A Coregulatory Role for the Mediator Complex in Prostate Cancer Cell Proliferation and Gene Expression

Ravi Vijayvargia, Michael S. May, and Joseph D. Fondell

Abstract

Androgen receptor (AR) signaling pathways are important for the survival and proliferation of prostate cancer cells. Because AR activity is facilitated by distinct coregulatory factors and complexes, it is conceivable that some of these proteins might also play a role in promoting prostate oncogenesis. The multisubunit Mediator complex is an important coactivator for a broad range of regulatory transcriptional factors including AR, yet its role in prostate cancer is unclear. Here, we used RNA interference to knock down the expression of two integral Mediator components, MED1/TRAP220 and MED17, in prostate cancer cells. MED1/TRAP220 plays a particularly important role in androgen signaling in that it serves as a direct binding target for AR. We found that the knockdown of either subunit markedly decreases transcription from transiently transfected androgen-responsive reporter genes, as well as inhibits androgen-dependent expression of endogenous AR target genes. We show for the first time that loss of either MED1/TRAP220 or MED17 in prostate cancer cells significantly decreases both AR-positive and -negative prostate cancer cells lines, as well as in 50% (10 of 20) of the clinically localized human prostate cancers we examined, thus suggesting that MED1/TRAP220 hyperactivity may have implications in prostate oncogenesis. In sum, our data suggest that Mediator plays an important coregulatory role in prostate cancer cell proliferation and survival, and therefore, may represent a new target for therapeutic intervention. [Cancer Res 2007;67(9):4034–41]

Introduction

Androgen receptor (AR) signaling pathways are important for the growth and survival of both normal and early stage neoplastic prostate cells (1). The primary treatment option currently available for patients with advanced prostate cancer is androgen ablation therapy, which initially results in tumor regression, but ultimately leads to cancer recurrence and progression to a fatal androgen-independent malignancy (2). Paradoxically, AR signaling pathways can remain active in androgen-independent prostate cancer and promote tumor growth, yet the molecular mechanisms are ill understood (3). Because AR activity in prostate cells is critically dependent on interactions with an assortment of coregulatory proteins and complexes (4), it has been proposed that overexpression or hyperactivation of distinct AR cofactors may facilitate important oncogenic events in both prostate cancer initiation and progression to the androgen-independent disease (3, 4).

Mediator is an evolutionarily conserved multisubunit coactivator complex (5–7) that acts as a molecular bridge between genespecific transactivators and the RNA polymerase II (RNA pol II)–associated basal transcription machinery. Although Mediator directly interacts with a diverse array of signal-activated transcription factors (8–13), the complex was originally identified in humans as a coactivator that bound nuclear receptors (NR) and was later shown to be essential for NR-dependent transcription (14). Most NRs bind Mediator via direct, ligand-dependent interactions with the MED1/TRAP220 subunit (14). Conditional knockout studies in mouse liver and mammalian epithelial tissue have confirmed the importance of MED1/TRAP220 in several NR signaling pathways in vivo (15–17). AR also binds MED1/TRAP220 in the presence of ligand (18) and a recent study using RNA interference in cultured prostate cancer cells found that MED1/TRAP220 was indispensable for AR-mediated transcription of the prostate-specific antigen (PSA) gene (19). Complete ablation of MED1/TRAP220 expression in mice is embryonic lethal (20, 21), and primary embryonic fibroblasts isolated from Med1/Trap220 null embryos exhibit retarded growth and cell cycle progression (21). Thus, in addition to NR coactivation, MED1/TRAP220 may also play a pivotal role in cellular growth and cell cycle regulation. Consistent with this notion, MED1/TRAP220 can serve as a binding target for other transactivators involved in cell growth and development including the GATA family proteins (22), p53 (23), BRCA-1 (24), and GABPα (25).

Several studies suggest that changes in the expression or activity of AR coregulatory factors can contribute to prostate oncogenesis. For example, members of the p160/steroid receptor coactivator (SRC) family of proteins were found to be amplified in breast, ovarian, and prostate cancers (26–28), and were further shown to be required for prostate cancer cell proliferation and tumor progression (29, 30). Similarly, the AR coactivator ARAT70 can facilitate AR-mediated transcription in the presence of AR antagonists and adrenal androgens (31), and is amplified in at least one androgen-independent prostate cancer xenograft (32). Interestingly, MED1/TRAP220 of the Mediator complex is amplified in several estrogen receptor–positive breast and ovarian cancer cell lines, thus suggesting that it might also play an oncogenic role in steroid hormone–dependent cancer progression (33).

In this study, we investigated the functional role of the Mediator complex in prostate cancer cells by using RNA interference to knock down the expression of two key Mediator subunits, MED1/
TRAP220 was chosen for knockdown given its pivotal role as a direct binding target for AR. MED17 was also chosen, primarily because it serves as an integral structural component within the core Mediator complex (34). Indeed, recent studies suggest that MED17 is a central binding hub for as many as six other Mediator subunits, and likely plays an essential role in maintaining the overall structural integrity of the Mediator complex (35). Importantly, we found that the knockdown of either Mediator subunit in prostate cancer cells markedly inhibits transcription from androgen-responsive AR target genes, decreases androgen-dependent and -independent cellular proliferation, and increases apoptosis. We also show that MED1/1/TRAP220 was overexpressed in both AR-positive and -negative prostate cancer cells lines, as well as in clinically localized prostate cancers, thus suggesting that MED1/1/TRAP220 hyperactivity might promote prostate oncogenesis. Taken together, our findings suggest that Mediator plays an important coregulatory role in prostate cancer progression, and therefore, may represent a new target for therapeutic intervention.

Materials and Methods

**Cell culture.** LNCaP, DU145, PC3, 1532N, and 1532T cells are from the American Type Culture Collection (see ref. 36 for immortalization of the 1532N and 1532T lines). DUPro cells were obtained from Dr. Allan Connery (Rutgers University). LAPC4 cells were obtained from Dr. Charles Sawyer (University of California at Los Angeles). DU145-EAR cells were generated by stably transfecting parental DU145 cells with recombinant retroviruses expressing a FLAG-tagged human AR cDNA essentially as previously described (37). LNCaP, 1532N, and 1532T cells were grown in RPMI 1640 (Invitrogen) containing 10% fetal bovine serum (FBS; Gemini BioProducts), DU145, PC3, and DUPro cells were grown in DMEM/10% FBS. LAPC4 cells were grown in I scove’s modified DMEM/10% FBS. In androgen starvation experiments, cells were grown in charcoal/dextran-stripped FBS (CDS-FBS; HyClone) containing phenol red–free medium.

**Materials.** Specific antibodies against MED1/TRAP220, MED17, AR, SRC-1, and tubulin were from Santa Cruz Biotechnology; antibodies against caspase 3 were from Calbiochem, and antibodies for cytochrome c were from BD PharMingen. Secondary anti-rabbit and anti-mouse IgG were from Cell Signaling, whereas anti-goat IgG was from Santa Cruz Biotechnology. Enhanced chemiluminescence reagents were from Amersham Pharmacia/GE Healthcare. The luciferase assay kit was from Promega Corp., whereas the PSA, KLK-2, and dehydrated and cleared before coverslipping with xylene.

**Immunohistochemistry.** Twenty clinically localized prostate cancer specimens (obtained from the Cancer Institute of New Jersey) were stained using antibodies against AR (Santa Cruz Biotechnology) or MED1/TRAP220 (Santa Cruz Biotechnology). Four-micrometer sections were mounted on slides and used to study both anti-AR and anti-MED1/TRAP220 staining from the same patient case. Anti-AR and anti-MED1/TRAP220 staining was first optimized using a Ventana Medical Systems Discovery automated immunostainer. Slides were deparaffinized and antigen retrieval was done using Cell Conditioning Solution (Ventana Medical Systems) with an extended protocol of 72 min. Ventana Universal Secondary antibody was applied for 12 min followed by chromogenic detection kit DABMap (Ventana Medical Systems). Slides were counterstained with hematoxylin and dehydrated and cleared before coverslipping with xylene.

**Results**

**Materials and Methods.** Luciferase assays. DU145-EAR or LNCaP cells were transfected with siRNA (see above) along with 250 ng of the pMMTV-Luc reporter gene and 100 ng of the pSV-luc plasmid as an internal transfection control. Three days posttransfection, the medium was replaced with or without 100 nmol/L of dihydrotestosterone as described previously (18, 19). Twenty-four hours later, cells were transfected and luciferase activity was determined via a luminometer. Luciferase activity was normalized for both protein concentration and β-galactosidase activity.

**Reverse transcription-PCR analysis.** LNCaP cells were androgen-starved in phenol red–free RPMI 1640/10% CDS-FBS for 3 days and then transfected with siRNA. Seventy-two hours posttransfection, fresh media with or without 100 nmol/L of final dihydrotestosterone was added and the cells incubated for an additional 24 h. Total RNA was then isolated using Trizol reagent (Invitrogen). First-strand cDNA was generated using 2 μg of total RNA via MuMLV-reverse transcriptase (New England Biolabs) in a final volume of 20 μL. The cDNA was then subjected to 25 cycles of PCR amplification using primers specific for either PSA, KLK-2, or β-actin as previously outlined (25, 40). The resulting PCR products were quantitated using NIH Image software.

**Cell proliferation assay.** DU145, PC3, and LNCaP were transfected with siRNA as described above with LNCaP cells being androgen-starved 72 h prior to transfection. Forty-eight hours posttransfection, cells were trypsinized and 2 × 10⁵ cells were seeded per well in 12-well plates. For DU145 or PC3 cells, fresh medium was then added, whereas for LNCaP cells, fresh medium with or without 1 nmol/L of dihydrotestosterone was added. Cells were allowed to proliferate for an additional 6 days with fresh media change after 3 days. Cell proliferation was measured by manually counting cell numbers. Experiments were done in triplicate.

**Cell cycle and cell death analyses.** DU145, PC3, and LNCaP were transfected with siRNA as described earlier with LNCaP cells being androgen-starved for 72 h prior to transfection. For LNCaP cells, fresh media with or without 1 nmol/L of dihydrotestosterone was added at 48 h posttransfection, and cells were harvested 60 h later. For DU145 and PC3 cells, fresh medium was added 48 h posttransfection and cells were harvested 18 h later. The cells were then fixed and stained with propidium iodide (20 μg/mL) and then analyzed using a Cytoemics FC500 flow cytometer (Beckman Coulter, Inc.). To assay for cell death, cells were processed as described above, except that harvesting was done 6 days post-siRNA transfection and the flow cytometry analyses included floating cells. Cell death was also determined by immunoblot probing for cleaved caspase 3 and release of cytochrome c in cytoplasm.

**Immunohistochemistry.** Twenty clinically localized prostate cancer specimens (obtained from the Cancer Institute of New Jersey) were stained using antibodies against AR (Santa Cruz Biotechnology) or MED1/TRAP220 (Santa Cruz Biotechnology). Four-micrometer sections were mounted on slides and used to study both anti-AR and anti-MED1/TRAP220 staining from the same patient case. Anti-AR and anti-MED1/TRAP220 staining was first optimized using a Ventana Medical Systems Discovery automated immunostainer. Slides were deparaffinized and antigen retrieval was done using Cell Conditioning Solution (Ventana Medical Systems) with an extended protocol of 72 min. Ventana Universal Secondary antibody was applied for 12 min followed by chromogenic detection kit DABMap (Ventana Medical Systems). Slides were counterstained with hematoxylin and dehydrated and cleared before coverslipping with xylene.
DU145-f:AR cells reduced dihydrotestosterone-dependent transcription by ~2.5- and 3.5-fold, respectively (Fig. 1A).

We next examined Mediator’s transcriptional coregulatory activity in androgen-responsive LNCaP prostate cancer cells that express AR endogenously. In these experiments, LNCaP cells were transfected with MED1/TRAP220 or MED17 siRNA, and as before, dihydrotestosterone-dependent transcription was measured from the MMTV-luciferase reporter gene. Similar to the findings in Fig. 1A, loss of either MED1/TRAP220 or MED17 reduced dihydrotestosterone-stimulated transcription by ~3- and 4-fold, respectively (Fig. 1B). As a positive control, we knocked down the expression of another AR coactivator, SRC-1, by using siRNA specific for the SRC-1 gene. Although loss of SRC-1 decreased dihydrotestosterone-dependent transcription by ~1.6-fold (Fig. 1C), the reduction in activation was not nearly to the extent observed following the loss of either Mediator subunit (Fig. 1B).

Whereas these findings clearly implicate Mediator in facilitating dihydrotestosterone-dependent transcription from transiently transfected reporter genes, it was important to further investigate Mediator’s role in regulating transcription from endogenous androgen-responsive genes in prostate cancer cells. PSA and human glandular kallikrien (KLK2) are two well-characterized AR target genes whose mRNA expression in LNCaP cells is markedly stimulated 16 to 24 h post–androgen stimulation (19, 40). To determine whether Mediator is required for endogenous PSA and KLK2 gene expression, MED1/TRAP220 or MED17 siRNA was transfected into LNCaP cells cultured with or without dihydrotestosterone. PSA and KLK2 mRNA expression was then measured via reverse transcription-PCR following normalization to β-actin expression. Strikingly, loss of MED1/TRAP220 or MED17 inhibited endogenous dihydrotestosterone-induced PSA gene expression ~4-fold (Fig. 2A and B), whereas KLK2 expression was reduced ~5- and 3-fold, respectively (Fig. 2C and D). These results are in close agreement with a previous study implicating MED1/TRAP220 in facilitating an essential transcriptional regulatory step in AR-mediated activation of the PSA gene (19). In sum, the data presented here strongly suggest that Mediator plays an important coregulatory role in androgen-dependent gene expression in prostate cancer cells.

Loss of MED1/TRAP220 or MED17 expression inhibits prostate cancer cell proliferation and cell cycle progression. Given that Mediator is important for androgen-dependent gene expression, we next asked whether the complex is required for other physiologic responses in prostate cancer cells. Accordingly, we first investigated whether Mediator is involved in androgen-dependent cellular proliferation. Equal numbers of androgen-starved LNCaP cells were transfected with MED1/TRAP220 or MED17 siRNA and then cultured with or without dihydrotestosterone for 6 days. As shown in Fig. 3A, loss of either subunit dramatically inhibited androgen-dependent cellular proliferation when compared with control siRNA–treated cells. Indeed, loss of MED1/TRAP220 decreased the average dihydrotestosterone-induced LNCaP cell number by 53%, whereas loss of MED17 decreased the cell number by 69%. The findings support the notion that Mediator plays an important coregulatory role in androgen-dependent prostate cancer cell proliferation.
Unexpectedly, we observed that loss of either Mediator subunit in LNCaP cells cultured in the absence of dihydrotestosterone still reduced the cell number by ~30% (Fig. 3A), thus suggesting a possible coregulatory role for Mediator in the androgen-independent proliferation. To address this issue more thoroughly, we knocked down the expression of MED1/TRAP220 or MED17 in the AR-negative DU145 and PC3 cell lines (Fig. 3B and C). Interestingly, we found that silencing MED1/TRAP220 or MED17 in DU145 cells inhibited proliferation by 47% and 52%, respectively (Fig. 3B), whereas silencing of MED1/TRAP220 or MED17 in PC3 cells inhibited proliferation by 43% and 53%, respectively (Fig. 3C). Thus, when androgen is limiting, or in the absence of AR, Mediator still seems to play an important coregulatory role in cell proliferation.
In view of the decreased proliferation of prostate cancer cells following the loss of specific Mediator subunits, it is predicted that silencing MED1/TRAP220 or MED17 will likewise inhibit cell cycle progression. To investigate this supposition, LNCaP cells transfected with either MED1/TRAP220 or MED17 siRNA were grown in steroid-depleted serum for 48 h and then stimulated with or without dihydrotestosterone for an additional 60 h. The percentage of cells that were dihydrotestosterone-induced into the G2-M phase of the cell cycle was then measured by flow cytometry. Consistent with the androgen-dependent proliferation studies, we found that loss of MED1/TRAP220 or MED17 expression decreased the number of cells that were dihydrotestosterone-induced into the G2-M phase between 4- and 5-fold (Fig. 4A and B). A similar decrease in the percentage of cells that were dihydrotestosterone-induced into the S phase of the cell cycle was also observed following Mediator siRNA treatment (data not shown). When either MED1/TRAP220 or MED17 was silenced in AR-negative PC3 cells, there was a >30% decrease in the number of cells in G2-M (Fig. 4C), consistent with the reduction in cell number observed in the proliferation assay (Fig. 3C). Taken together, these findings provide the first direct evidence that Mediator is important for both androgen-dependent and -independent prostate cell proliferation and cell cycle progression.

Loss of MED1/TRAP220 or MED17/TRAP80 expression increases prostate cancer cell apoptosis. The observed inhibition of prostate cancer cell proliferation and cell cycle progression following the loss of MED1/TRAP220 or MED17 suggests that Mediator might function, at least in part, by promoting cell survival. To address this issue more closely, LNCaP, PC3, and DU145 cells were transfected with MED1/TRAP220 or MED17 siRNA and cell death was measured by flow cytometry. When compared with cells transfected with control siRNA, loss of MED1/TRAP220 or MED17 increased apoptosis by ~4-fold in LNCaP cells, ~7-fold in PC3 cells, and between 2.5- and 3-fold in DU145 cells (Fig. 5A–C). Furthermore, immunoblot analyses revealed increased levels of cleaved caspase 3 and cytoplasmic cytochrome c in PC3 cells transfected with either MED1/TRAP220 or MED17 siRNA (see Supplementary Fig. S2A and B). Thus, the findings suggest that in addition to fostering androgen-dependent and -independent cell proliferation, Mediator also promotes prostate cancer cell survival, possibly by serving as an antiapoptotic coregulatory complex. AR expression does not seem to be a critical determinant for the antiapoptotic function of Mediator, as both AR-positive (LNCaP) and AR-negative (PC3 and DU145) prostate cancer cell lines were similarly affected by the loss of MED1/TRAP220 and MED17.

MED1/TRAP220 overexpression in human prostate cancer cell lines and tissues. MED1/TRAP220 is required for estrogen-dependent transcription (41–43) and was found to be amplified in estrogen receptor–positive breast cancer tissues and cell lines (33) thus suggesting a possible oncogenic role for MED1/TRAP220 in steroid hormone–dependent cancer. To evaluate MED1/TRAP220 expression in prostate cancer, several human prostate cell lines were probed by immunoblot using an antibody specific for MED1/TRAP220 (Fig. 6A). 1532T and 1532N are AR-positive prostate cell lines derived from a primary adenocarcinoma and normal peripheral epithelium, both from the same patient (36). LAPC4 is an AR-positive metastatic prostate cancer cell line, whereas DUPro, PC3, and DU145 are all AR-negative metastatic prostate cancer cell lines (44). MED1/TRAP220 expression in each line was normalized against α-tubulin immunoblot signals from the same blot. When compared with normal 1532N cells, MED1/TRAP220 expression was ~1.5-fold higher in adenocarcinoma 1532T cells, and ~2- to 3-fold higher in the metastatic prostate cancer cell lines, with DU145 and PC3 showing the highest expression (Fig. 6A).

To determine which specific types of prostate cells express MED1/TRAP220, immunohistochemistry using an anti-MED1/TRAP220 antibody was carried out on human prostate sections obtained from patients undergoing radical prostatectomy (Fig. 6B).
Antibodies specific for AR were used as a positive control. Consistent with its presumptive role as an AR coactivator, both AR and MED1/TRAP220 were clearly present in basal and luminal epithelial cells with staining predominantly confined to the nucleus (Fig. 6B). Interestingly, MED1/TRAP220 expression was also variably detected in both prostate stromal and endothelial cells. This finding contrasts the situation observed with other AR coregulatory factors like SRC-1, the expression of which seems to be limited to basal and luminal epithelial cells (30) and is suggestive of a broader physiologic role for MED1/TRAP220 and the Mediator complex throughout the prostate gland.

To evaluate MED1/TRAP220 expression in prostate cancer more thoroughly, immunohistochemistry was done on 20 clinically localized human prostate cancers using the MED1/TRAP220 antibody. The prostate cancer sections from each patient contained normal peripheral zones that allowed us to compare MED1/TRAP220 expression in normal versus neoplastic epithelium. Analogous to the scoring system used in similar prostate immunohistochemistry studies (29, 30), MED1/TRAP220-stained sections were scored for both extent of staining (scale, 0–3) and intensity of staining (scale, 0–3). A staining index was then determined by multiplying the extent score by the intensity score such that the final staining index ranged from 0 (no staining) to 9 (strong and extensive staining). For both neoplastic and normal prostate tissue, only basal and luminal epithelial cells were scored. Interestingly, we found that the MED1/TRAP220 staining indices for the normal prostate epithelium were quite variable, ranging from 3 to 9. For any given patient, the staining index of the normal epithelium was consistent throughout the entire section, thus suggesting that the variability reflects variation between patients rather than heterogeneity within a given specimen. Nonetheless, we observed that half of the patients with prostate cancer (10 of 20 or 50%) exhibited a higher MED1/TRAP220 staining index in the neoplastic epithelium (average index, 8.05) than in the surrounding normal epithelium (average index, 5.30; Fig. 6C). Indeed, nearly two-thirds of the adenocarcinomas examined (12 of 20 or 60%)
exhibited strong and extensive MED1/TRAP220 staining (index, 7–9), whereas only about a third of the normal prostate epithelia examined (7 of 20 or 35%) showed similar staining levels (P < 0.01, t test).

Discussion

The Mediator complex plays an essential role in mammalian transcriptional regulation by serving as a functional adaptor between DNA-bound regulatory factors and the RNA pol II–associated basal transcription machinery (5–7). MED1/TRAP220 is a variably associated subunit of the complex that acts as a direct binding target for NRs, as well as a broad range of other types of activators. Although previous studies implicated Mediator, particularly the MED1/TRAP220 subunit, in breast cancer (33, 41), the pathologic importance of Mediator in prostate cancer is unclear. Here, we show that disruption of the Mediator complex in prostate cancer cells, either by silencing MED1/TRAP220 expression, or by silencing the structurally important MED17, significantly decreased both androgen-dependent and -independent cell proliferation, inhibited cell cycle progression, and increased apoptosis (Figs. 3–5). We also show that MED1/TRAP220 was overexpressed in 50% of the clinically localized human prostate cancers we examined, as well as in several prostate cancer cell lines (Fig. 6). Our data thus suggests that Mediator may play an important coregulatory role in prostate cancer cell proliferation and survival.

Prostate cancer cells initially depend on androgens for growth and survival (1). We previously showed that MED1/TRAP220 binds AR in an androgen-dependent manner and that overexpression of various Mediator subunits could coactivate AR-dependent transcription (18). Here, we found that loss of either MED1/TRAP220 or MED17 expression in prostate cancer cells markedly decreased transcription from a transfected androgen-responsive reporter gene (Fig. 1) and from two endogenous AR target genes, PSA and KLK2 (Fig. 2). Wang et al. (19) recently showed that MED1/TRAP220 silencing in LNCaP cells significantly decreased AR-dependent recruitment of both RNA pol II and the general transcription factor TATA-binding protein to the PSA gene promoter. Thus, Mediator seems to serve as an AR coactivator by facilitating recruitment of RNA pol II and other basal factors at AR target genes.

Consistent with the transcription assays, MED1/TRAP220 or MED17 silencing inhibited androgen-dependent proliferation and cell cycle progression in LNCaP cells, yet unexpectedly, MED1/TRAP220 or MED17 silencing in prostate cells lacking AR (PC3 and DU145) also led to a decrease in proliferation and cell cycle progression. Furthermore, MED1/TRAP220 or MED17 silencing in LNCaP, DU145, or PC3 cells led to an increase in cell death. These findings suggest that in addition to AR-coactivation, Mediator plays alternative coregulatory roles in other cellular growth and survival pathways. Consistent with this notion, we recently showed that Mediator regulates the gene expression of Aurora-A, a centrosome kinase critical for cell cycle progression (25). In this connection, it is notable that MED1/TRAP220 and MED17 are direct binding targets for a number of regulatory transcription factors involved in cell growth and differentiation, including GATA family members (22), PIT-1 (45), BRCA-1 (24), GABPα (25), and p53 (23). Moreover, because the knockdown of MED17 likely disrupts the entire Mediator complex, the involvement of other Mediator subunits in facilitating transcriptional responses to other diverse signal transduction pathways cannot be ruled out. For example, MED12 is a transducer of Wnt/β-catenin signaling (11), MED14 is functionally required for IFN responses (13), MED15 mediates transforming growth factor-β signaling (12), whereas MED23 can facilitate activated Ras signaling (9, 10).

Importantly, we found that MED1/TRAP220 protein expression was elevated in half of the human prostate cancers we examined. Although a larger number of prostate cancers will need to be screened, the present study suggests that MED1/TRAP220 could play a role in prostate cancer progression. Amplification of chromosomal region 17q12, which contains the human MED1/TRAP220 gene locus, is thought to be the predominant mechanism by which MED1/TRAP220 becomes amplified in primary breast cancers (33). However, this chromosomal region is rarely amplified in prostate neoplasia, and a recent study found no significant increase in MED1/TRAP220 mRNA levels in 43 primary prostate cancers (46). An alternative mechanism is that MED1/TRAP220 amplification in prostate cancer cells may occur at the level of stabilized protein expression. Indeed, we recently reported that MED1/TRAP220 is a specific substrate for mitogen-activated protein kinase (MAPK) and that MAPK phosphorylation significantly increases the stability and half-life of the MED1/TRAP220 protein in vivo (38). Given that MAPK signaling pathways are commonly activated constitutively in prostate cancer cells (47–49), particularly in hormone-refractory diseases (50, 51), it is conceivable that hyperactivated MAPKs might promote MED1/TRAP220 overexpression during prostate oncogenesis. Notable in this regard, MAPK activity is abnormally high in PC3 and DU145 cells (52, 53), both of which display unusually high levels of MED1/TRAP220 (Fig. 6A).

In summary, our findings confirm that Mediator is important for androgen-dependent gene expression in prostate cancer cells. More significantly, our data suggests that Mediator facilitates important coregulatory roles in both androgen-dependent and -independent prostate cancer cell proliferation and survival. Thus, Mediator and its associated polypeptides may represent novel targets for therapeutic intervention. Finally, our data showing that MED1/TRAP220 is overexpressed in some primary prostate cancers suggests that this subunit may play a role in promoting prostate cancer progression, and as such, could serve as a diagnostic marker for clinical investigations.

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