Clusterin Is a Critical Downstream Mediator of Stress-Induced YB-1 Transactivation in Prostate Cancer

Masaki Shiota¹, Amina Zoubeidi³, Masafumi Kumano¹, Eliana Beraldi¹, Seiji Naito³, Colleen C. Nelson¹,⁴, Poul H.B. Sorensen², and Martin E. Gleave¹

Abstract

Clusterin is a stress-activated, cytoprotective chaperone that confers broad-spectrum treatment resistance in cancer. However, the molecular mechanisms mediating CLU transcription following anticancer treatment stress remain incompletely defined. We report that Y-box binding protein-1 (YB-1) directly binds to CLU promoter regions to transcriptionally regulate clusterin expression. In response to endoplasmic reticulum stress inducers, including paclitaxel, YB-1 is translocated to the nucleus to transactivate clusterin. Furthermore, higher levels of activated YB-1 and clusterin are seen in taxane-resistant, compared with parental, prostate cancer cells. Knockdown of either YB-1 or clusterin sensitized prostate cancer cells to paclitaxel, whereas their overexpression increased resistance to taxane. Clusterin overexpression rescued cells from increased paclitaxel-induced apoptosis following YB-1 knockdown; in contrast, however, YB-1 overexpression did not rescue cells from increased paclitaxel-induced apoptosis following clusterin knockdown. Collectively, these data indicate that YB-1 transactivation of clusterin in response to stress is a critical mediator of paclitaxel resistance in prostate cancer. Mol Cancer Res; 1–12. ©2011 AACR.

Introduction

Prostate cancer is the most common noncutaneous cancer and the second leading cause of cancer-related death in men of Western countries. The incidence of prostate cancer is increasing as a result of aging populations and high-fat diet in the Western world (1). While advanced prostate cancer is initially sensitive to androgen deprivation therapy (ADT), most eventually recur in a castration-resistant prostate cancer (CRPC). At present, only second-line ADT with abiraterone (2), and the taxanes docetaxel (3), and cabazitaxel (4) have 4-month gains in survival before treatment resistance, progression, and death occurs. Many strategies used to kill cancer cells, including ADT or taxane chemotherapy, induce a treatment-resistant phenotype that is the underlying basis for most cancer deaths. Improved understanding of the molecular basis underlying metastasis and resistance to ADT or chemotherapy will facilitate to design new therapeutic strategies to inhibit the emergence of this CRPC phenotype.

CRPC progression is a complex process by which cells acquire the ability to both survive and proliferate in the absence of gonadal androgens and involves mechanisms attributed to reactivation of the androgen receptor (AR) axis (5), alternative growth factor pathways (6, 7), stress-induced prosurvival genes (8–10), and cytoprotective chaperone networks (11, 12). Clusterin is a stress-induced cytoprotective chaperone expressed in many human cancers. In prostate cancer, clusterin levels increase following castration and in CRPC models (11). Clusterin levels are low in untreated, favorable grade tissues but increase with higher Gleason grade (13) and within weeks after treatment stress with ADT (14) or taxane chemotherapy (15). Overexpression of clusterin in human prostate cancer LNCaP cells accelerates progression after ADT (11) or chemotherapy (16), identifying CLU as an antia apoptotic gene upregulated by treatment-related stress that confers therapeutic resistance when overexpressed. Miyake and colleagues first reported that antisense treatment against clusterin significantly enhanced paclitaxel sensitivity in human prostate cancer PC-3 cells (10). Moreover, clusterin knockdown reduced tumor growth and sensitized to chemotherapeutic agents in various cancer cells including renal cell cancer (17), bladder cancer (18), lung cancer (19), and breast cancer (20).

Y-box binding protein-1 (YB-1) is a stress-activated transcription factor implicated in and linked to unfavorable clinical outcome in various cancers (21). In prostate cancer, YB-1 is induced by ADT (22) and its overexpression confers...
castration resistance (23). Moreover, YB-1 is associated with drug resistance to both cisplatin (24, 25) and paclitaxel (26, 27). YB-1 confers paclitaxel resistance in PC-3 cells (27), whereas its knockdown sensitized breast cancer SUM149 cells to paclitaxel (28).

While the above findings functionally link YB-1 and clusterin to treatment resistance in prostate cancer, the precise mechanisms mediating these effects remain undefined. Here, we define functional links between stress-induced increases in YB-1 and clusterin and analyze their biological relevance, as related to taxane resistance in prostate cancer.

Materials and Methods

Cell culture and transfection

The human prostate cancer cell lines, PC-3 and DU145, were purchased from the American Type Culture Collection (2008 and 1989, ATCC authentication by isoenzymes analysis) and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM; Thermo Scientific) supplemented with 5% FBS. LNCaP cells were kindly provided by Dr. Leland W.K. Chung (1992, MD Anderson Cancer Center, Houston, TX), tested and authenticated by whole-genome and whole-transcriptome sequencing on Illumina Genome Analyzer Ix platform in 2009. LNCaP cells were maintained in RPMI-1640 (Thermo Scientific) supplemented with 5% FBS. Docetaxel-resistant derivatives of LNCaP and DU145 cells (LNCaP/DTX and DU145/DTX cells) were established by long-term culture under the appropriate medium containing gradually increasing concentrations of docetaxel and maintained under media containing 3 ng/mL and 2 ng/mL of docetaxel, respectively. The LNCaP/DTX and DU145/DTX cells were found to be about 2.5-fold more resistant to docetaxel than their parental cells. Cells were transfected with the indicated siRNA or the indicated plasmid as described previously (29).

Antibodies and reagents

Antibodies against ATF4 (sc-200), Myc (9E10, sc-815), GRP78 (sc-1051), and clusterin (sc-6419) were purchased from Cell Signaling Technology. Anti-phosphorylated YB-1 (p-YB-1, #2900), anti-cleaved PARP (#9541), anti-PARP (#9542), and anti-cleaved caspase-3 (#9661) antibodies were purchased from Cell Signaling Technology. Anti-YB-1, anti-caspase-3, and anti-β-actin antibodies were purchased from Epitomics, BD Biosciences, and Sigma, respectively. Paclitaxel and docetaxel were obtained from Sigma and R&D Systems, respectively. Paclitaxel and docetaxel were obtained from Sigma and R&D Systems, respectively.

Plasmids

The pCMV-Tag2B/YB-1 plasmid expressing the N-terminal, myc-tagged YB-1 protein, as well as the pCMV/YB-1-myc-nuc and pCMV/YB-1-myc-cyt plasmids expressing the C-terminal, myc-tagged YB-1 protein [with or without nuclear-localizing signaling (NLS), respectively], were constructed as described previously (23). The pRC/CMV-Clusterin plasmid expressing the clusterin protein was kindly provided from Dr. Martin P. Tenniswood (State University of New York at Albany, Rensselaer, NY; ref. 30).

Various lengths of the promoter and partial first exon of the wild-type CLU gene were amplified by PCR using genomic DNA and the following primer pairs: 5’-CTCGACATGGCAGTTAGTGAGCTCCCTG-3’ and 5’-AGATCTGTGTCCAGAAGGGTTTGCT-3’ for clusterin–Luc–1,998/–707/+254 and 5’-AGATCTGTGTTCCTAACTGGAAGG-3’ and 5’-AAAGCTTAGCTGTTGACATCCCCCTCTG-3’ for clusterin–Luc–707/+254. The resultant PCR products were cloned and ligated into the pGL3-basic vector (Promega). Clusterin–Luc–1,998/+254 was constructed by inserting clusterin–Luc–707/+254 fragment digested by BglII and HindIII into BglII and HindIII site of clusterin–Luc–1,998/–702. Clusterin–Luc–1,116/–702 was constructed from clusterin–Luc–1,198/–702 by deletion of the Smal and PshAI fragments.

siRNAs

The following double-stranded 25-bp siRNA oligonucleotides were commercially generated (Invitrogen): 5’-UUGCUGUAUUUGCCGAGGACCT-3’ for YB-1 siRNA #1; 5’-UGGAUAGGCUCUUAAUGGUACGG-3’ for YB-1 siRNA #2. The sequence of siRNA corresponding to the human clusterin initiation site in exon II was 5’-GACGAGAGCUCUUCAGAU-3’ (Dharmacon Research Inc.). Stealth RNAi Negative Control Medium GC Duplex #2 (Invitrogen) was used as a control siRNA.

Quantitative reverse transcriptase PCR

RNA extraction and reverse transcriptase PCR (RT-PCR) were carried out as described previously (31). Real-time monitoring of PCR amplification of CDNA was carried out using the following primer pairs and probes: YB-1 (Hs00898625_g1), CLU (Hs00156548_m1), and GAPDH (Hs03929097_g1; Applied Biosystems) on the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) with TaqMan Gene Expression Master Mix (Applied Biosystems). Target gene expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels in respective samples as an internal control. The results are representative of at least 3 independent experiments.

Western blot analysis

Whole-cell extracts were obtained by lysis of cells in an appropriate volume of ice-cold RIPA buffer composed of 50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 0.5% sodium deoxycholate, 1% Nonidet P-40, 0.1% SDS containing 1 mmol/L Na2VO4, 1 mmol/L NaF, 1 mmol/L phenylmethylsulfonylfluoride, and protease inhibitor cocktail tablets (Complete; Roche Applied Science). Nuclear and cytoplasmic extracts were obtained using CellLytic NuCLEAR Extraction Kit (Sigma) according to manufacturer’s protocol. Cellular extracts were clarified by centrifugation.
after incubation for 48 hours, cell growth was measured using
plates and treated with paclitaxel at indicated concentration.

Luciferase reporter assay
Prostate cancer cells were transfected with the indicated reporter
plasmid, expression plasmid, or siRNA, and 0.05 μg
of pRL-TK as an internal control. After 48 hours, the luci-
ferase activities were measured using a Dual-Luciferase
Reporter Assay System (Promega) and a microplate lumin-

Immunofluorescence
Prostate cancer cells were grown on coverslips and treated
with 10 nmol/L of paclitaxel for 1 hour. After media were
refreshed, cells were recovered for 1 hour, and fixed in
parafomaldehyde for 10 minutes at room temperature.
Immunofluorescence was carried out as described previously
(32) using YB-1 (1:100) antibody.

Cell growth assay
Prostate cancer cells transfected with the indicated amount
of siRNA and expression plasmid were plated in 96-well
plates and treated with paclitaxel at indicated concentration.
After incubation for 48 hours, cell growth was measured using
the crystal violet assay as described previously (31). The results
are representative of at least 3 independent experiments.

Flow cytometric analysis
Prostate cancer cells transfected with the indicated
amount of siRNA and expression plasmid were plated in
6-well plates, and cells were treated with 10 nmol/L of

Figure 1. YB-1 regulates clusterin expression in prostate cancer cells. LNCaP (A) and PC-3 (B) cells were transfected with 40 nmol/L of the
indicated siRNA. At 48 hours after transfection, qRT–PCR was carried out
using the primer pairs and probes for YB-1, CLU, and GAPDH. Each
transcript level from cells transfected with control siRNA was set as 1.
Boxes, mean; bars, ± SD. * P < 0.05 (compared with cells transfected
with control siRNA). A representative of at least 3 independent experi-
ments.
paclitaxel. After incubation for 48 hours, cell-cycle fraction was analyzed as described previously (33). The results are representative of at least 3 independent experiments.

Statistical analysis

All data were assessed using the Student $t$-test. Levels of statistical significance were set at $P < 0.05$.

Results

YB-1 regulates clusterin expression in prostate cancer cells

YB-1 is known to regulate the expression of various genes through transcriptional and translational mechanisms. To investigate the functional link between YB-1 and clusterin, we examined clusterin expression levels after YB-1

---

**Figure 2.** YB-1 binds to CLU promoter region and regulates CLU transcription. A, schematic representation of the promoter region and 5’ end of the CLU gene. Double bars, Y-boxes (5’-ATTG-GA-3’); gray box, CLE; white box, AP-1-binding site; rail, primer pairs used in (D). Clusterin–Luc plasmids (−1,998/−254, −1,998/−702, −1,116/−702, and −707/−254) used in (B) and (C) are shown. B, LNCaP and PC-3 cells were cotransfected with 0.5 μg/mL of the various clusterin–Luc plasmids, 0.5 μg/mL of myc, or myc-YB-1 expression plasmid and 0.05 μg/mL of pRL-TK. The luciferase activity of clusterin–Luc=−1,998/−254 alone was set as 1. Boxes, mean; bars, ±SD. *, $P < 0.05$ (compared with cells transfected with myc expression plasmid). C, LNCaP and PC-3 cells were cotransfected with 0.5 μg/mL of the various clusterin–Luc plasmids, 20 nmol/L of the indicated siRNA, and 0.05 μg/mL of pRL-TK. The luciferase activity of clusterin–Luc=−1,998/−254 alone was set as 1. Boxes, mean; bars, ±SD. *, $P < 0.05$ (compared with cells transfected with control siRNA). D, ChIP assays were conducted on nuclear extracts from LNCaP and PC-3 cells using 2.0 μg of the indicated antibodies and 20 μL of Protein G agarose. The qRT-PCR was carried out using immunoprecipitated DNAs, soluble chromatin, and specific primer pairs for the CLU and RPL30 genes. The results of immunoprecipitated samples were corrected for the results of the corresponding soluble chromatin samples. Boxes, mean; bars, ±SD. *, $P < 0.05$ (compared with immunoprecipitants by IgG). CLU, clusterin.
knockdown. Clusterin expression, both at transcript and protein levels, decreased after YB-1 silencing in prostate cancer LNCaP and PC-3 cells (Fig. 1A and B). Conversely, YB-1 overexpression increased clusterin mRNA and protein levels in both LNCaP and PC-3 cells (Fig. 1C and D).

**YB-1 binds to clusterin promoter region and regulates clusterin transcription**

Because YB-1 has been reported to act both transcriptionally (21) and translationally (34), to begin defining mechanisms by which YB-1 regulates clusterin expression, we searched for putative YB-1 binding sites (Y-boxes) in the CLU promoter region between −2,000 bp and +500 bp from transcription start site. Ten Y-boxes were identified in CLU promoter regions (Fig. 2A). Subsequently, we cloned the CLU promoter region and constructed various lengths of clusterin reporter plasmids (clusterin−Luc−1,998/+254, −1,998/+702, −1,116/+702, and −707/+254) as shown in Fig 2A and then conducted reporter assays with YB-1 overexpression. The results indicate increased luciferase activity with clusterin−Luc−1,998/+702 and clusterin−Luc−702/+254, but not clusterin−Luc−1,116/+702, in response to YB-1 overexpression in both LNCaP and PC-3 cells (Fig. 2B). These findings suggest that the cis-element of CLU promoter region containing −702/+254 bp and −1,998/−1,116 bp was activated by YB-1. Conversely, YB-1 knockdown reduced CLU transcriptional activity in the clusterin−Luc−1,998/+254 constructs in both LNCaP and PC-3 cells (Fig. 2C). These data indicate that YB-1 transcriptionally activates clusterin expression.

Next, we set out to determine whether YB-1 binds to CLU promoter by ChIP assay and the primer pairs against CLU gene. As shown in Fig. 2D, YB-1 bound to CLU gene regions around −1,400 and −400 bp from transcription start site represented by clusterin−02 and clusterin−01, respectively, in both LNCaP and PC-3 cells. In contrast, YB-1 did not bind to the CLU gene around +6,600 bp region from transcription start site or PRL30 gene in either cell lines (Fig. 2D).

**Nuclear YB-1 regulates clusterin transcription**

To further confirm transcriptional regulation of clusterin by YB-1, we used different YB-1 expression plasmid that expresses YB-1 proteins with or without a NLS (23). As shown in Fig 3A and in corroboration with data in Fig 2, reporter gene assays indicate that YB-1−NLS+ plasmids increased clusterin−Luc luciferase activity more effectively than YB-1−NLS− in both LNCaP and PC-3 cells (Fig. 3A). Similarly, CLU transcription was induced more effectively by YB-1−NLS+ plasmids compared with YB-1−NLS− in both LNCaP and PC-3 cells (Fig. 3B), finding also confirmed at the protein level (Fig. 3C).
**Endoplasmic reticulum stress inducers increase clusterin expression through YB-1**

Clusterin expression is induced by various stresses including proteasome inhibitors like MG132 (35), Hsp90 inhibitors (31), and chemotherapeutics like paclitaxel (15). We next investigated whether these stressors induced clusterin expression via YB-1 in prostate cancer cells.

MG132 treatment induced time-dependent expression of endoplasmic reticulum (ER) stress markers ATF4 and GRP78 in both LNCaP and PC-3 cells (Supplementary Fig. S1A and S1B); similar time-dependent increases in clusterin mRNA and protein expression were also observed, whereas YB-1 was not altered at either mRNA or protein levels. On the basis of the functional links between YB-1 and clusterin, we hypothesized that YB-1 could be a regulator of clusterin expression in response to ER stress.

**Figure 4.** Paclitaxel treatment increases clusterin expression through YB-1. A, LNCaP and PC-3 cells were cultured with 10 nmol/L of paclitaxel for 1 hour, then changed into fresh media, and further incubated for the indicated duration. After the cells were harvested, whole-cell extracts were analyzed by SDS-PAGE and Western blot analysis with specific antibodies. B, LNCaP and PC-3 cells cultured as described in (A). After the cells were harvested, qRT-PCR was carried out using the primer pairs and probes for YB-1, CLU, and GAPDH. Each transcript level from cells treated with vehicle was set as 1. Boxes, mean; bars, ±SD. *P < 0.05 (compared with cells treated with vehicle). C, LNCaP and PC-3 cells were transfected with 40 nmol/L of the indicated siRNA and incubated for 48 hours. Then, cells were cultured with 10 nmol/L of paclitaxel for 1 hour, then changed into fresh media, and further incubated for the indicated duration. After the cells were harvested, qRT-PCR was carried out using the primer pairs and probes for YB-1, CLU, and GAPDH. Each transcript level from cells transfected with control siRNA and treated with vehicle was set as 1. Boxes, mean; bars, ±SD. *P < 0.05 (compared with cells treated with vehicle). D, LNCaP and PC-3 cells were transfected and cultured as described in (C). After the cells were harvested, whole-cell extracts were analyzed by SDS-PAGE and Western blot analysis with specific antibodies. CLU, clusterin.
clusterin expression (Figs. 2 and 3), we next measured changes in clusterin expression following ER stress with MG132 when YB-1 was silenced with siRNA. YB-1 knockdown attenuated clusterin transcript (Supplementary Fig. S1C) and protein (Supplementary Fig. S1D) induction after MG132 treatment compared with control siRNA. Interestingly, induction of the ER stress markers ATF4 and GRP78 were not affected by YB-1 silencing, suggesting that YB-1 was not a general upstream activator of these markers of ER stress but rather specifically upregulated clusterin.

Similarly, ER stress induction using the Hsp90 inhibitor, 17-AAG, also increased clusterin protein and mRNA expression concurrently with ER stress markers ATF4 and GRP78 in both LNCaP and PC-3 cells; again YB-1 expression was not affected (Supplementary Fig. S2A and S2B). When YB-1 was silenced, however, clusterin transcript and protein

![Figure 5](https://www.aacrjournals.org/mcr/doi/10.1158/1541-7786.MCR-11-0379)

**Figure 5.** Paclitaxel treatment induces YB-1 nuclear translocation and transactivation. A, LNCaP and PC-3 cells were cultured with 10 nmol/L of paclitaxel for 1 hour, then changed into fresh media, and further incubated for the indicated duration. After the cells were harvested, whole-cell extracts were analyzed by SDS-PAGE and Western blot analysis with specific antibodies. B, LNCaP and PC-3 cells cultured with 10 nmol/L of paclitaxel for 1 hour, then changed into fresh media, and further incubated for 1 hour. After the cells were fixed, immunofluorescence was conducted for YB-1 (green). Then, cells were stained with 4',6-diamidino-2-phenylindole (DAPI; blue) and observed by fluorescence microscopy. Representative images of high magnification fields (400×) are shown. Bars, 10 μm. For Western blotting, after cells were harvested and fractioned into nuclear and cytoplasmic extracts, extracts were analyzed by SDS-PAGE and Western blot analysis with specific antibodies. C, ChIP assays were conducted on nuclear extracts from LNCaP and PC-3 cells cultured as described in (B) using 2.0 μg of the indicated antibodies and 20 μL of Protein G agarose. The qRT-PCR was carried out using immunoprecipitated DNAs, soluble chromatin, and specific primer pairs for the CLU gene. Results of immunoprecipitated samples were corrected for the results of the corresponding soluble chromatin samples. Boxes, mean; bars, ±SD. * P < 0.05 (compared with immunoprecipitants from cell treated with vehicle). CLU, clusterin.
induction by 17-AAG was attenuated compared with control siRNA; again, induction of the ER stress markers ATF4 and GRP78 were not affected by YB-1 silencing (Supplementary Fig. S2C and S2D).

Similar patterns of ER stress and clusterin induction, without changes in YB-1 levels, were seen following treatment with paclitaxel in both LNCaP and PC-3 cells (Fig. 4A and B); however, YB-1 silencing again attenuated clusterin transcript and protein induction by paclitaxel, without affecting induction of ER stress markers ATF4 and GRP78 (Fig. 4C and D). Collectively, these data indicate that clusterin induction following ER stress is mediated, in part, by YB-1.

Paclitaxel treatment induces YB-1 nuclear translocation and transactivation

Because treatment stress did not increase expression levels of YB-1, we next examined the effects of treatment stress with paclitaxel on YB-1 intracellular localization and transactivation. We examined phosphorylation status of YB-1 after paclitaxel treatment. As shown in Fig. 5A, phosphorylated YB-1 was increased by paclitaxel treatment. Next, we investigated YB-1 localization with or without paclitaxel treatment. Then, the translocation into nucleus was revealed by immunofluorescence against endogenous YB-1 protein and using Western blot analysis after LNCaP or PC-3 cell fractionation (Fig. 5B). To confirm that paclitaxel treatment leads to transactivation of YB-1 and clusterin expression, we conducted ChIP assay after paclitaxel stress. As shown in Fig.5C, YB-1 binding to clusterin promoters (clusterin −02 and clusterin −01) was enhanced after paclitaxel treatment in both LNCaP and PC-3 cells. These data confirm that paclitaxel-induced stress leads to nuclear translocation of YB-1 with binding to CLU promoter.

YB-1 and clusterin are augmented in docetaxel-resistant cells and involved in paclitaxel resistance

We next examined the expression levels of YB-1 and clusterin in prostate cancer cells resistant to doctaxel (LNCaP/DTX and DU145/DTX). As shown in Fig. 6, higher expression levels of both YB-1 and clusterin mRNA (Fig. 6A) and protein (Fig. 6B) were seen in both docetaxel-resistant cell lines.

To determine the biological significance of the functional link between YB-1 and clusterin, we evaluated the effects of YB-1/clusterin knockdown and overexpression on stress induced prostate cancer cell death. As shown in Supplementary Fig. S3, YB-1 and clusterin siRNAs potentially and specifically silenced their target gene in intact and overexpressed cell lines. As expected, YB-1 overexpression led to higher clusterin levels, which could be silenced by clusterin siRNA; in contrast, clusterin overexpression did not affect YB-1 levels. As shown in Fig. 7A, both YB-1 and clusterin knockdown sensitized LNCaP and PC-3 cells to paclitaxel, whereas both YB-1 and clusterin overexpression conferred paclitaxel resistance to both cell lines. Importantly, when clusterin was silenced, YB-1 overexpression did not significantly rescue cell survival, suggesting that clusterin is a critical downstream mediator of stress-induced YB-1 transactivation. Consistent with this view, clusterin overexpression almost completely rescued cell survival following YB-1 knockdown. These findings were confirmed by other methods shown in Fig. 7B where knockdown of either YB-1 or clusterin plus paclitaxel stress led to activation of caspase-3 and increased cleavage of PARP. Clusterin overexpression with YB-1 knockdown attenuated caspase-3 activation and PARP cleavage whereas YB-1 overexpression with clusterin knockdown did not. Similarly, paclitaxel induction of apoptosis, as measured by sub-G0 to -G1 fraction using flow cytometry, increased after either YB-1 or clusterin knockdown, an effect that was rescued only by overexpression of clusterin but not by YB-1 (Fig. 7C). Collectively, these data confirm that clusterin is a critical downstream mediator of stress-induced YB-1 transactivation.

Discussion

YB-1 and clusterin are both stress-activated survival factors functionally associated with anticancer treatment resistance (21, 36). In prostate cancer, both are induced by castration and confer castration resistance when overexpressed (10, 22, 23). In addition, both YB-1 and clusterin are linked to cytotoxic resistance including cisplatin (18, 24, 25) and taxanes (16, 19, 20, 26–28). YB-1 is a transcription factor that binds to inverted CCAAT “Y” boxes present in the promoter region of a variety of genes, including MHC class II gene, epidermal growth factor receptor (EGFR), proliferating cell nuclear antigen (PCNA), DNA polymerase A, topoisomerase IIα, MDR-1, and matrix metalloproteinase (MMP)-2. The cascade of gene...
expression regulated by YB-1 have been linked to genotoxic stress, drug resistance, and metastatic invasion (21).

While YB-1 and clusterin are functionally linked to treatment resistance in cancer, the role of YB-1 in stress-induced clusterin transactivation and cytoprotection is undefined. Promoter regions of CLU gene are conserved during evolution and include stress-associated sites like activator protein-1 (AP-1), AP-2, stimulatory element (SP-1), heat shock element (HSE), cAMP response element (CRE), and a "clusterin-specific element" (CLE) recognized by HSF-1/HSF-2 heterocomplexes (37) as well as steroid response elements including glucocorticoid and androgen response element sites (38). Our initial analysis of the CLU promoter region identified 10 putative YB-1 binding sites (Y-boxes), which led us to investigate the role of YB-1 in stress-induced clusterin transactivation.

YB-1 is known to be activated by many varied stressors via phosphorylation by AKT (39) and p90RSK (40),...
stimulating its translocation to the nucleus. Stresses linked to activation of YB-1 include genotoxic stress with radiation (41, 42) and cisplatin (43) as well as paclitaxel (26, 28). In this study, we show that ER stress associated with paclitaxel, proteasome inhibition, or Hsp90 inhibition led to YB-1 nuclear translocation. Because clusterin is a potent inhibitor of protein aggregation and functions like small Hsps to chaperone and stabilize proteins under stress, we used these ER stress inducers to define the role of YB-1 in regulation of CLU transcription. We further focused on paclitaxel activation of YB-1 and clusterin as taxane-based chemotherapy is the standard first-line therapy for men with metastatic CRPC (3).

Although it was previously reported that clusterin is overexpressed in taxane-resistant cells (44, 45), the status of YB-1 in taxane-resistant cells was unknown. This study revealed that YB-1 is overexpressed in taxane-resistant prostate cancer cells. Furthermore, we show that both YB-1 and clusterin are functionally involved in paclitaxel resistance, findings compatible with previous reports (16, 26, 27). For example, because YB-1 regulates P-glycoprotein (P-gp) expression, and paclitaxel is a substrate of P-gp, it is reasonable to speculate that YB-1 may affect paclitaxel sensitivity via P-gp. However, prostate cancer cell lines like LNCaP and PC-3 cells express P-gp at undetectable levels (46), and hence, it is likely that YB-1 affects paclitaxel sensitivity through factors other than P-gp. Clusterin is an extracellular chaperone which directly interacts with paclitaxel and confers paclitaxel resistance (47). Our studies indicate that clusterin acts as a critical mediator of stress-induced YB-1 activity and paclitaxel resistance. Clusterin overexpression reversed increases in paclitaxel-induced apoptosis following YB-1 knockdown; in contrast, however, YB-1 overexpression did not rescue cells from increased paclitaxel-induced apoptosis following clusterin knockdown. In addition, these data indicate that both YB-1 and clusterin confer taxane resistance irrespective of p53 and AR expression status because LNCaP cells express wild-type p53 as well as AR, whereas PC-3 cells express neither p53 nor AR.

Many strategies used to kill cancer cells induce stress responses that promote the emergence of a treatment resistant phenotype, and novel therapeutic approaches targeting these stress responses are being developed. With respect to YB-1, a small-molecular inhibitor of integrin-linked kinase (ILK), QLT0267, was found to inhibit YB-1 with suppression of breast cancer cell growth (48). Alternatively, a molecular decoy to YB-1 also inhibited both breast cancer and prostate cancer cell growth without affecting normal immortalized breast epithelial cells and primary breast epithelial cells (49). Clusterin is being targeted using the antisense oligonucleotide (OGX-011), now in phase III clinical trials, in CRPC after randomized phase II studies reported a significant survival benefit when OGX-011 was added to docetaxel (50). Previously, we reported that clusterin downregulation using OGX-011 re sensitized docetaxel-resistant prostate cancer cells to docetaxel and mitoxantrone and sensitized breast cancer cells to paclitaxel (20). Although inhibition of either YB-1 or clusterin sensitizes prostate cancer cells to paclitaxel, clusterin overexpression can reverse increases in paclitaxel-induced apoptosis following YB-1 knockdown; in contrast, however, YB-1 overexpression did not rescue cells from increased paclitaxel-induced apoptosis following clusterin knockdown, suggesting that clusterin may be a more relevant therapeutic target.

In summary, YB-1 transcriptionally enhances clusterin expression following ER stress in prostate cancer cells, resulting in inhibition of treatment-induced apoptosis and taxane resistance. Although inhibition of either YB-1 or clusterin sensitized prostate cancer cells to paclitaxel, clusterin seems to play a more dominant role. Collectively, these data indicate that YB-1 transactivation of clusterin in response to stress is a critical mediator of paclitaxel resistance in prostate cancer.

Disclosure of Potential Conflicts of Interest

The University of British Columbia has submitted patent applications, listing Dr. Gleave as inventor, on the antisense sequence targeting CLU described in this article. This IP has been licensed to OncoGenex Technologies, a Vancouver-based biotechnology company in which Dr. Gleave has founding shares.

Acknowledgments

The authors thank Dr. Martin P. Tenniswood (State University of New York at Albany, Rensselaer, NY) for providing the pRC/CMV-clusterin expression plasmid and Dr. Bob Shukin for sequencing cloned CLU promoter region.

Grant Support

This study was supported by the Terry Fox New Frontiers Program, the Canadian Institutes of Health Research, the Pacific Northwest Prostate Cancer SPORE, and the Japanese Postdoctoral Fellowship for Research Abroad.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 10, 2011; revised September 27, 2011; accepted October 3, 2011; published OnlineFirst October 10, 2011.

References


Published OnlineFirst October 10, 2011; DOI: 10.1158/1541-7786.MCR-11-0379
Clusterin Mediates Taxane Resistance by YB-1


Molecular Cancer Research

Clusterin Is a Critical Downstream Mediator of Stress-Induced YB-1 Transactivation in Prostate Cancer

Masaki Shiota, Amina Zoubeidi, Masafumi Kumano, et al.

Mol Cancer Res  Published OnlineFirst October 10, 2011.

Updated version  Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-11-0379

Supplementary Material  Access the most recent supplemental material at:
http://mcr.aacrjournals.org/content/suppl/2011/10/10/1541-7786.MCR-11-0379.DC1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.