-1/PD-L1 pathway is now recognized as a target for monoclonal antibody directed immunotherapy used to treat various solid tumors, particularly melanoma and lung tumors.

We employed RT-PCR in previous and current experiments to determine alterations in gene expression associated with androgen sensitivity, specifically evaluating expression of the androgen receptor (AR) its co-activators (p300 and IL-6), the enzyme 5-alpha reductase (5AR), associated with conversion of testosterone to dihydrotestosterone, an androgen know to fuel the progression of androgen sensitive prostate cancer cells, like LNCaP. Although more sophisticated techniques are available we find this method both sensitive enough to identify those genes involved with compensation, and able to identify non-targeted genes which are particularly affected and could provide combination targets for bcl-2 suppressive therapy.

To further evaluate adaptive compensatory mechanisms related to androgen sensitivity we now evaluate the level of expressed TMPSS22 (transmembrane protease, serine 2) and ERG (human v-ets erythroblastosis virus E26 oncogene homologue), and their contribution to tumor aggressiveness. TMPSS22 is associated with prostatic carcinogenesis and its expression is enhanced by over expression of ERG. It is up regulated by androgen in prostate cancer cells and down regulated when tissue becomes androgen insensitive, which makes it of interest in this model, where androgen sensitivity seems to increase following oligo suppression of bcl-2.

The TMPSS22-ERG gene fusion is found in 40-80% of human prostate tumors, and ERG over expression is thought to disrupt AR signaling contributing to the development of androgen independent tumors. Targeting such fusion proteins by antisense oligos could be therapeutic in prostate cancer patients as well, by providing a more selective target than traditional chemotherapy, as in the bcr-abl (Philadelphia chromosome) target in chronic myelogenous leukemia.

Methods

Oligonucleotides

Oligos (mono- or bispecific) were purchased from Eurofins MWG Operon (Huntsville, AL). Each was phosphorothioated on three terminal bases at 5' and 3' positions. Stock solutions were made to a final concentration of 625 μ M in sterile Dulbecco PBS.

Base Sequences

Each oligo contained at least one **CAT** sequence and targeted the area adjacent to the mRNA AUG initiation codon for the respective targeted protein (EGFR or bcl-2).

MR₄ (monospecific targeting bcl-2) T-C-T-C-C-C-A-G-C-G-T-G-C-G-C-C-A-T

MR₂₄ (bispecific targeting EGFR/bcl-2) G-A-G-G-G-T-C-G-C-A-T-C-G-C-T-G-C-T-C-T-C-T-C-C-A-G-C-G-T-G-C-G-C-C-A-T

MR₄₂ (bispecific targeting bcl-2/EGFR) T-C-T-C-C-C-A-G-C-G-T-G-C-G-C-C-A-T-G-A-G-G-G-T-C-G-C-A-T-C-G-C-T-G-C-T-C

Cell Culture

LNCaP cells were grown in RPMI 1640 supplemented with 10% bovine serum, 1% L-glutamine and 1% penicillin/streptomycin in a 5% CO₂ incubator. Log phase cells were harvested using EDTA/trypsin and equally distributed into 75 cm² flasks (Corning, NY). At intervals media were either supplemented or replaced with fresh.

Oligo Treatment Prior to PCR

Fours days prior to oligo addition, when cell density approached 75% confluence, 10 ml of fresh media was added. Cells were incubated for an additional 3 days before 5 ml of media was replaced with fresh the day before oligos were added. 100 μ l of stock oligos were added to bring the final concentration to 6.25 μ M. Incubation proceeded for an additional 24 hours in the presence or absence of monospecific MR₄, or the MR₂₄ and MR₄₂ bispecifics in a nanoparticle suspension with lipofectin.

RNA Extraction

Following treatment, media was removed, a single ml of cold (4°C) RNAzol B was added to each 75 cm² culture flask and the monolayer lysed by repeated passage through a pipette. All procedures were performed at 4°C. The lysate was removed, placed in a centrifuge tube to which 0.2 ml of chloroform was added, and shaken. The mixture stayed on ice for 5 min, was spun at 12,000 g for 15 min, and the upper aqueous volume removed and placed in a fresh tube. An equal volume of isopropanol was added, the tube shaken, and allowed to stay at 4°C for 15 min before similar centrifugation to pellet the RNA. The supernatant was removed, the pellet washed in a single ml of 75% ethanol, then spun for 8 min at 7500 g. The ethanol was pipetted off and the formed pellet air dried at -20°C.

RNA Quantitation

RNA was resuspended in 250 μ l of DEPC treated H₂O, and quantitated using a Qubit fluorimeter and Quant-iT RNA assay kit (Invitrogen). DEPC is an inhibitor of RNase activity.

RT-PCR

Extracted RNA was diluted in DEPC treated water to 40 µg/µl. 1-4 µl of this RNA was added to 1 µl of both sense and antisense primers (forward and reverse sequences) for actin, bcl-2, AR, p300, IL-6 and 5AR. From a kit purchased from Invitrogen the following reactants were added for RT-PCR: 25 µl of 2X reaction mixture, 2 µl SuperScript III RT / platinum *Taq* mix, tracking dye, and 3 µl MgSO₄ (of a 5mM stock concentration). DEPC treated water was added to yield a final volume of 50 µl. RT-PCR was performed for 2 X 25 cycles using the F54 program in a

Sprint PCR Thermocycler. As a control for RT-PCR product production, human actin expression was tested in RNA extracted from HeLa cells which was provided in a kit purchased from Invitrogen (in the reaction mixture, no MgSO₄ was included, the difference compensated for by 3 µl of DEPC treated water).

Primers: Primer sequences were obtained from the National Center Biotechnology Information (NCBI) website: http://www.ncbi.nlm.nih.gov/nuccore/NM_031966

They were designed to amplify 100-300 bp fragments and anneal between 58-60°C.

Actin

Forward primer sequence: 5' CAA ACA TGA TCT GGG TCA TCT TCT C 3'

Reverse primer sequence: 5' GCT CGT CGT CGA CAA CGG CTC

PCR product produced was 353 base pairs in length

Bcl-2

Forward primer sequence: 5' GAG ACA GCC AGG AGA AAT CA 3'

Reverse primer sequence: 5' CCT GTG GAT GAC TGA GTA CC 3'

PCR product produced was 127 base pairs in length.

Androgen Receptor (AR)

Forward primer sequence: 5' CGG AAG CTG AAG AAA CTT GG 3'

Reverse primer sequence: 5' ATG GCT TCC AGG ACA TTC AG 3'

PCR product produced was 155 base pairs in length.

p300

Forward primer sequence: 5' CGC TTT GTC TAC ACC TGC AA 3'

Reverse primer sequence: 5' TGC TGG TTG TTG CTC TCA TC 3'

PCR product produced was 167 base pairs in length.

IL-6

Forward primer sequence: 5' ATG CAA TAA CCA CCC CTG AC 3'

Reverse primer sequence: 5' GAG GTG CCC ATG CTA CAT TT 3'

PCR product produced was 167 base pairs in length.

5-alpha reductase (5AR)

Forward primer sequence: 5' ATG GCG CTT CTC TAT GGA CT 3'

Reverse primer sequence: 5' TGG CAA GAC ATA GCC AAA AG 3'

PCR product produced was 249 base pairs in length.

TMPRSS22

Forward primer sequence: 5' TGA CAA CCT GAT CAC ACC AG 3'

Reverse primer sequence: 5' TCA CAT TCC CGT ACA CTC CT 3'

PCR product produced was 185 base pairs in length.

ERG

Forward primer sequence: 5' AAC GAG CGC AGA GTT ATC GT 3'

Reverse primer sequence: 5' GTG AGC CTC TGG AAG TCG TC 3'

PCR product produced was 179 base pairs in length.

Detection and Quantitation of Product

Agarose Gel Electrophoresis

1.5% agarose gels were prepared in a 50 ml volume of TBE buffer (1X solution: 0.089 M Tris borate and 0.002M EDTA, pH 8.3), containing 3 µl of ethidium bromide in a Fisher Biotest electrophoresis system. Samples were run for 2 hours at a constant voltage of 70 using a BioRad 1000/500 power supply source. To locate the amplified PCR product, 3 µl of a molecular marker (Invitrogen) which contained a sequence of bases in 100 base pair increments (Invitrogen) as well as 2 µl of a sucrose based bromophenol blue tracking dye were run in each gel.

Quantitation

Gels were visualized under UV light and photographed using a Canon 800 digital camera. Photos were converted to black and white format and bands quantitated using Medical Image Processing and Visualization (Mipav) software provided by the National Institute of Health. Means and standard deviations were compared using Student *t*-tests to determine significance.

Results

Bcl-2 Expression

As a control (data not shown) for RT-PCR product production, human actin expression was tested in RNA extracted from HeLa cells [1].

LNCaP cells incubated for 24 hours in the presence of 6.25 µM of oligos suppressed Bcl-2 expression, and support the finding of comparable biologic activity in both mono- and bispecific oligos measured in the *in vitro* cell growth inhibition experiments [1]. When photographs of the identified product bands were scanned on agarose gels and quantitated using Mipav software, in a series of runs, the greatest expression of bcl-2 was always found in untreated LNCaP cells. Those treated with oligos, whether mono- or bispecific, produced bands which indicated obvious (to the naked eye) suppression. For each oligo evaluated, the greatest amount of suppression measured approached 100% for the mono-specific MR₄; and for the bispecifics MR₂₄ and MR₄₂, 86% and 100%, respectively. Suppression was found in both repeat PCR runs with bcl-2 primers, as well as in repetitive agarose gel quantifications [14] (Figure 1).

Figure 1: Bcl-2 Expression is suppressed by Oligos as Indicated in a Representative Agarose Gel

200 base pairs



100 base pairs

Untreated Treated Treated Treated
 MR₄ MR₂₄ MR₄₂

bcl-2 is a 127 base pair product

Androgen Receptor (AR) Expression

Comparable amounts of extracted RNA from LNCaP cells treated with either mono- or bispecific oligos directed against bcl-2 (and EGFR in the bispecifics) were then evaluated by RT-PCR using primers directed against AR. When background intensity was subtracted, the relative intensity of all bands corresponding to AR representing cells

treated with MR₄, MR₂₄ and MR₄₂ compared to controls were enhanced 31.2% ± 26.0 (P = 0.015), 58.5% ± 51.4 P = 0.019) and 53.1% ± 45.9 (P = 0.019). These results were pooled from both duplicate PCR runs and multiple gels (a total of six gels were evaluated), and indicate similar (significant) enhancement of AR activity is produced by each oligo type. A representative band is depicted in Figure 2.

Figure 2: Androgen Receptor Expression is enhanced by Oligos as Indicated in a Representative Agarose Gel



Untreated	Treated MR ₄	Treated MR ₂₄	Treated MR ₄₂
_____Percent Enhancement_____			
	31.2±26.0	58.5±51.4	53.1±45.9
	P = 0.015	0.019	0.019

Androgen Receptor is a 155 base pair product

p300 Expression

Comparable amounts of extracted RNA from LNCaP cells treated with either mono- or bispecific oligos directed against bcl-2 (and EGFR in the bispecifics) were then evaluated by RT-PCR using primers directed against p300. When background intensity was subtracted, the relative intensity of all bands corresponding to p300

representing cells treated with MR₄, MR₂₄ and MR₄₂ compared to controls were increased 82.9% ± 51.9 (P = 0.006), 93.0% ± 87.3 P = 0.044) and 105.4% ± 65.9 (P = 0.007). These results were pooled from both duplicate PCR runs and multiple gels (a total of six gels were evaluated), and indicate similar (significant) enhancement of p300 activity is produced by each oligo type. A representative band is depicted in Figure 3.

Figure 3: p300 Expression is enhanced by Oligos as Indicated in a Representative Agarose Gel



Untreated	Treated MR ₄	Treated MR ₂₄	Treated MR ₄₂
_____Percent Enhancement_____			
	82.9±5.9	93.0±87.3	105.4±65.9
	P = 0.006	0.044	0.007

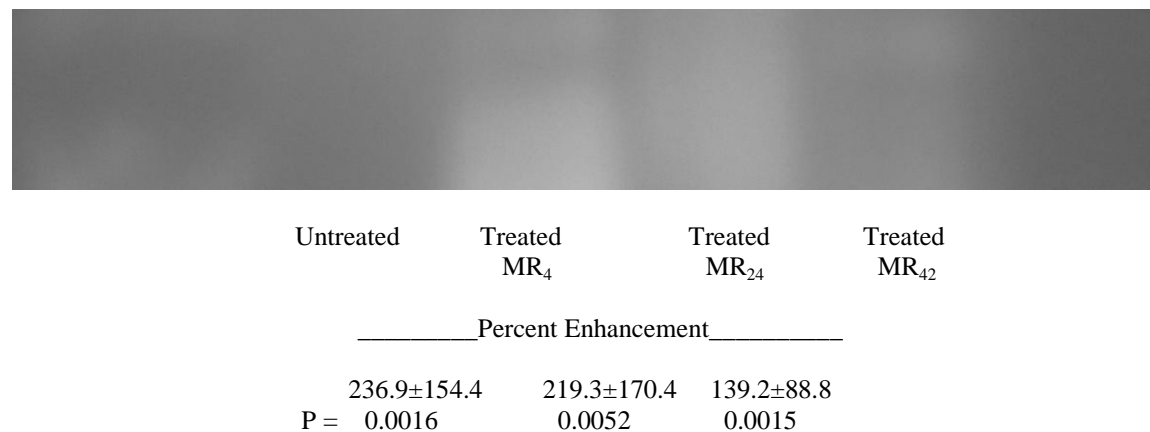
p300 is a 167 base pair product

IL-6 Expression

Comparable amounts of extracted RNA from LNCaP cells treated with either mono- or bispecific oligos directed against bcl-2 (and EGFR in the bispecifics) were then evaluated by RT-PCR using primers directed against IL-6. When background intensity was subtracted, the relative intensity of all bands corresponding to IL-6

representing cells treated with MR₄, MR₂₄ and MR₄₂ compared to controls were increased 236.9% ± 154.4 (P = 0.001585), 219.3% ± 170.4 P = 0.005231) and 139.2% ± 88.8 (P = 0.001537). These results were pooled from both duplicate PCR runs and multiple gels (a total of seven gels were evaluated), and indicate similar (significant) enhancement of p300 activity is produced by each oligo type. A representative band is depicted in Figure 4.

Figure 4: IL-6 Expression is enhanced by Oligos as Indicated in a Representative Agarose Gel



IL-6 is a 167 base pair product

5-alpha Reductase Expression

Comparable amounts of extracted RNA from LNCaP cells treated with either mono- or bispecific oligos directed against bcl-2 (and EGFR in the bispecifics) were then evaluated by RT-PCR using primers directed against 5-AR. When background intensity was subtracted, the relative intensity of all bands corresponding to 5-AR representing

cells treated with MR₄, MR₂₄ and MR₄₂ compared to controls were within 14.2% ± 13.9, 29.3% ± 28.0 and -8.9% ± 28.9 respectively from the untreated controls. None of these changes are statistically significant and we conclude that 5-AR activity does not contribute to the increased androgen sensitivity seen in treated LNCaP cells. A representative band is depicted in Figure 5.

Figure 5: 5-alpha Reductase (5-AR) Expression is unchanged by Oligos as Indicated in a Representative Agarose Gel



5-AR is a 249 base pair product

TMPRSS22

Comparable amounts of extracted RNA from LNCaP cells treated with either mono- or bispecific oligos directed against bcl-2 (and EGFR in the bispecifics) were then evaluated by RT-PCR using primers directed against

TMPRSS22. When background intensity was subtracted, the relative intensity of all bands corresponding to TMPRSS22 representing cells treated with MR₄, MR₂₄ and MR₄₂ compared to controls were increased 29.8% ± 21.2 (P = 0.002939), 19.0% ± 66.2 P = 0.022816) and 45.4% ± 52.1 (P = 0.039764). A representative band is depicted in Figure 6.

Figure 6: TMPRSS22 Expression is enhanced by Oligos as Indicated in a Representative Agarose Gel



Untreated	Treated MR ₄	Treated MR ₂₄	Treated MR ₄₂
<u>Percent Enhancement</u>			
29.8±21.2	19.0±66.2	45.4±52.1	
P = 0.002939	0.022816	0.039764	

TMPRSS22 is a 185 base pair product

ERG

Comparable amounts of extracted RNA from LNCaP cells treated with either mono- or bispecific oligos directed against bcl-2 (and EGFR in the bispecifics) were then evaluated by RT-PCR using primers directed against ERG. In contrast to TMPRSS22, no PCR product was detected.

Discussion

Gene therapy is often promoted as a highly specific and deliverable treatment to control aberrant gene expression by tumor cells; particularly when growth factors, their receptors or apoptosis inhibitors are excessively produced and therefore subject to knockdown therapy including translational inhibition initiated by antisense oligos. However, it's now apparent that it's not as specific as previously thought. Antisense oligos consist of nucleotide bases synthesized complimentary in sequence to mRNA. When hybridized to mRNA, they produce a translational arrest of the targeted gene's mRNA expression. Now in clinical trials against a variety of solid tumors, this method is an effective, relatively non-toxic and inexpensive form of therapy and various types of antisense RNA have been constructed for this purpose, including first generation phosphorothioated oligos used in these evaluations and later formulations like 2'-MOE-RNA, morpholinos, siRNA, miRNA etc. Such types often include modifications to the

oligo backbone and base structures are used to prevent nuclease degradation, increase systemic half-life or enhance distribution and delivery. Some of these derivatives have been evaluated clinically, but all are directed against single gene transcriptional (mRNA) products. In contrast, the oligos discussed in this paper included both mono- and bispecific forms, each having a base sequence complementary to and directed against mRNA encoding the apoptosis inhibitor bcl-2, (bispecifics included an additional site directed against epidermal growth factor receptor [EGFR]). We evaluate bispecific oligos because it would be naïve to believe targeting a single mRNA would be sufficient to produce a clinical response in most tumors, and activity at one site does not affect binding at a second [16], therefore administration of a single oligo having two mRNA targets could have an additional suppressive benefit. Furthermore, we have shown that mono- and bispecific oligos have comparable activity suppressing bcl-2 [14].

While it's understandable that genes which share sequence homology would also be susceptible to antisense oligos, when directed at common sequences, what is not expected are the effects on non-targeted genes, many of which control additional growth regulating pathways. We have also shown that certain complementary stretches of base sequences within the oligo could also produce unanticipated effects on the expression of cell surface antigens (and differentiation proteins). In an early evaluation of bispecifics we reported the enhanced expression of prostate specific membrane antigen (PSMA) [15] when oligos were directed against bcl-2.

The unique capacity to produce such changes by these bispecifics (and not a similarly directed monospecific) is attributable to an unusual double strand conformation present in bispecifics and interferon induction (an enhancer of surface antigen expression) [16]. Such expression could enable more specific recognition and targeting by cytotoxic T cells [16].

Tumors are a mass of genetically unstable heterogeneous cells capable of both rapid mutation and selection. Just as bacterial and viral agents develop resistance to chemotherapeutics, tumor cells have a similar capability. In prostate cancer it's thought that hundreds of genes (including those listed here) drive tumor cells to grow, in addition to the effects of androgen acting through the AR (as a transcriptional factor), AR coactivating proteins (p300, IL4, IL6), regulators of apoptosis (bcl-2, clusterin, AKT-1), transcriptional factors (MED-12, STAT-3) and various autocrine loops (involving transforming growth factor-alpha [TGF- α], its EGFR binding site, insulin like growth factor [IGF1] and its receptor [IGF-1R]).

Initial evaluation of protein expression associated with compensation regulating the traditional mode of apoptosis (mitochondrial mediated) focused on the bcl-2, bax, bad, clusterin etc. However, more recent work evaluated proteins associated with tumor cell destruction, via apoptosis, mediated by a secondary route for activation, involving direct signal transduction. This is a process of initiating apoptosis through the binding of activating proteins (ligands) to cell surface receptors. When ligands bind to these receptors they activate a destructive cascade of protein interactions which lead to cell death. These receptors are structurally similar to the tumor necrosis factor receptor (CD95) and also regulate the immune system's cytotoxic T cell response. As mediators of the immune system their expression can have unanticipated effects on certain types of therapy since these proteins, particularly PD-1 and PD-L1, are now being targeted by monoclonal antibodies to treat cancer patients via immune checkpoint blockade, particularly those with melanoma, mesothelioma and lung cancer [17]. Our studies found that PD-1 its ligand PD-L1 and fas-ligand, were all significantly enhanced following bcl-2 suppression and therefore we now recognize immune regulation, as an additional pathway for compensatory based resistance, when apoptosis is targeted [9].

Oligos (produced by Oncogenex Pharmaceuticals) have reached clinical trials for the treatment of prostate cancer (OGX-011), while others remain in preclinical development (OGX-225). Often administered in combination with traditional chemotherapy, these oligos target bcl-2, clusterin (OGX-011 in Phase II testing), heat shock protein 27 (OGX-427) or insulin growth factor

binding proteins (OGX-225) [20]. Genta conducted a phase 3 test using oligos (Genasense; oblimersen) directed against bcl-2 for treating melanoma, chronic lymphocytic leukemia and various solid tumors [18], but compensatory effects produced by this agent were not reported. Many represent efforts to restore tumor apoptosis by eliminating suppressive bcl-2 [2-4] associated with treatment resistance. Similar approaches are directed at clusterin, but compensatory mechanisms activated by these oligos have not been evaluated. Since derivatives of antisense oligos (siRNA, miRNA) continue to be developed and tested, and while some directed against bcl-2 are in clinical trials, it is important to identify compensatory changes that result.

These experiments evaluated compensatory alterations in gene expression involving TMPSS22 and ERG. TMPSS22 expression is associated with prostatic carcinogenesis and enhanced by over expression of ERG. The TMPSS22-ERG gene fusion is found in 40-80% of human prostate tumors, and ERG over expression is thought to disrupt AR signaling contributing to the development of androgen independent tumors. Targeting such fusion proteins by antisense oligos could be therapeutic in prostate cancer patients as well, by providing a more selective target than traditional chemotherapy, as in the bcr-abl (Philadelphia chromosome) target in chronic myelogenous leukemia. Such targeting of tumor specific fusion proteins would make antisense approaches even more efficacious and should be evaluated in a clinical setting for better treatment of prostate cancer patients.

This year (2016) the American Cancer Society (ACS) estimated that in spite of early detection, screening for prostate specific antigen (PSA) and effective treatments for localized disease, in the United States there will be 26,120 deaths from prostate cancer with 180,890 newly diagnosed cases [19]. New types of treatment, possibly targeting prostate cancer associated fusion proteins using oligo mediated translational inhibition, must be developed and employed (probably in combination with traditional androgen ablation).

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