Universitätsklinikum Hamburg-Eppendorf

Institut für Pathologie

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CHD1 is a 5q21 Tumor Suppressor required for ERG-Rearrangement in Prostate Cancer

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
an der Medizinischen Fakultät der Universität Hamburg.

vorgelegt von:

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aus Hamburg

Hamburg 2013

Angenommen von der
Medizinischen Fakultät der Universität Hamburg am: 25.02.2014.

Veröffentlicht mit Genehmigung der
Medizinischen Fakultät der Universität Hamburg.

Prüfungsausschuss, der/die Vorsitzende: Prof. Dr. G. Sauter

Prüfungsausschuss, zweite/r Gutachter/in: PD Dr. T. Schlomm

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Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

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Cancer Res 2013;73:2795-2805. Published OnlineFirst March 14, 2013.

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

Molecular and Cellular Pathobiology

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

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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); 2795–805. ©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

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-12-1342

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

(14, 15). Furthermore *CHD*8, 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 *CHD*1 deletion, we analyzed more than 3,200 prostate cancers with molecular, pathologic, and clinical follow-up data for *CHD*1 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 *CHD*1 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 *CHD*1, and functional data showing a growth arrest in *CHD*1-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–May 2009) and to define the minimally overlapping region of deletion (19). In addition, Genomic Identification of Significant Targets in Cancer (GISTIC) analysis was carried out to identify significant peaks of deletion (20).

FISH

Four micrometer TMA sections were used for FISH. For proteolytic slide pretreatment, a commercial kit was used (paraffin pretreatment reagent kit; Vysis). TMA sections were deparaffinized, air-dried, and dehydrated in 70%, 85%, and 100% ethanol, followed by denaturation for 5 minutes at 74°C in 70% formamid 2× SSC solution. The FISH probe set consisted of a spectrum green-labeled *CHD1* probe (made from a mixture of BAC RP11-533M23 and BAC RP11-422M08, RZPD) covering the *CHD1* gene and repulsive guidance molecule B (*RGMB*; Supplementary Fig. S1), and a spectrum orange-labeled commercial centromere 10 probe (#06J36-090; Abbott, Wiesbaden) as a reference. Centromere 10 was selected for 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 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 μmol/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 #4368814). 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 $\Delta\Delta C_t$ 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

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

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*: GCGGTTTATCAAGAGCTATAA, *PTEN*: CCACAGCTAGAACTTATCAAA, *RB1*: GTGCGCTCTTGAGGTTGTAAT and *GFP*: CAACAAGATGAAGAGCACCAA as well as *Escherichia coli* DNA polymerase TTATCGCGCATATCACGCG (24). Lentivirus supernatants were prepared after cotransfection into HEK-293T cells of a lentivirus vector plasmid with pVSV-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 GFP (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 precipitated with immunoglobulin G (IgG; Sigma-Aldrich), AR (PG-21, Millipore), and 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 1×10^5 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 radioimmunoprecipitation 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 2×10^5 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 5×10^3 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 1×10^5 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 filters 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 (*TMPRSS2*: Hs01122331_m1; *KLK4*: Hs00191772_m1; *FKBP5*: Hs01561001_m1; *SLC45A3*: Hs00263832_m1; *PPARγ*: Hs00234592_m1; *P53*: 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 $\Delta\Delta C_t$ method (26).

Statistical analysis

Statistical calculations were carried out with JMP 9 software (SAS Institute Inc.). Contingency tables and the χ^2-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

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

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

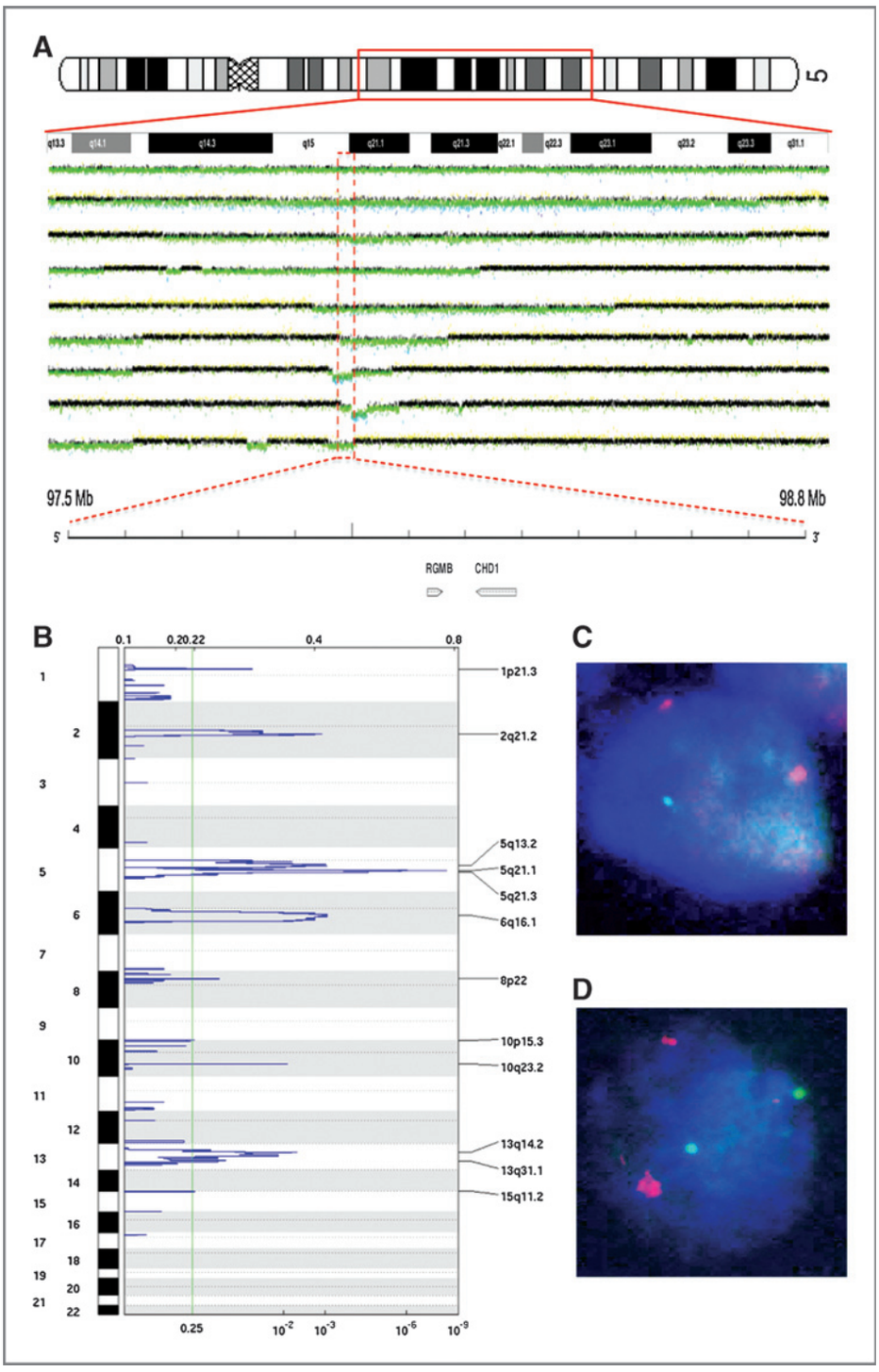

Figure 1. A, architecture of 5q21 deletions in 9 primary prostate cancers determined by Affymetrix SNP V6.0 array analysis. Green color indicates chromosomal areas with deletion and black color indicates normal copy numbers. The smallest commonly deleted region encompasses 1.3 megabases containing only 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*.

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

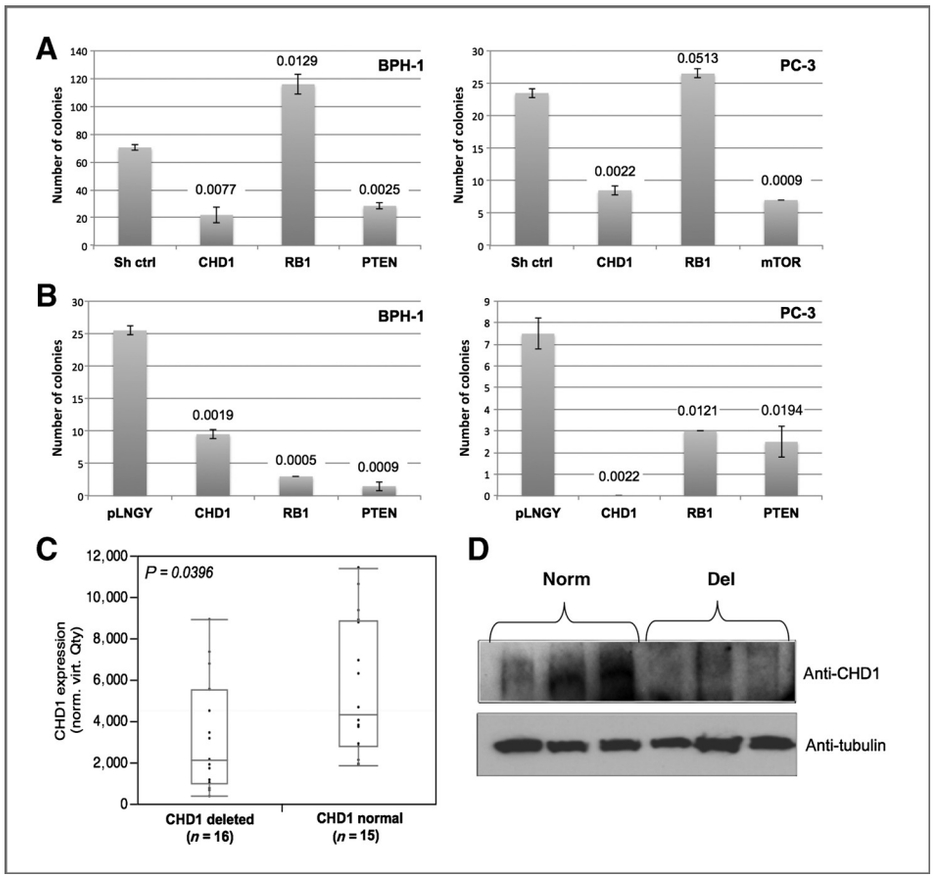

Figure 2. Effect of both *CHD1* depletion and overexpression cell growth. A, effect of *CHD1* depletion on cell growth. BPH-1 and PC-3 cells were transfected with a *CHD1*-targeting shRNA vector. The control group consisted of shGFP-expressing lentiviral vector pLKO (shGFP) and shRNA constructs against the known tumor suppressor genes *RB1* and *PTEN* (*PTEN* and *RB1-KO*). In PC-3 cells, which carry a PTEN deletion (35), we used mTOR as a surrogate control for an essential gene (36). Cells were selected with puromycin and cultured for 2 weeks, and colonies were fixed with methanol and stained with Giemsa. B, effect of *CHD1* overexpression on cell growth. BPH-1 and PC-3 cells were transfected with expression vectors encoding *CHD1*. The control group consisted of EYFP-expressing lentiviral vector pLKO PLNGY (control) and the known tumor suppressor genes *RB1* and *PTEN*. C, impact of *CHD1* deletions on *CHD1* mRNA expression in clinical prostate cancer specimens. D, impact of *CHD1* deletions on CHD1 protein expression in clinical prostate cancer specimens. Norm, CHD1 normal; Del (CHD1 deleted) by immunoblot analysis for CHD1 and tubulin.

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 *CHD*1 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

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

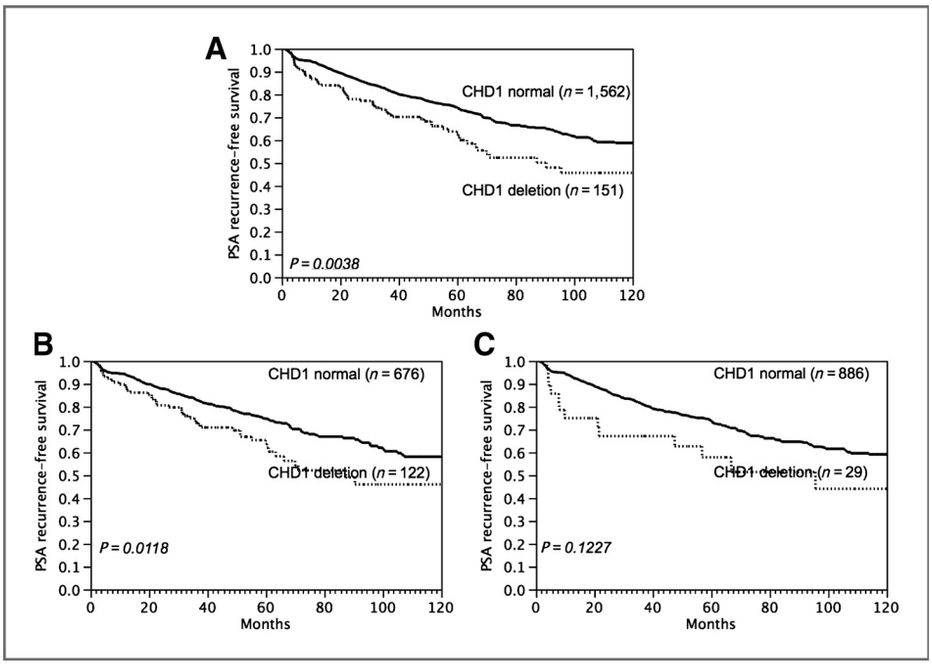

Figure 3. Impact of *CHD1* deletions on biochemical recurrence in all cancers (A) and in the subsets of ERG fusion-negative (B) and fusion-positive cancers (C).

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

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 ERG fusion-negative cancers (n = 985)				ERG fusion-positive cancers (n = 1,108)			
		n	Hetero. del. (%)	Homo. del. (%)	*P*	*n*	Hetero. del. (%)	Homo. del. (%)	*P*	*n*	Hetero. del. (%)	Homo. del. (%)	*P*
All samples		2093	6.7	2.0		985	10.9	4.1		1108	3.0	0.2	
Tumor stage	pT2	1268	5.0	2.0	0.0064	643	8.4	3.6	0.0549	625	1.4	0.3	0.0002
	pT3a	422	6.9	2.1		164	15.2	5.5		258	1.6	0.0	
	pT3b	268	11.6	2.2		114	14.9	5.3		154	9.1	0.0	
	pT4	29	13.8	0.0		14	14.3	0.0		15	13.3	0.0	
Gleason score	≤3+3	848	4.6	0.9	<0.0001	445	7.4	1.4	<0.0001	403	1.5	0.5	0.0123
	3+4	897	5.9	2.1		364	11.0	5.2		533	2.4	0.0	
	4+3	207	13.0	5.8		100	18.0	12.0		107	8.4	0.0	
	≥4+4	37	21.6	2.7		26	26.9	3.9		11	9.1	0.0	
Nodal stage	pN0	1064	7.7	2.9	0.145	477	13.0	6.3	0.1508	587	3.4	0.2	0.7578
	pN > 0	65	9.2	0.0		30	13.3	0.0		35	5.7	0.0	
PSA level (ng/μL)	< 4	297	6.4	2.0	0.4406	139	9.4	4.3	0.5697	158	3.8	0.0	0.6786
	4–10	1087	5.7	1.8		502	9.8	3.4		585	2.2	0.3	
	10–20	415	7.7	3.1		208	12.0	6.3		207	3.4	0.0	
	>20	149	8.1	1.3		67	13.4	3.0		82	3.7	0.0	
Surgical margin	Negative	1553	5.8	2.1	0.1821	744	9.5	4.0	0.2623	809	2.4	0.3	0.2235
	Positive	434	8.3	1.8		190	13.7	4.2		244	4.1	0.0	

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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* downregulation, 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

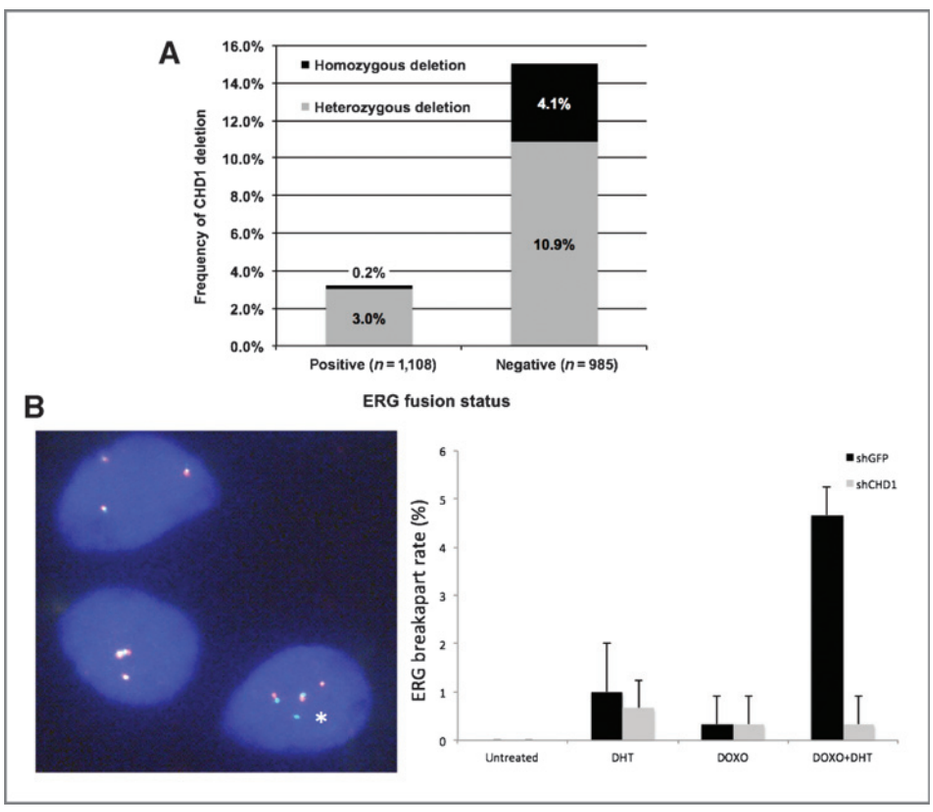

Figure 4. A, association between heterozygous and homozygous *CHD1* deletions and the ERG fusion status. B, induction of ERG translocation in LNCaP cells by the combined and single treatment with DHT and doxorubicin (DOXO). Presence of ERG translocation in the Chr 21-trisomic cell line LNCaP after dihydrotestosterone/doxorubicin treatment in one nucleus (asterisk, bottom right). The frequency of ERG translocations in shGFP and *CHD1*-depleted LNCaP (shCHD1) cells after treatment with dihydrotestosterone or doxorubicin alone (control) and with combined treatment (bottom right).

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

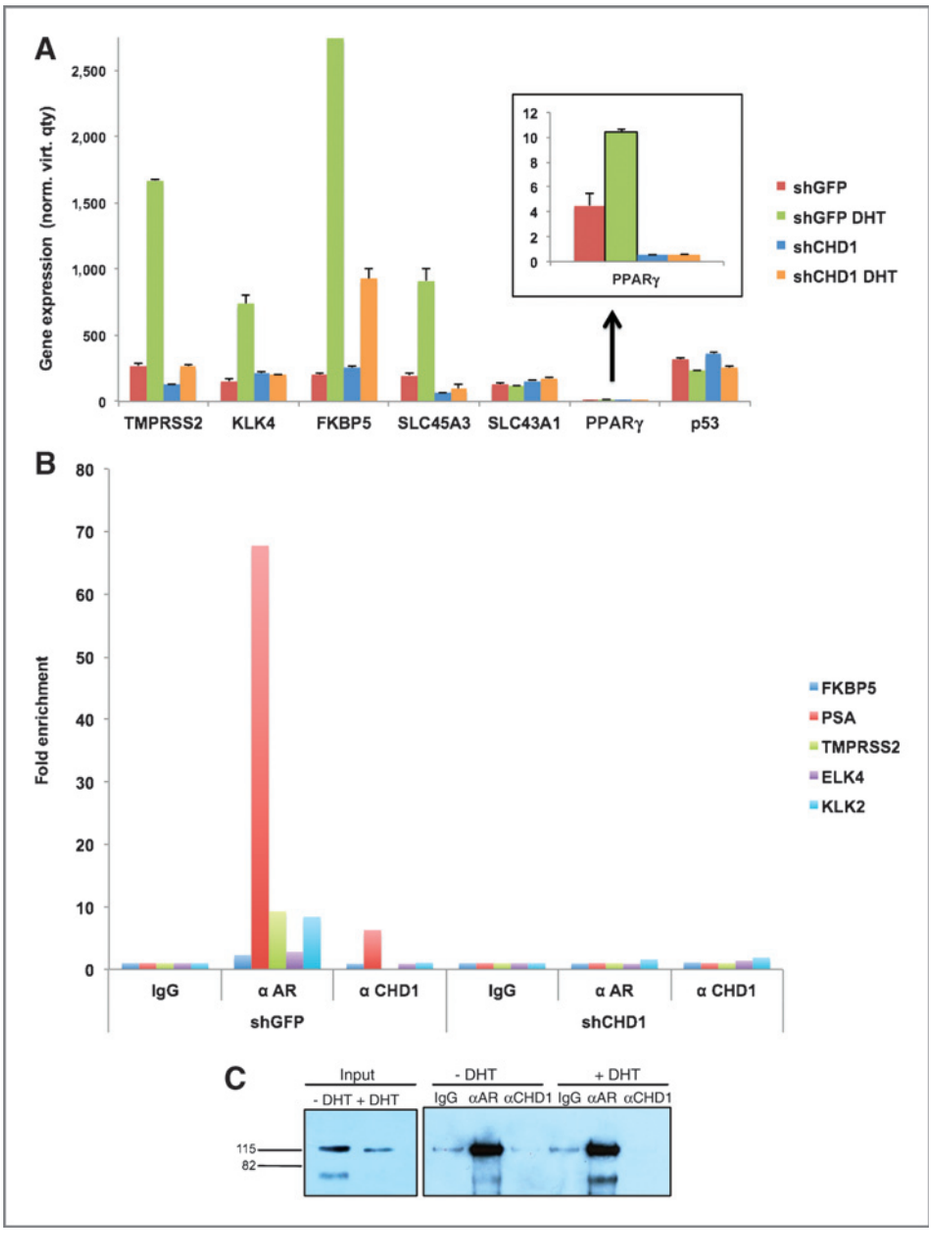

Figure 5. A, effect of *CHD1* depletion on the transcription of AR-dependent genes. To determine the expression status of AR-responsive genes as a function of *CHD1*, *CHD1*-depleted (shCHD1) and control (shGFP) LNCaP cells were treated with DHT, and gene expression was determined by TaqMan PCR. For example, the dihydrotestosterone stimulation in LNCaP cells leads as expected to a strong induction of AR-dependent genes known as *FKBP5* and *TMPRSS2 SCL45A3*, but not of non-AR–regulated genes such as *SLC45A1*. B, AR recruitment to promoters of AR-responsive genes requires *CHD1*. LNCaP-shGFP and -sh*CHD1* cells were stimulated for 12 hours with or without dihydrotestosterone. Chromatin was cross-linked, cells were lysed, and then subjected to ChIP with indicated antibodies. The bound DNA was analyzed with primers specific for the presence of promoter sequences of indicated genes by PCR. IgG precipitates served as a control. Binding to the indicated promoters is presented as fold enrichment above the level of the IgG control (100%). C, interaction of CHD1 with the AR. AR and CHD1 (or IgG as control) was immunoprecipitated from untreated or dihydrotestosterone-treated LNCaP lysates and blotted for AR. Total lysates were used as input control.

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

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

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 ($P = 0.0009$), 2q21.2 ($P = 0.001$), 5q13.1 ($P = 0.001$), and 13q14.2 ($P = 0.005$). Our results are in line with previous studies reporting codeletion 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 the invasive cancer in our *CHD1*-deleted cancers. Four tumors matched this criterion. Large section FISH analysis revealed *CHD1* deletion already in the PIN of 2 of these tumors, showing that *CHD1* deletion may in fact develop early (Supplementary Fig. S6). This is consistent with an early and causal role of *CHD1* loss for development of *ERG* fusion-negative tumors.

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*, *PPARγ*, *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 coresiding with AR at the respective promoters (Fig. 5B). To test whether CHD1 mediate 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).

Taken 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* depletion caused a significant downregulation of both the constitutive and the AR-inducible expression of well-known tumor suppressors in prostate cancer, such as *NKX3.1*, *FOXO1*, and *PPARγ*, paralleled by an increase of dedifferentiation as shown

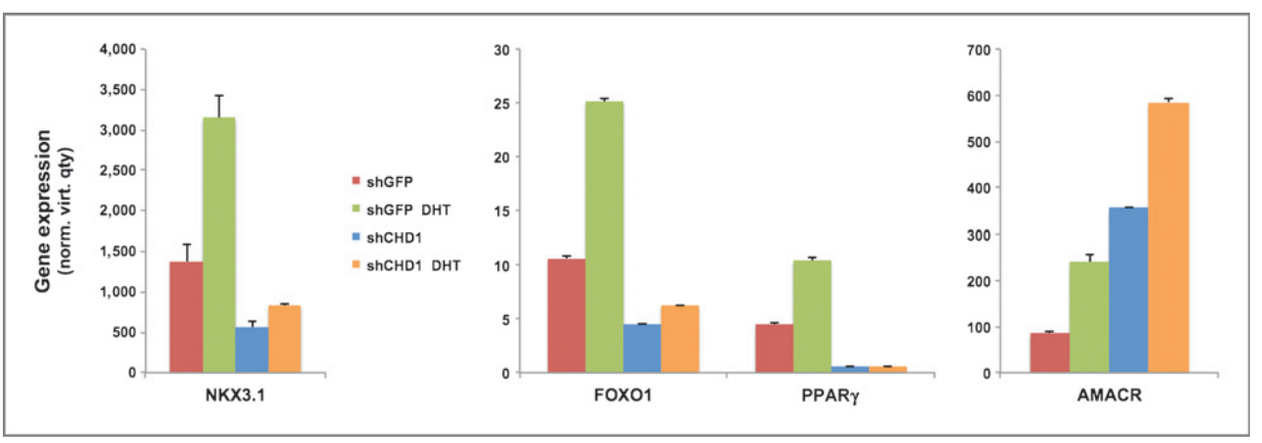

Figure 6. Effect of *CHD1* on expression of known tumor suppressor genes of prostate cancer (*NKX3.1*, *FOXO1*, and *PPARγ*) and cell differentiation (AMACR) in LNCaP cells. The gene expression was determined by quantitative qRT-PCR and normalized against a reference gene (*GAPDH*; normalized virtual quantity).

Published OnlineFirst March 14, 2013; DOI: 10.1158/0008-5472.CAN-12-1342

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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Conception and design: A. Krohn, M. Graefen, T. Schlomm, G. Sauter, H. Sirma, R. Simon
Development of methodology: A. Krohn, H. Sirma
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Burkhardt, S. Fuchs, A. Krohn, S. Masser, M. Kluth, F. Bachmann, T. Steuber, M. Graefen, T. Schlomm, S. Minner, R. Simon
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Burkhardt, S. Fuchs, A. Krohn, M. Mader, M. Kluth, F. Bachmann, T. Steuber, T. Schlomm, S. Minner, H. Sirma, R. Simon
Writing, review, and/or revision of the manuscript: L. Burkhardt, M. Graefen, T. Schlomm, G. Sauter, H. Sirma, R. Simon
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Graefen, T. Schlomm, R. Simon
Study supervision: H. Huland, H. Sirma, R. Simon

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Received April 9, 2012; revised December 21, 2012; accepted January 28, 2013; published OnlineFirst March 19, 2013.

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2. Darstellung der Publikation

Einleitung

Das Prostatakarzinom ist mit 63.400 Neuerkrankungen im Jahre 2008 die häufigste Krebserkrankung des Mannes in der Bundesrepublik Deutschland (1). Es ist mit einem mittleren Erkrankungsalter von 70 Jahren (1) vor allem eine Erkrankung des älteren Mannes. Mit einer Mortalität von 30 Fällen pro 100.000 Einwohner (1) stellt es die dritthäufigste Todesursache unter den malignen Erkrankungen dar. Durch seinen langsamen Verlauf kann das Prostatakarzinom lange Zeit klinisch stumm bleiben und erst im fortgeschrittenen Stadium symptomatisch werden. Die Therapie der Wahl in frühen Stadien (T1 und T2) ist die radikale Prostatektomie mit den einhergehenden Komplikationen der postoperativen Impotenz und Harninkontinenz. Aufgabe der Früherkennung sollte es demnach sein, diejenigen Patienten, die von einer solchen radikalen Operation profitieren, von denjenigen zu unterscheiden, denen durch eine Operation mehr Schaden zugefügt wird als durch das Karzinom selbst. Prostatakarzinome haben unbehandelt oft einen langsamen Verlauf, so dass Menschen oft nicht an, sondern mit einem Prostatakarzinom sterben. Anerkannte prognostische Marker sind klinische und pathologische Parameter wie präoperatives PSA, der Gleason-Grad und das TNM-Stadium. Es ist demnach von hoher klinischer Relevanz, molekulare Marker zu finden, die eine Unterscheidung zwischen niedrig malignen und aggressiven Tumoren zulassen, um eine Überbehandlung der Patienten zu vermeiden. Es wurden bisher eine Reihe von genetischen Veränderungen entdeckt, welche eventuell eine individuelle Voraussage zulassen (2). Die Fusion des androgenregulierten Gens TMPRSS2 mit dem Transkriptionsfaktor ERG ist die häufigste chromosomale Alteration beim Prostatakarzinom. Sie kann in der Hälfte aller Prostatakarzinome nachgewiesen werden (3). Diese auf Chromosom 21 liegende Genfusion spielt eine wichtige Rolle bei Zellproliferation, Differenzierung, Angiogenese, Entzündung sowie Apoptose (4). Deletionen im chromosomalen Bereich 5q21 gehören mit einer Deletionsfrequenz von 13-26% zu den häufigsten genetischen Veränderungen im Prostatakarzinom. Es konnte eine kleine gemeinsam deletierte Region auf diesem chromosomalen Bereich identifiziert werden, die das für chromodomain helicase DNA binding protein 1 (CHD1) kodierende Gen enthält (5-7). CHD1 unterstützt die Anordnung, Umlagerung und Entfernung von Nucleosomen von der DNA Doppelhelix, so dass diese in einer offenen und transkriptionsbereiten Form bleibt (8). Das Protein CHD1 ist somit essenziell für das Chromatinremodelling. Mehrere der anderen Mitglieder der CHD Familie wurden schon mit Krebserkrankungen in Verbindung gebracht. CHD2 wurde als mutmaßlicher Tumorsuppressor vorgeschlagen, der eine Rolle bei der DNA Reparatur während der Lymphomentwicklung spielen könnte (9). Heterozygote frameshift Mutationen oder Verlust der Heterozygotität von CHD1, CHD2, CHD3, CHD4, CHD7 und CHD8 wurden in Magen- und Darmkrebs gefunden (10). Eine Mutation von CHD7 wurde in einer Lungenkrebszelllinie entdeckt (11). CHD5 ist ein bekannter Tumorsuppressor, der die Apoptose mit Hilfe der p19-p53 Signalkaskade einleitet (12, 13). CHD8 spielt eventuell eine Rolle in der androgenrezeptorabhängigen Transkriptionsregulation in Prostatakarzinomen (14). Prostatakarzinome zeigen häufig eine Hochregulation von Androgenrezeptoren im Vergleich zum Normalgewebe, so dass Testosteron das Tumorwachstum fördert (15). Die vorliegende Studie soll an einem Kollektiv von über 3.200 Prostatakarzinomen mit klinischen Verlaufsdaten klären, wie häufig die 5q21 Deletion tatsächlich vorkommt und ob sie mit klinisch-pathologischen Parametern dieses Tumortypes assoziiert ist. Außerdem soll geklärt werden, inwieweit eine CHD1 Deletion mit einer ERG-Fusion assoziiert ist und ob CHD1 direkt auf die Fähigkeit, ERG-Fusionen zu entwickeln, Einfluss nimmt.

Material und Methoden

Patientenkollektiv und Gewebemikroarray

Die Studie schließt Patienten ein, die zwischen den Jahren 1992 und 2005 in der Martini-Klinik des Universitätsklinikums Hamburg-Eppendorf eine radikale Prostatektomie bei Prostatakarzinom erhalten haben. Für insgesamt 2.891 Patienten waren weitere klinische Parameter wie Alter, PSA-Verlauf, TNM-Stadium, Gleason-Score und Überlebenszeit vorhanden. Die makroskopischen Prostatektomiepräparate wurden standardisiert analysiert und zugeschnitten. Ein repräsentativer 0,6 mm durchmessender Zylinder wurde aus den so ermittelten Indextumoren ausgestanzt und auf einen Gewebemikroarray transferiert. Die knapp 3.000 Tumorproben konnten so auf 7 Objektträger verteilt werden (16).

Fluoreszenz *in-situ*-Hybridisierung

Die Fluoreszenz *in-situ*-Hybridisierung (FISH) ist eine molekularbiologische Methode zum Nachweis pathologischer Genstrukturen. Spezifische DNA-Abschnitte können hierbei mit Hilfe fluoreszenzmarkierter Sonden nachgewiesen werden. Die Auswertung erfolgte an einem Fluoreszenzmikroskop. In dieser Studie wurde eine orangemarkierte CHD1 Sonde und eine grün markierte CEP 10 Sonde verwendet. Ein normaler Status wurde definiert als das Vorhandensein zweier grüner Signale für die Zentromerregion und zweier orangefarbener Signale für beide CHD1 Genregionen. Eine heterozygote Deletion wurde definiert als das Vorhandensein zweier grüner und nur eines orangefarbenen Signals. Bei einer homozygoten Deletion ist im Tumor kein orangenes CHD1- Signal zu sehen, in den Stromazellen hingegen schon. Um ERG-Fusionen nachzuweisen, wurde eine ERG break apart Sonde mit einer grünen Sonde am 5´Ende und einer orangefarbenen Sonde am 3´Ende benutzt. Die Zellen wurden als normal eingestuft, wenn die grünen und orangefarbenen Signale unter dem Mikroskop übereinander liegend zu sehen waren. Ein Bruch wurde angenommen, wenn wenigstens ein einzelnes grünes oder orangenes Signal pro Zellkern identifiziert werden konnte. Eine genauere Beschreibung der Methoden findet sich in der beiliegenden Publikation.

Resultate

Um die klinische Relevanz von CHD1 Deletionen zu untersuchen, wurden über 3.200 Prostatakarzinome mittels FISH analysiert und der so bestimmte Deletionsstatus mit klinischen Parametern korreliert. Es konnte eine Deletionsrate von 8,7% bei 2.093 interpretierbaren Tumoren gefunden werden, darunter 6,7% heterozygote und 2,0% homozygote Deletionen. Die Ergebnisse der Arbeit sind in der eingefügten Publikation zusammengefasst. Die wesentlichen Befunde sind:

1. Der CHD1-Deletionsstatus korreliert mit der Prognose des Karzinoms.
2. Der CHD1-Deletionsstatus korreliert mit der Abwesenheit einer ERG-Fusion.
3. Die Inaktivierung von CHD1 in-vitro verhindert eine ERG-Translokation.

Diskussion

CHD1 ist in der kleinsten gemeinsam deletierten Region der untersuchten Prostatakarzinome lokalisiert. Expressionsanalysen von Tumoren mit CHD1 Deletionen und solchen ohne CHD1 Deletionen zeigten eine signifikante deletionsbedingte Herabregulierung von CHD1 auf mRNA- und Proteinebene.

Um weitere Beweise für die tumorsuppressiven Eigenschaften von CHD1 zu finden, haben wir den Einfluss von CHD1 auf die Fähigkeit, Kolonien in benignen (BPH-1)) sowie malignen (PC-3, DU-145) Zellreihen zu formen, analysiert. Alle drei Zellreihen exprimieren CHD1 auf mRNA und Proteineben. Als Kontrolle wurden die bekannten Tumorsuppressorgene PTEN und RB1 benutzt. Eine Depletion, d.h. Eine Unterdrückung der Expression von CHD1 verhinderte die Bildung von Kolonien in BPH-1, PC-3 und DU-145. Es wird deutlich, dass CHD1 essenziell für Wachstum und Überleben von Prostatazellen ist. Die Überexpression resultierte in der Aufhebung des Wachstums in den BPH-1- Zellen sowie in reduzierter Koloniebildung in den malignen Zelllinien. CHD1 besitzt also tumorsuppressive Eigenschaften.

Um die klinische Relevanz von CHD1 Deletionen zu untersuchen, wurden über 3.200 Prostatakarzinome mittels FISH analysiert und der so bestimmte Deletionsstatus mit klinischen Parametern korreliert. Es konnte eine Deletionsrate von 8,7% in 2.093 interpretierbaren Tumoren gefunden werden, darunter 6,7% heterozygote und 2,0% homozygote Deletionen. Das Auftreten einer CHD1 Deletion ist mit einem frühen Wiederanstieg des PSA- Wertes, einem hohen Gleason-Score und einem fortgeschrittenem Tumorstadium assoziiert. Zusätzlich zeigte sich eine Assoziation zwischen CHD1 Deletionen und einer erhöhten Zellproliferationsrate anhand des Ki67-Index.

Bemerkenswert ist, dass CHD1 Deletionen dramatisch häufiger in Tumoren ohne ERG-Fusion (15%) als in Tumoren mit ERG-Fusion (3,2%) gefunden wurden. Insbesondere homozygote Deletionen wurden nur in ERG-negativen Tumoren gefunden. Diese Erkenntnis legt die Vermutung nahe, dass ein Verlust von CHD1 entweder einen Selektionsvorteil für ERG-negative Tumorzellen darstellt, oder dass eine CHD1 Deletion einen direkten Einfluss auf die Entwicklung von chromosomalen Veränderungen hat. Dies wiederum suggeriert die Existenz zweier von einander unabhängiger molekularbiologischer Tumorsubtypen, die entweder durch CHD1 Deletion oder ERG-Fusion charakterisiert sind. Um die Hypothese zu testen, dass CHD1 ERG-Fusionen verhindert, haben wir die Fähigkeit, DNA-Doppelstrangbrüche, eine wichtige Vorraussetzung für die Entstehung von Tumoren, zu induzieren, bei CHD1 deletierten und nicht deletierten Zellen verglichen. Die Doppelstrangbrüche wurden mit dem interkalierenden Zytostatikum Doxorubicin induziert und mittels einer ERG-break apart-Sonde nachgewiesen. Die Kontrollzellen zeigten im Durchschnitt 4,7 ERG-Brüche pro 100 Zellkernen. In den CHD1 deletierten Zellen war diese Rate mit 0,3 Brüchen pro 100 Kernen signifikant vermindert. Um herauszufinden, ob der Bruch der ERG-Region auch durch unspezifische chromosomale Schädigungen hervorgerufen werden kann, die nicht androgenrezeptorabhängig (AR) sind, haben wir noch eine Kontrolle mit AR-negativen Zellen durchgeführt. Es wurden nur in 0,5-1,5% der AR- negativen Zellen, aber in 4% der AR-positiven Zellen ERG-Brüche gefunden, was die Rolle der Androgenrezeptoren bei der ERG-Fusion unterstreicht. Die Zusammenschau dieser Daten zeigt, dass CHD1 eine wichtige Rolle bei der Entstehung der ERG-Fusion spielt.

Es wird angenommen, dass die Entwicklung einer ERG-Fusion im Rahmen der Kanzerogenese ein frühes Ereignis ist und somit schon in prostatischer intraepithelialer Neoplasie (PIN) nachgewiesen werden kann. Aufgrund der Annahme, dass CHD1 Deletionen eine ERG-Fusion verhindern, ist davon auszugehen, dass die Entstehung einer CHD1 Deletion auch ein frühes Ereignis und damit in PIN nachzuweisen ist. Um dies zu untermauern, haben wir in den CHD1 deletierten Tumoren nach PIN, die an invasive Karzinome angrenzen, gesucht. Vier Tumoren konnte dies Kriterium erfüllen. Die FISH-Analyse zeigte, dass schon in zwei dieser PIN eine CHD1-Deletion nachzuweisen war. Es ist also davon auszugehen, dass der Verlust eines CHD1 Allels ein frühes Ereignis ist.

CHD1 bindet an Chromatin, so dass dieses offen und für Transkriptionsfaktoren zugänglich ist. Wir stellten die Hypothese auf, dass CHD1 direkten Einfluss auf die Bindung zwischen AR und den Promotern der AR Zielgene nimmt. Wir haben den Einfluss von CHD1 auf die Aktivierung der AR responsiven Gene TMPRSS2, SLC45A3, PPARgamma, FKBP5 und KLK4 sowie der AR unabhängigen Gene SLC43A1 und TP53 analysiert. Es zeigte sich, dass eine CHD1 Depletion die Expression aller AR responsiven Gene abschwächt, die Expression der AR unabhängigen Gene aber nicht beeinflusst. Die Beeinflussung der AR vermittelten Transkription wurde schon für CHD8 beschrieben (17).
Um zu testen, ob dieser Effekt auf der verminderten Rekrutierung des AR zu den Promotern zurückzuführen ist, haben wir eine ChIP-Analyse durchgeführt und die Besetzung von AR an responsiven Promotern in CHD1 depletierten Zellen sowie einer Kontrolle mit normaler CHD1-Expression bestimmt. In der Kontrolle konnte AR an den Promotern nachgewiesen werden, hingegen war AR an den Promotern CHD1 depletierter Zellen vermindert oder nicht vorhanden. Alles in allem zeigt unsere Studie, dass CHD1 für eine effiziente Aktivierung der AR-abhängigen Transkription, welche wiederum Chromatinbewegungen verursacht, die für chromosomale Translokationen bestimmter Loci prädisponieren, benötigt wird.

Um weitere tumorsuppressive Eigenschaften von CHD1 zu ermitteln, haben wir seinen Einfluss auf die Expression bekannter Tumorsuppressorgene in Prostatakarzinomen untersucht. CHD1-Depletionen verursachten eine signifikante Herabregulierung sowohl der konstitutiven als auch der AR-abhängigen Expression der bekannten Tumorsuppressorgene NKX3.1, FOXO1 und PPARgamma. Zusätzlich konnte mit Hilfe der Bestimmung der Alpha-methylacyl-CoA racemase (AMACR) eine Zunahme der Entdifferenzierung nachgewiesen werden. AMACR ist ein bei Prostatakarzinomen überexprimierter Biomarker (18). Die Datenauswertung legt ein für die Expression von prostataspezifischen Tumorsuppresorgenen wichtiges Zusammenspiel zwischen CHD1 und Transkriptionsfaktoren nahe. Diese Erkenntnis mag auch die ungünstigen biologischen Eigenschaften, die CHD1 deletierte Tumoren in der Klinik aufweisen, erklären. CHD1 scheint kein klassischer Tumorsuppressor zu sein, da es keinen direkten Einfluss auf das Zellwachstum hat. Viel eher spielt es eine Rolle bei der Anordnung des Chromatins und nimmt somit Einfluss auf Differenzierung, Proliferation und Tumorsuppression.

Zusammenfassend wurde CHD1 in unserer Studie als wichtiger Tumorsuppressor der 5q21 Deletion in Prostatakarzinomen identifiziert. Inaktivierung von CHD1 verhindert die Bindung von AR an responsive Promoter, so dass AR-responsive Gene herabreguliert werden. Hierdurch werden AR-abhängige Chromatinbewegungen vermindert, so dass potentiell AR- abhängige Rearrangments wie die ERG-Fusion nicht mehr entstehen können.

Der Einsatz von CHD1 als neuer Biomarker beim Prostatakarzinom steckt noch in den Anfängen. Um zu einer ernstzunehmenden Alternative bei der Krebsfrüherkennung zu werden, muss es zur klinisch-praktischen Anwendung kommen. Die Patienten profitieren von einer Früherkennung, wenn die Methode nicht invasiv, für die zu untersuchende Krankheit spezifisch ist sowie eine Behandlungskonsequenz mit sich führt. Es bietet sich jedoch an, den CHD1- Deletionsstatus in bioptisch diagnostizierten PIN zu bestimmen. Da eine CHD1- Deletion eine frühe molekularbiologische Veränderung auf dem Weg zum invasiven Karzinom zu sein scheint, kann so potentiell vorhergesagt werden, ob eine PIN die Tendenz hat, sich schnell zu einem invasiven Karzinom zu entwickeln. So könnten diejenigen Patienten, denen eine radikale Operation das Überleben sichert von denjenigen unterschieden werden, bei denen der Tumor eher langsamer wächst und denen somit mit einer Therapie des „active surveillance“ am Besten gedient wäre.

Zusammenfassung

Deletionen im chromosomalen Bereich 5q21 gehören zu den häufigsten genetischen Veränderungen im Prostatakarzinom. Wir konnten dort eine 1.3 Megabasen umfassende Region identifizieren, die das Gen für den Tumorsupressor CHD1 enthält. Die erfolgreiche FISH-Analyse von 2.093 Prostatakarzinomen zeigte eine starke Assoziation zwischen CHD1 Deletionen und schlechter Prognose für den Patienten ($p=0.0038$) sowie Abwesenheit der ERG-Fusion ($p<0.0001$). Es zeigte sich weiter, dass die Inaktivierung von CHD1 *in vitro* durch Beeinträchtigung der AR-abhängigen Transkription eine ERG-Translokation verhindert. Unsere Studie identifiziert CHD1 als wichtigen Tumorsuppressor im Bereich 5q21 und weist ihm eine Schlüsselrolle beim Chromatinremodelling zu.

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3. Erklärung des Eigenanteils an der Publikation

- Analyse und Auswertung der Fluoreszenz-*in-situ*-Hybridisierung
- Literaturrecherche
- Schreiben der Publikation zusammen mit Ronald Simon, Prof. Sauter und seinem Team
- Schreiben der Doktorarbeit

4. Danksagung

An dieser Stelle danke ich Herrn Professor Sauter für die Möglichkeit, in seinem Institut zu promovieren. Professor Sauter war stets ansprechbar und hat mein Projekt zuverlässig begleitet. Ohne sein wertvolles Fachwissen wäre diese Arbeit nicht entstanden. Mein herzliches Dankeschön auch an alle Personen im Institut für Pathologie, die hier nicht namentlich erwähnt werden, aber zur Realisierung dieses Projektes beigetragen haben.

Mein besonderer Dank gilt dem gesamten TMA-Laborteam. Ich danke Lia Burkhardt und Martina Kluth für die Tage und Nächte, die wir gemeinsam vor den Mikroskopen verbracht und diskutiert haben, Antje Krohn für ihr Wissen über die FISH Auswertung, Bianca Kelp für die zuverlässige Herstellung der Slides, Ronald Simon für seine konstruktive Kritik an meinen Ergebnissen und Sawinee Masser für schöne Gespräche bei einer Tasse Tee.

Ich danke auch besonders meinen Eltern, die mich während meines Studiums und der Promotion stets unterstützt haben. Sie haben mir einen Ort gegeben, an dem ich zur Ruhe kommen konnte und mir diesen Lebensweg ermöglicht.

Vielen Dank auch an Sandra Prues, die mir während einer sehr schwierigen Lebensphase beigestanden hat.

5. Eidesstattliche Erklärung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe. Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....