**CHD1 Is a 5q21 Tumor Suppressor Required for ERG Rearrangement in Prostate Cancer**

Lia Burkhardt1, Sarah Fuchs1, Antje Krohn1, Sawinee Masser1, Malte Mader1, Martina Kluth1, Frederik Bachmann1, Hartwig Huland2, Thomas Steuber2, Markus Graefen2, Thorsten Schlommm2, Sarah Minner1, Guido Sauter1, Hüseyin Sirma1, and Ronald Simon1

**Abstract**

Deletions involving the chromosomal band 5q21 are among the most frequent alterations in prostate cancer. Using single-nucleotide polymorphism (SNP) arrays, we mapped a 1.3 megabase minimally deleted region including only the repulsive guidance molecule B (RGMB) and chromodomain helicase DNA-binding protein 1 (CHD1) genes. Functional analyses showed that CHD1 is an essential tumor suppressor. FISH analysis of 2,093 prostate cancers revealed a strong association between CHD1 deletion, prostate-specific antigen (PSA) biochemical failure ($P = 0.0038$), and absence of ERG fusion ($P < 0.0001$). We found that inactivation of CHD1 in vitro prevents formation of ERG rearrangements due to impairment of androgen receptor (AR)-dependent transcription, a prerequisite for ERG translocation. CHD1 is required for efficient recruitment of AR to responsive promoters and regulates expression of known AR-responsive tumor suppressor genes, including NKX3-1, FOXO1, and PPARγ. Our study establishes CHD1 as the 5q21 tumor suppressor gene in prostate cancer and shows a key role of this chromatin remodeling factor in prostate cancer biology. *Cancer Res; 73(9); 1–11. ©2013 AACR.*

**Introduction**

Prostate cancer is the most frequent malignancy in men worldwide. The clinical behavior ranges from slowly growing indolent tumors to highly aggressive, metastatic cancers. It is believed that a significant fraction of patients with localized prostate cancer may be followed up safely even without radical prostatectomy and its related side effects including urinary and sexual dysfunction. Thus, the search for new molecular markers distinguishing low malignant tumors from the aggressive ones is a major challenge of current prostate cancer research. Molecular analysis of prostate cancer has highlighted a large number of candidate genome and gene alterations that may predict prognosis of individual patients (1, 2). The TMPRSS2:ERG fusion is the most frequent alteration affecting about half of all prostate cancers (3), but the clinical impact of this alteration and its downstream effects remains to be clarified. DNA copy number alterations have been comprehensively characterized in prostate cancer, and identified numerous chromosomal regions of recurrent deletions harboring known tumor suppressor genes like RB1 at 13q14, CDKN1B at 12p13, or the PTEN gene at 10q23, but the target genes of most other recurrently deleted regions remain to be identified. Deletion of 5q21 belongs to the most frequent alterations in prostate cancer. After its first description in 1995 (4), this alteration has gained considerable interest, as recent studies reported a high deletion frequency (13%–26%), and identified a small commonly deleted region including the gene encoding chromodomain helicase DNA-binding protein 1 (CHD1; refs. 5–7). Two groups reported independently that CHD1 has tumor-suppressive features in prostate cancer (8, 9). CHD1 is involved in assembly, shifting, and removal of nucleosomes from the DNA double helix to keep it in an open and transcriptionally active state (10). CHD1 is essential to maintain the open chromatin of pluripotent embryonic stem cells (10). CHD1 associates with the promoters of active genes by the cooperative action of its 2 chromodomains, which specifically bind to the H3K4-trimethylated histones. Several of the 8 other members of the CHD family have been implicated in cancer before. CHD2 has been suggested as a putative tumor suppressor that might play a role in DNA damage response and lymphoma development (11). Heterozygous frameshift mutation or loss of heterozygosity of CHD1, CHD2, CHD3, CHD4, CHD7, and CHD8 have been reported from gastric and colon cancers (12). Mutation of CHD7 was detected in a lung cancer cell line (13). CHD5 is a known tumor suppressor controlling apoptosis via the p19–p53 pathway and which is often inactivated by deletions involving the chromosomal band 1p36.
Furthermore CHD8, another member of the CHD gene family, has been suggested to play a role in androgen receptor (AR)-dependent transcription regulation in prostate cancer (16).

To estimate the frequency and clinical impact of CHD1 deletion, we analyzed more than 3,200 prostate cancers with molecular, pathologic, and clinical follow-up data for CHD1 deletion by means of FISH. The results of our study suggest that CHD1 is the tumor suppressor gene at 5q21 in prostate cancer. This notion is based on the localization of CHD1 within the smallest commonly deleted region (8, 9) at 5q21, the association of the deletion with adverse features of prostate cancer, the link between 5q21 deletion and reduced expression of CHD1, and functional data showing a growth arrest in CHD1-overexpressing prostate cancer cells.

Materials and Methods

Patients and tissue microarray

The prostate cancer prognosis tissue microarray (TMA) used in this study has been described in detail before (18). In brief, patients were consecutively treated by radical prostatectomy in our center between 1992 and 2005. Clinical follow-up data were available for 2,891 of the 3,261 arrayed tumors and were last updated in 2010. Median follow-up was 68.9 months ranging from 1 to 209 months. None of the patients received neoadjuvant or adjuvant therapy. Additional (salvage) therapy was initiated in case of a biochemical relapse. In all patients, prostate-specific antigen (PSA) values were measured quarterly in the first year, followed by biannual measurements in the second and annual measurements after the third year following surgery. Recurrence was defined as a postoperative PSA of 0.2 ng/mL and rising thereafter. The first PSA value above or equal to 0.2 ng/mL was used to define the time of recurrence. Patients without evidence of tumor recurrence were censored at the time of the last follow-up.

SNP-array analysis

A total of 72 snap-frozen prostate cancer samples with at least 70% tumor cell content and 5 prostate cell lines (LNCaP, VCaP, RWPE-1, PC-3, BPH-1) were selected for single-nucleotide polymorphism (SNP) array analysis. DNA was isolated using a commercial kit (QiAamp DNA Mini Kit, Qiagen). Affymetrix SNP V6.0 arrays were used for copy number analysis. Fragmentation, labeling, and hybridization of the DNA to the SNP arrays was carried out exactly as described in the Affymetrix V6.0 SNP array manual. We used our own genomic browser (FISH Oracle) to map all 5q21 deletions to the human genome reference sequence (Archive EnsEMBL release 54 – browser (FISH Oracle) to map all 5q21 deletions to the human genome reference because a centromere 5-speciﬁc FISH probe is not available. A 2-color ERG break-apart FISH probe consisting of 2 BAC clones one at each at 5’ ERG (spectrum-green labeled RP11-95I21 and RP11-360N24) and the other at 3’ ERG (spectrum-orange labeled RP11-720N21 and RP11-315E22) with approximately a 55-kb genomic gap between the 2 sets was made. The stained slide was manually interpreted with an epifluorescence microscope. In LNCaP cells, signals were defined as “normal” when 3 pairs of overlapping red and green signals were seen per cell nucleus. An ERG translocation was assumed if at least one split signal consisting of separate red and green signals was observed per cell nucleus. An interstitial deletion of 5’ ERG sequences was assumed if at least one green signal per cell nucleus was lost. Hybridization was conducted overnight at 37°C in a humidified chamber. Slides were subsequently washed and counterstained with 0.2 μm/L 4’-6-diamidino-2-phenylindole in antifade solution. Each spot was evaluated and the predominant signal numbers were recorded for each FISH probe.

CHD1 Expression analysis in tissue samples

For comparison of CHD1 expression levels in tumors with and without genomic CHD1 deletion, tissue blocks containing 70% or more tumor cells were selected that had been used for TMA manufacturing before. For RNA isolation, one 0.6 mm tissue core was taken from each tumor block. The deparaffinized and air-dried cores were grinded in liquid nitrogen before total RNA was isolated using a commercial kit (RNeasy FFPE kit #744044, QIAGEN) following the manufacturers instructions except for prolonged (overnight) proteinase digestion. cDNA was synthesized from 0.5 to 1 μg total RNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems #4366814). Quantitative reverse transcriptase PCR (qRT-PCR) was carried out in duplicate using combinations of primer pairs and TaqMan probes targeting mRNA sequences of CHD1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers were obtained from Applied Biosystems (Darmstadt). The GAPDH gene served as an internal control for the normalization of CHD1 RT-PCR products. The PCR program included a 10 minute denaturation at 95°C followed by 40 cycles of 15 seconds at 95°C, and 1 minute at 60°C. Relative quantification results were calculated according to the ΔΔCt method (26).

Cell culture, treatments, constructs, and lentivirus production

The LNCaP, BPH-1, PC-3, DU-145, RWPE-1, and VCaP prostate cell lines were cultured according to the supplier’s (LG Promochem, Wesel) instructions. Following expression constructs were used: pCMV6-XL4-CHD1 (Origene), PSG5L-HA-RB1 (21), PSG5L-HA PTEN (22), pLNGY (23). For depletion experiments, shRNA-expressing vectors based on lentiviral
pLKO.1 construct and part of the RNAi Consortium (TRC) vector collection were purchased from Sigma-Aldrich. The shRNAs used included the mature sense sequences for CHD1: GCCGGTTATACAGAGCTTAA, PTEN: CCACAGCTAGACCTATCAA, RB1: GTGGGCTTGGAGGTGTAAT and GAPDH: CCAACAGTGAAGACACCAA as well as Escherichia coli DNA polymerase TTATCCGCCATATATCGCG (24). Lentivirus supernatants were prepared after cotransfection into HEK-293T cells of a lentivirus vector plasmid with pSVS-G [expressing the vesicular stomatitis virus (VSV) envelope protein] pRev and pRRE and (expressing lentivirus helper functions), as described previously (23). Prostate cancer cells were transduced with lentiviruses expressing shRNAs directed against either shNeg (as a control) or GAPDH (as a control) or CHD1. Transduced target cells were selected with puromycin (1.5 μg/mL). For induction of chromosomal breaks, cells were treated dihydrotestosterone (DHT; 100 nmol/L), doxorubicin (1 μmol/L), or in combination for 48 hours. Thereafter cells were fixed with ice-cold methanol/acetone (1:1) for 10 minutes and subjected to FISH analysis. For gene expression analysis, LNCaP cells and its derivates were treated for 48 hours with DHT (100 nmol/L).

ChIP analysis

Chromatin immunoprecipitation (ChIP) analysis was conducted as described (25). In brief, cells were fixed in 1% formaldehyde for 10 minutes. Cells were lysed and sonicated to fragmentate. Insoluble debris was removed by centrifugation. The supernatant was diluted in ChIP buffer and equivalent amounts of input DNA incubated with primary antibody followed by the collection of immune complexes on magnetic beads. Immune complexes were washed in low and high salt ChIP buffer, the protein–DNA complex eluted, and the DNA–protein cross-links reversed by addition of NaCl, and heating at 65°C for 2 hours. After proteinase K digestion, DNA was transferred to nitrocellulose membranes by Western blotting. Proteins were resolved on SDS-PAGE gels and then transferred to nitrocellulose membranes by Western blotting. The following antibodies were used: anti-α-tubulin (Sigma-Aldrich), anti-AR (PG-21, Millipore), anti-CHD1 (C-8, Santa Cruz) antibodies, subjected to PCR, and then quantitated.

Western blot analysis and immunoprecipitation

Cells were seeded into 12-well dishes at 1 × 10⁵ cells per well. The following day, the cells were washed with PBS before harvesting in SDS-PAGE loading buffer. Tissue sections (2 × 10 μm thickness) from frozen prostate cancer specimens with a CHD1 deletion (n = 3) and with normal CHD1 copy numbers (n = 3) were lysed in SDS-PAGE loading buffer. Proteins were resolved on SDS-PAGE gels and then transferred to nitrocellulose membranes by Western blotting. The following antibodies were used: anti-α-tubulin (Sigma-Aldrich), anti-AR (PG-21, Millipore), anti-CHD1 (C-8, Santa Cruz), anti-PTEN (138G6, Cell Signaling), anti-RB1 (4H1, Cell Signaling), and anti-mTOR (Ab2732, Abcam). To test whether AR interacts with CHD1, LNCaP cells were lysed in radiolabeled immunoprecipitation assay buffer. Aliquots of lysates were subjected to immunoprecipitation using antibodies against AR and CHD1. Control IgG served as control. Immunocomplexes were captured from lysates by using DYNAL magnetic beads (Invitrogen). Immunoprecipitates were resuspended in loading buffer and subjected to SDS-PAGE.

Colony formation assay

This assay is based on the principle that expression of certain genes induces either cell-cycle arrest or cell death, hence result in a reduction in the colony number. BPH-1, DU-145, and PC-3 prostate cells were plated at about 2 × 10⁵ in 6-well plates. Cells were transfected with 4 μg of indicated plasmids using Lipofectamine 2000 (Life Technologies). Thirty six hours after transfection, cells were cultured in medium containing puromycin (1.5 μg/mL). Medium was replaced every 2 to 3 days with fresh medium containing the selection drug. Drug-resistant colonies appearing about 2 weeks later were fixed with methanol, stained with Giemsa, and counted.

Soft agar assay

A layer of 0.6% low-melting agarose in standard culture medium was prepared in 6-well plates. On top, a layer of 0.3% agarose containing 5 × 10⁵ PC-3-shCHD1 or shNeg control cells were plated. At day 14, cells were stained with crystal violet and colonies were counted.

Invasion assay

For invasion assay 1 × 10⁵ cells were resuspended in 0.5 mL of RPMI-1640 medium containing no serum and placed into the top chamber of Matrigel-coated Transwell inserts (BD Falcon). The bottom wells contained 0.75 mL medium supplemented with 10% FBS. After 24 hours, cells on the top surface of the fillers were removed with a cotton swab. Thereafter, filters were fixed and stained with crystal violet and photographed.

Total RNA extraction and TaqMan qPCR

Total RNA was extracted using Trizol and RNeasy system (Macherey-Nagel). RNA was reverse transcribed using the High Capacity cDNA Archive Kit (Applied Biosystems). Real-time reverse transcriptase PCR (RT-PCR) was carried out as described previously (26). For all other genes, Assay-on-Demand primer/probe sets supplied by Applied Biosystems were used (TMRPSS2: Hs01122331_m1; KLK4: Hs00191772_m1; FKBP5: Hs01561001_m1; SLC45A3: Hs02638322_m1; PPARγ: Hs00234592_m1; PS3: Hs01034249_m1; CHD1: Hs00154405_m1; RB1: Hs01078066_m1; PTEN: Hs02621230_s1; mTOR: Hs00234508_m1; AMACR: Hs01091294_m1; FOXO1: Hs01054576_m1; NKX3.1: Hs00171834_m1; ERG: Hs01554630_m1). Relative expression was calculated by normalization to a selected housekeeper mRNA (GAPDH) by the ΔΔCt method (26).

Statistical analysis

Statistical calculations were carried out with JMP 9 software (SAS Institute Inc.). Contingency tables and the χ²-test were conducted to search for associations between CHD1 deletion and tumor phenotype or other molecular markers. Survival curves were calculated according to Kaplan–Meier. The log-rank test was applied to detect significant survival differences between groups. ANOVA test was applied to compare CHD1 mRNA expression levels between tumors with and without
CHD1 deletion. COX proportional hazards regression analysis was conducted to test the statistical independence of CHD1 deletions.

Results and Discussion
Deletions of 5q21 have been reported to occur in 13% to 26% of prostate cancers (5–7). We determined the size of 5q21 deletions in 72 primary prostate cancers by SNP array analysis and found that the smallest commonly deleted region (8, 9) encompassed a 1.3 Mb interval containing the 2 genes RGMB and CHD1 (Fig. 1A). GISTIC analysis including all 9 cancers with 5q21 deletion confirmed CHD1 as the only significantly deleted gene inside the 5q21.1 region (P < 0.0001 at a cutoff q value of 0.25, Fig. 1B and Supplementary Table 1A). Of note, the

Figure 1. A, architecture of 5q21 deletions in 9 primary prostate cancers determined by Affymetrix SNP V6.0 array analysis. Green color indicates chromosomal areas with deletion and black color indicates normal copy numbers. The smallest commonly deleted region encompasses 1.3 megabases containing only the 2 genes RGMB and CHD1. B, chromosomal regions showing significant associations with 5q21 deletion by SNP array profiling generated by GISTIC analysis. The significance threshold (q value 0.25) is indicated by the green line. C, prostate cancer cell nucleus showing 2 red FISH signals for chromosome 10 and 1 green FISH signal for CHD1, corresponding to a heterozygous deletion of CHD1. D, prostate cancer cell nucleus showing normal copy numbers of centromere 10 and CHD1.
CHD1 Deletion in Prostate Cancer

GISTIC analysis excluded APC and MCC, which had been suggested as potential target genes before (4). The 5q21.1 deletion region identified in our study is virtually identical to the minimally deleted regions identified by Taylor and colleagues (5) and Huang and colleagues (9). Berger and colleagues (27) revealed mutations and intragenic breaks of CHD1 in 3 of 7 prostate cancers by means of deep sequencing, and Huang and colleagues reported one additional CHD1-mutated prostate cancer (9). Recent deep sequencing studies carried out by us (17) and others (27, 30, 31) including 191 prostate cancers revealed mutation CHD1 in only 4 (2%) tumors. Taken together, these findings strongly suggest that CHD1 mutation occurs only rarely, whereas genomic deletion is the major mode of CHD1 inactivation, and that gene dosage-dependent effects must be relevant in this tumor type. Further in support of CHD1 being the 5q21 tumor suppressor gene in prostate cancer, expression analysis of 16 prostate cancers with CHD1 deletion and 15 tumors with normal CHD1 copy numbers identified by FISH analysis in our study revealed significant downregulation of CHD1 mRNA and protein levels in tumors having 5q21 deletion (Fig. 2C and D).

To obtain further evidence for a tumor suppressor function of CHD1, we analyzed the effects of both shRNA-mediated CHD1 knockdown and forced overexpression on the ability to form colonies in one benign (BPH-1) and 2 highly malignant (PC-3 and DU-145) prostate cell lines. All 3 cell lines express CHD1 on mRNA and protein level (Supplementary Fig. S2). Known essential (PTEN) and nonessential (RB1) tumor suppressor genes were included for control. Depletion of CHD1 strongly inhibited colony formation in BPH-1, PC-3, and DU-145 cells (Fig. 2A and Supplementary Fig. 5A). In this assay, the effect was comparable with knockdown of PTEN in BPH-1 cells, showing that CHD1 is essential for prostate cell survival and growth. Likewise, overexpression of CHD1 resulted in complete growth abolishment in BPH-1 cells and in reduced colony formation in PC-3 and DU-145 cells, comparable with the effects seen with both PTEN and RB1 (Fig. 2B and Supplementary Fig. 5A). Similarly, depletion of
CHD1 in PC-3 cells resulted in reduced growth in soft agar and invasiveness (Supplementary Fig. 5B and 5C). Taken together, our findings are directly supported by 2 recent studies by Liu and colleagues and Huang and colleagues, who reported tumor suppressor properties of CHD1 while our study was under review (8, 9).

Although the loss of invasiveness and reduced growth in CHD1-depleted cells seem paradoxical for a tumor suppressor at first glance, also these findings are supported by previous work. Gaspar-Maia and colleagues (10) showed that acute CHD1 depletion strongly inhibits cell proliferation and colony formation. In addition, Nijhawan and colleagues identified

![Figure 3. Impact of CHD1 deletions on biochemical recurrence in all cancers (A) and in the subsets of ERG fusion-negative (B) and fusion-positive cancers (C).](image)

| Table 1. Association between heterozygous and homozygous CHD1 deletions and prostate cancer phenotype in all cancers as well as in the subsets of ERG-negative and ERG-positive cancers |
|-----------------|-----------------|-----------------|-----------------|
|                 | All cancers (N = 2,093) | CHD1 deletion (n = 985) | ERG fusion-positive cancers (n = 1,108) |
|                 | Hetero. del. (%) | Homo. del. (%) | P  | Hetero. del. (%) | Homo. del. (%) | P   | Hetero. del. (%) | Homo. del. (%) | P   |
| All samples     | 2093 6.7 2.0  | 1108 3.0 0.2  |    | 985 10.9 4.1  | 625 1.4 0.3 0.0002 |    | 403 1.5 0.0 0.0123 |
| Tumor stage     |                  |                  |    |                  |                  |    |                  |                  |    |
| pT2             | 1268 5.0 2.0  | 164 15.2 5.5  | 0.0064  | 643 8.4 3.6  | 533 2.4 0.0 |    | 403 1.5 0.0 0.0123 |
| pT3a            | 422 6.9 2.1  | 14 14.3 0.0  |    | 114 14.9 5.3  | 154 9.1 0.0 |    |                  |                  |    |
| pT3b            | 268 11.6 2.2  | 14 14.3 0.0  |    | 114 14.9 5.3  | 154 9.1 0.0 |    |                  |                  |    |
| pT4             | 29 13.8 0.0  | 14 14.3 0.0  |    | 114 14.9 5.3  | 154 9.1 0.0 |    |                  |                  |    |
| Gleason score   |                  |                  |    |                  |                  |    |                  |                  |    |
| 3–4             | 897 5.9 2.1  | 364 11.0 5.2  |    | 444 7.4 1.4  | 403 1.5 0.0 0.0123 |    | 533 2.4 0.0 |    |
| 4–6             | 207 13.0 5.8  | 100 18.0 12.0 |    | 364 11.0 5.2  | 533 2.4 0.0 |    | 533 2.4 0.0 |    |
| Nodal stage     |                  |                  |    |                  |                  |    |                  |                  |    |
| pN0             | 1064 7.7 2.9 | 26 26.9 3.9 | 0.145 | 477 13.0 6.3 | 107 8.4 0.0 |    | 26 26.9 3.9 |    |
| pN>0            | 65 9.2 0.0  | 30 26.9 3.9 |    | 477 13.0 6.3 | 107 8.4 0.0 |    | 477 13.0 6.3 |    |
| PSA level (ng/mL) |                  |                  |    |                  |                  |    |                  |                  |    |
| <4              | 297 6.4 2.0  | 208 12.0 6.3 | 0.4406 | 139 9.4 4.3 | 403 1.5 0.0 0.0123 |    | 403 1.5 0.0 0.0123 |    |
| ≥4–10           | 1087 5.7 1.8 | 208 12.0 6.3 |    | 139 9.4 4.3 | 403 1.5 0.0 0.0123 |    | 403 1.5 0.0 0.0123 |    |
| ≥20             | 415 7.7 3.1 | 67 13.4 3.0 |    | 208 12.0 6.3 | 403 1.5 0.0 0.0123 |    | 403 1.5 0.0 0.0123 |    |
| Surgical margin |                  |                  |    |                  |                  |    |                  |                  |    |
| Negative        | 1553 5.8 2.1 | 744 9.5 4.0 | 0.1821 | 190 13.7 4.2 | 244 4.1 0.0 |    | 244 4.1 0.0 |    |
| Positive        | 434 8.3 1.8  | 190 13.7 4.2  |    | 744 9.5 4.0  | 244 4.1 0.0 |    | 244 4.1 0.0 |    |
CHD1 as one of a large set of genes required for cell proliferation or survival, termed "Cyclops" (copy number alterations yielding cancer liabilities owing to partial loss; ref. 28). Taken together, these findings show that certain levels of CHD1 are essential for cell viability. The ability of clinical prostate cancers to survive and proliferate in the presence of 5q21 deletion may depend on the actual level of CHD1 down-regulation, but suggests adaptive mechanisms that provide a selection advantage to CHD1-defective tumors particularly in cancers carrying homozygous deletions. This notion is consistent with the finding that CHD1-depleted prostate cancers harbor additional collaborative genetic alterations (8). An analogous situation has been reported for PTEN, where complete inactivation triggers a p53-dependent fail-safe response inducing growth arrest and senescence both in vitro and in vivo, and which is overcome by defective p53 pathway (29).

To investigate the clinical relevance and biologic effects of CHD1 deletions, we carried out FISH analysis in more than 3,200 clinical prostate cancer specimens with full histopathologic and clinical follow-up data in a tissue TMA format. A commercial centromere 10 probe was used to control for hybridization, as a chromosome 5-specific reference probe is not available. We applied a stringent threshold of at least 60% of tumor cells showing absolute or relative CHD1 signal losses to define CHD1 deletion. Examples of CHD1 deleted and normal tumor cell nuclei are shown in Fig. 1C and D. CHD1 losses were present in 8.7% of 2,093 interpretable tumors, including 6.7% heterozygous and 2.0% homozygous deletions. This frequency is comparable with the 12.5% deletions found in our SNP array study and to the results of previous studies reporting 13% to 26% 5q21 deletions in prostate cancer (5–7). Because the vast majority (88.5%) of tumors with heterozygous CHD1 deletion showed one CHD1 gene copy in our study, it seems unlikely that the usage of a centromere 10 reference probe might have caused a significant number of false deletion calls.

CHD1 deletion was strongly linked to early PSA recurrence (P = 0.0038, Fig. 3) in univariate, however, not in multivariate analysis (P = 0.9530, Supplementary Table 2). Smaller studies correlating array-based comparative genomic hybridization data yielded inconclusive results (5, 9) with respect to the clinical significance of 5q21 deletion. In addition, CHD1 deletion was strongly linked to high Gleason grade (P < 0.0001) and advanced tumor stage (P = 0.0064), further supporting a role of CHD1 deregulation for tumor progression (Table 1). We also observed an association between the presence of CHD1 deletion and increased cell proliferation as measured by the Ki67 labeling index (P = 0.0002, Supplementary Table 3).

Most strikingly, CHD1 deletions were significantly more frequent in ERG fusion-negative (15%) as compared with fusion-positive (3.2%) cancers. In particular, homozygous deletions were almost exclusively found in fusion-negative tumors (Fig. 4A). Similar observations have been made first by Taylor and colleagues and confirmed Liu and colleagues, Barbieri and colleagues, and Grasso and colleagues (5, 8, 30, 31), who reported negative associations between 5q21 deletions and
deletions of 21q22 causing ERG fusion. Further in support of a negative association between CHD1 deletions and ERG fusion, Demichelis and colleagues failed to find 5q21 deletions in ERG fusion-positive prostate cancer (32). These findings suggest that CHD1 loss either might provide a selection advantage to ERG-negative tumor cells, or that CHD1 deletion causally prevents tumor cells from developing certain genomic alterations including ERG fusions, providing evidence for the existence of 2 mutually exclusive tumor subsets characterized by either CHD1 deletion or ERG fusion. To test the hypothesis that CHD1 prevents ERG rearrangements, we compared the capability of inducing DNA double-strand breaks leading to ERG rearrangements in control and CHD1-depleted LNCaP cells by doxorubicin/dihydrotestosterone treatment (33, 34) and ERG break-apart FISH (3). While control cells averaged 4.7 ± 0.3 ERG rearrangements per 100 nuclei, this rate was significantly reduced to 0.3 ± 0.3 per 100 nuclei in CHD1-depleted cells (P = 0.0008, Fig. 4B). To test whether ERG breakage may also result from nonspecific chromosomal damage independently from AR signaling, which is an important prerequisite for incidental chromatin breaks leading to TMPRSS2:ERG and other AR-dependent translocations (33, 34), we conducted analogous control experiments using AR-negative prostate cells. These experiments revealed incidental ERG breakage in only 0.5% to 1.5% of AR-negative BPH-1, DU-145, and PC-3 cells as compared with 12 of 300 (4%) breaks in AR-positive LNCaP
cells (BHP-1: \( P = 0.016, \) DU-145: \( P = 0.077, \) PC-3: \( P = 0.108)\) underscoring the role of AR for ERG fusion development and the specificity of ERG rearrangement. Taken together, these data show that a functional CHD1 supports ERG fusion development.

CHD1 deletions were also linked to other molecular alterations based on our GISTIC analysis, including deletions of 6q15 (\( P = 0.0009)\), 2q12.2 (\( P = 0.001)\), 5q13.1 (\( P = 0.001)\), and 13q14.2 (\( P = 0.005)\). Our results are in line with previous studies reporting codelletion of 5q21 and 6q15 (5, 8, 30, 31), including the putative target genes MAP3K7 (5–7) and CASP8AP2 (5, 7). Liu and colleagues also found a link between 5q21 and 2q22.1, which is, however, slightly different from the peak identified in our analysis (2q21.2; ref. 8). Together with the strong inverse association to ERG rearrangements, these findings pinpoint toward the existence of a distinct subgroup of ERG-negative prostate cancers, development of which is driven by the possible cooperative effects from inactivation of genes located at 5q21, 6q15, and 2q21. Of note, 5q21 deletions are not limited to prostate cancer, but have also been reported from lung, ovarian, and colorectal cancers (Tumorscape, http://www.broadinstitute.org/tumorscape/pages/portalHome.jsf), suggesting that this alteration has a broad tumor-relevant function in multiple human cancer types.

ERG fusion has been suggested to be an early molecular event, which might develop already in prostatic intraepithelial neoplasia (PIN), a precursor lesion of invasive cancer. Assuming that CHD1 deletion prevents the formation of ERG rearrangement, one would expect that CHD1 deletion is also an early event and, thus, detectable already in PIN lesions. To address this issue, we searched for high-grade PIN adjacent to early event and, thus, detectable already in PIN lesions. To further address the tumor suppressor function of CHD1, we studied its impact on the expression of well-known tumor suppressor genes in our analysis (2q21.2; ref. 8). Together with the strong inverse association to ERG rearrangements, these findings pinpoint toward the existence of a distinct subgroup of ERG-negative prostate cancers, development of which is driven by the possible cooperative effects from inactivation of genes located at 5q21, 6q15, and 2q21. Of note, 5q21 deletions are not limited to prostate cancer, but have also been reported from lung, ovarian, and colorectal cancers (Tumorscape, http://www.broadinstitute.org/tumorscape/pages/portalHome.jsf), suggesting that this alteration has a broad tumor-relevant function in multiple human cancer types.

Because CHD1 associates with chromatin to maintain it in an open and for transcription factors accessible state, we further hypothesized that CHD1 could be directly involved in AR binding to the promoters of AR target genes. We studied the effects of CHD1 on the activation of AR-responsive genes including TMPRSS2, SLC45A3, PPAR\(_g\), FKBP5, and KLK4 as well as AR-independent genes like SLC43A1 and TP53. This analysis revealed that CHD1 depletion strongly attenuated the DHT-induced expression of all tested AR-dependent, but not of the AR-independent target genes (Fig. 5A). In line with these findings, modulation of the AR-mediated transcription was recently reported for CHD8, another member of the CHD family (16). To test whether this effect was due to the impaired recruitment of AR to the promoters of AR-responsive genes, we carried out ChIP analysis and determined the AR occupancy at responsive promoters (PSA, TMPRSS2, FKBP5, ELK4, and KLK2) in control and CHD1-depleted LNCaP cells upon stimulation with DHT. AR was detectable at all tested promoters in control cells, but was completely absent at the promoters of TMPRSS2 and PSA, and reduced at the promoters of FKBP5 (1.5-fold decrease), ELK4 (5.5-fold), and KLK2 (1.8-fold) in CHD1-depleted cells (Fig. 5B). CHD1 was not detectable at promoters probed indicating that CHD1 is not coexisting with AR at the respective promoters (Fig. 5B). To test whether CHD1 mediates AR signaling through interaction, we immunoprecipitated endogenous AR from DHT- and nontreated LNCaP cells and blotted for CHD1. As shown in Fig. 5C there was no interaction between CHD1 and AR. This finding confirms the previous observation of Grasso and colleagues who did not find any interaction between CHD1 and AR (31).

Together, our data show that CHD1 is required for efficient activation of AR-dependent transcription, which in turn causes chromatin movements that predispose specific chromosomal loci to translocations (34).

To further address the tumor suppressor function of CHD1, we studied its impact on the expression of well-known tumor suppressor genes in prostate cancer. Importantly, CHD1 deletion caused a significant downregulation of both the constitutive and the AR-inducible expression of well-known tumor suppressor genes in prostate cancer, such as NKX3.1, FOXO1, and PPAR\(_g\), paralleled by an increase of dedifferentiation as shown.

Figure 6. Effect of CHD1 on expression of known tumor suppressor genes of prostate cancer (NKX3.1, FOXO1, and PPAR\(_g\)) and cell differentiation (AMACR) in LNCaP cells. The gene expression was determined by quantitative qRT-PCR and normalized against a reference gene (GAPDH; normalized virtual quantity).
by increasing AMACR expression (Fig. 6). Our data suggest an important interplay between CHD1 and transcription factors required for prostate-specific and tumor-suppressive gene expression patterns. These findings may also provide a biologic explanation for the adverse effects of CHD1 deletions observed in clinical cancer specimens. CHD1 does not seem to be a “classical” tumor suppressor, as it does not directly regulate cell growth. Rather, it acts as a “guardian of the chromatin conformation” that governs expression of genes controlling differentiation, proliferation, and tumor suppression.

In summary, our study identifies CHD1 as the tumor suppressor targeted by genomic deletions at 5q21 in prostate cancer. CHD1 inactivation abolishes recruitment of AR to responsive promoters, resulting in downregulation of AR-responsive genes. As an additional consequence, chromatin movements are abandoned which potentially cause AR-dependent rearrangements, including TMPRSS2:ERG fusion, in prostate cancer. Our study supports an important role of chromatin remodeling in prostate cancer biology.

References


CHD1 Is a 5q21 Tumor Suppressor Required for ERG Rearrangement in Prostate Cancer

Lia Burkhardt, Sarah Fuchs, Antje Krohn, et al.

Cancer Res  Published OnlineFirst March 14, 2013.