Prostate Cancer Genetic-susceptibility Locus on Chromosome 20q13 is Amplified and Coupled to Androgen Receptor-regulation in Metastatic Tumors

David P. Labbé1,2, David G. Nowak4, Geneviève Deblois1,3, Laurent Lessard6, Vincent Giguère1,2,3, Lloyd C. Trotman4, and Michel L. Tremblay1,2,3

Abstract

The 20q13 chromosomal region has been previously identified as the hereditary prostate cancer genetic-susceptibility locus on chromosome 20 (HPC20). In this study, the 20q13 region was shown to be significantly co-amplified with the androgen receptor (AR) in metastatic prostate cancer. Furthermore, the AR signaling axis, which plays an essential role in the pathogenesis of prostate cancer, was demonstrated to be central to the regulation of the 20q13 common amplified region (CAR). High-resolution mapping analyses revealed hot spots of AR recruitment to response elements in the vicinity of most genes located on the 20q13 CAR. Moreover, amplification of AR significantly co-occurred with CAR amplification on 20q13 and it was confirmed that the majority of AR-bound genes on the 20q13 CAR were indeed regulated by androgens. These data reveal that amplification of the AR is tightly linked to amplification of the AR-regulated CAR region on 20q13. These results suggest that the cross-talk between gene amplification and gene transcription is an important step in the development of castration-resistant metastatic disease.

Implications: These novel results are a noteworthy example of the cross-talk between gene amplification and gene transcription in the development of advanced prostate cancer.

Visual Overview: http://mcr.aacrjournals.org/content/early/2014/02/07/1541-7786.MCR-13-0477/F1.large.jpg. Mol Cancer Res; 12(2); 184–9. ©2013 AACR.

Introduction

Prostate cancer is the most frequent cancer in North American men and the second leading cause of cancer-related deaths. Age, African ancestry, and diet are among the known risk factors contributing to prostate cancer development. In addition, evidence based on case-control, cohort, twin, and family studies demonstrates that prostate cancer is also a genetic disease. Men with a history of familial or hereditary prostate cancer have a 2- to 7-fold increased risk of developing the disease (1). In fact, a positive family history is one of the strongest risk factors for prostate cancer and it is linked to approximately 10% to 15% of cases (2).

At least 15 different loci located on 10 distinct chromosomes have been linked to hereditary prostate cancer (3), but there is no single highly penetrant prostate cancer susceptibility gene identified to date. Instead, the heredity of prostate cancer is attributable to a large number of genes that have small effect(s) on their own, further illustrating the heterogeneity of the disease (2). In addition, recent genome-wide association studies revealed a minimum of 30 common genetic loci associated with prostate cancer risk, making this disease the most prolific of all cancers in terms of common susceptibility loci. However, there is no clear evidence that the various loci associated with the risk of developing prostate cancer are also associated with either aggressiveness or mortality (4).

Current techniques enable the detection and treatment of most early stage tumors. Still, androgen-deprivation therapy targeting androgen receptor (AR) transcriptional activity has remained the first line of treatment for advanced disease since its description in 1941, although it ultimately leads to incurable castration-resistant metastatic prostate cancer (CRPC). Strikingly, most CRMPCs still rely on the AR transcriptional activity due to different adaptive mechanisms, such as AR mutation, ligand-independent AR activation, endogenous androgen synthesis, or even AR amplification (5).
In this study, we report that a region of the previously identified hereditary prostate cancer genetic-susceptibility locus located on chromosome 20 (HPC20; 6) is frequently coamplified with the AR in metastatic prostate cancer tumors. Interestingly, we found that this region is also a hot spot for AR recruitment to chromatin. We show that AR binds and regulates most genes within the common amplified region (CAR), suggesting that the coordinated copy number gain and increased transcriptional output of the 20q13 CAR might be an important event leading to the development of CRMPC.

Materials and Methods

Copy number alteration in prostate cancer
Analysis of copy number alteration on the 20q chromosomal arm is based on the published copy number profiles from 181 primary and 37 metastatic prostate tumors (7) using the Nexus Copy Number software v6.0 (Biodiscovery Inc.). Circos graph was performed using the R software and RCircos package (8).

Cell culture
LNCaP cells were purchased from the American Type Culture Collection and maintained in RPMI-1640 medium (Wisent) supplemented with 10% FBS, 1-glutamine, and 50 μg/mL gentamycin. The synthetic androgen analog R1881 was obtained from PerkinElmer. For androgenic stimulation assays, cells were first androgen deprived in phenol-free RPMI 1640 supplemented with 5% charcoal-stripped FBS, 1-glutamine, and 50 μg/mL gentamycin. After 48 hours, medium was refreshed and R1881 or ethanol (vehicle) was added for the indicated period.

ChIP assays and ChIP-on-chip analysis on chr.20 tiled array
Chromatin was prepared from LNCaP cells exposed to 1 nmol/L R1881 or vehicle for 4 hours. Chromatin immunoprecipitation (ChIP) was performed as described previously (9) using antibodies specific to AR (mouse monoclonal anti-AR from Lab Vision and BD Biosciences). Amplification and labeling of AR-bound ChIP fragments was performed as described previously (9). Hybridization was carried out on a custom-designed tiled array from Agilent covering the q arm of chr.20 at a resolution of 150 bp and analyzed from assembly hg18, using the Feature Extraction 10 alignment program and ChIP Analytics 3.1 program for peak detection (Agilent).

Analysis of gene expression
Total RNA extraction, reverse transcription, and quantitative real-time PCR were performed as already described (9). For MIR645 reverse transcription, the qScript microRNA cDNA synthesis kit was used (Quanta Biosciences). The primer sequences used can be found in Supplementary Table S1. Threshold cycle numbers were calculated using the second derivative maximum obtain with the LightCycler 480 software version 3.5 (Roche). Data were normalized according to RPLP0 (Supplementary Table S1). mRNA expression Z-scores were obtained from the cBIO portal (www.cbioportal.org) using the dataset by Taylor and colleagues (7). mRNA expression was represented as the Z-score of prostate cancer samples versus normal prostate samples. Then, the average Z-scores from primary samples were subtracted from the average Z-scores from metastatic samples.

Statistical analysis
Statistical analyses were performed with the Prism 5.0 GraphPad Software. The significance of gene expression modulation following R1881 treatment was assessed by the Mann-Whitney test. The differences in the risk of biochemical relapse were computed using the log-rank test.

Results

Given the addiction of prostate cancer cells to AR transcriptional activity, androgen-deprivation therapy invariably results in adaptive mechanisms that maintain AR signaling. Accordingly, AR gene amplification was observed exclusively in CRMPC samples (7). In addition, metastatic tumors displayed an array of DNA copy number alterations (CNA) scattered throughout the genome that can possibly synergize with AR gene amplification. Analysis of metastatic prostate cancer samples revealed frequent coamplification (≥35%) of several genomic regions on eight different chromosomes with the AR gene (Fig. 1). Surprisingly, our analysis uncovered that the AR is significantly coamplified with a region located on the 20q chromosome arm, previously identified as HPC20 (6), uniquely in metastatic tumors (Supplementary Fig. S1). We determined that the CAR on 20q13 is located within the HPC20, which is flanked by the markers D20S887 and D20S196 (Figs. 2A and B, orange bars).

![Figure 1. AR is coamplified with a region within the HPC20 in metastatic prostate cancer. Circos plot of genome-wide coamplification events in AR amplified metastatic samples at P ≤ 0.0125. The 20q13, within the HPC20 locus, is the sole region associated with AR amplification on chromosome 20. A total of eight different chromosomes possess at least one locus significantly associated with AR amplification.](www.aacjr.org/molcancerres;12(2)february2014/185/fig1.jpg)
Figure 2. The 20q13 CAR is rich in AR binding sites and is coamplified with the AR. A, representation of the amplified (blue) and deleted (red) regions on the entire 20q chromosomal arm for patients demonstrating amplification of the 20q13 CAR (orange bars) in primary and metastatic tumors. Localization of the AR-bound segments identified by ChIP-on-chip analysis is also indicated (vertical red lines, \( P < 10^{-5} \)). B, zoomed representation of panel A. C, overview of the 20q12–20q13.33 locus revealed that the genomic region significantly associated with AR coamplification in metastatic cancer falls within the AR-dense binding region identified within the HPC20 (green).
Owing to the frequent coamplification of the 20q13 CAR with the AR in metastatic prostate cancer, we investigated whether this region was subjected to AR-transcriptional regulation. We performed genomic location analysis of AR recruitment to chromat in the androgen-sensitive LNCaP cell line, using a tiled array covering the q arm of human chromosome 20. Cells were maintained in a steroid-depleted medium for 48 hours before stimulation with the synthetic androgen analog R1881 for 4 hours. ChIP-on-chip analyses reveal 245 segments bound by the AR, mostly in nonpromoter regions (Supplementary Table S2, P ≤ 10^{-13}). Strikingly, the 20q13 CAR is a local hot spot in AR binding sites (Figs. 2A and B, vertical red lines). Refined analysis of this locus uncovered that AR coamplification with the 20q13 is restricted to the CAR in metastatic prostate cancer, which also corresponds to a region rich in AR binding sites (Fig. 2C).

Fourteen genomic segments bound by AR in LNCaP cells were associated with 10 of the 16 genes (62.5%) present within the 20q13 CAR (Fig. 3A). To validate the relevance of this AR binding profile, we examined the impact of AR activation on the expression of amplicon-resident genes. R1881 treatment resulted in the significant modulation of 7 of the 10 genes that we found associated with AR-bound segments (Fig. 3B, P < 0.01). The three remaining genes were either not affected by R1881 treatment (PARD6B), were not expressed in LNCaP cells (LOC284751), or were impossible to distinguish from a chimeric transcript with specific primers (UBE2V1 vs. TMEM189-UBE2V1; Fig. 3B). Interestingly, we found that the expression level of most of the genes within the 20q13 CAR was increased in metastatic samples (Fig. 3C). In addition, analysis of the three metastatic samples positives for AR and CAR coamplification for which clinical survival data were collected (7) demonstrate significant earlier biochemical recurrence compared with patients without AR and CAR amplification (Fig. 3D). Together, these results suggest that AR, through its amplification/activation, could synergistically enhance the expression of genes within the 20q13 CAR in advanced disease and significantly alter patient’s prognosis when coamplified with the CAR.

Discussion

Genetic linkage studies identified several susceptibility loci for type II diabetes (10, 11) and obesity (12) mapping to the D20S196 marker located on the 20q13 chromosomal region. In addition, this region, flanked by the markers D20S196 and D20S887 (HPC20), has been described as a hereditary prostate cancer susceptibility locus (6). Interestingly, we and others have shown that the protein tyrosine phosphatase 1B (encoded by PTPN1), which is located in the vicinity of the D20S196 marker and is part of the CAR, is indeed implicated in type II diabetes and obesity (13) and plays a tumor-promoting role in prostate cancer (9).

In this study, we further demonstrate that the 20q13 chromosomal region is significantly amplified in metastatic prostate cancer. Notably, this region encodes two transcription factors, namely SNAI1 and CEBPB. SNAI1 is an important mediator of the epithelial-mesenchymal transition, a critical event in the metastatic process (14), and whose expression mediates cell survival and inhibits cellular senescence in metastatic prostate cancer cell lines (15). On the other hand, CEBPB is a recognized oncogene in Ras-mediated tumorigenesis (16). In addition, the 20q13 CAR encodes MIR645, a member of the microRNA family that regulates gene expression posttranscriptionally. Beside a single report suggesting that the coexpression of MIR410 and MIR645 is negatively associated with overall survival in advanced serous ovarian cancer (17), the role of MIR645 in cancer remains unknown. Similarly, a recurrent lung cancer amplicon located at 14q13.3 was found to encode three transcription factors, namely TTF1/NKX2-1, NKX2-8, and PAX9. Remarkably, although the overexpression of a single transcription factor did not modify the proliferation of premalignant lung epithelial cell, overexpression of any pairwise combination led to a major increase in their tumorigenic potential (18). Together, the altered transcriptional output mediated by the coordinated DNA copy number gain of SNAI1, CEBPB, and MIR645 might contribute to a global transcriptional rewiring process, ultimately resulting in the increased tumor aggressiveness observed in patients bearing the 20q13 CAR.

A noteworthy feature of the 20q13 CAR is its extensive regulation by AR. ChIP-on-chip analysis using the androgen-sensitive LNCaP cell line revealed AR binding in the vicinity of a large proportion of genes within the 20q13 CAR (Fig. 3A). Importantly, this result was not biased by chromosomal abnormalities because LNCaP cells do not have CNA in the 20q13 region (19). Surprisingly, most protein-coding transcripts from AR-bound genes were sensitive to R1881 treatment (Fig. 3B). As previously described, PTPN1 mRNA expression was strongly regulated by the AR (9). We also identified several novel AR-regulated genes within the 20q13 CAR with functions yet to be described in the prostate, including the solute carrier family 9, member 8 (SLC9A8), the spermatogenesis-associated protein 2 (SPATA2), the ring finger protein 114 (RNF114), and the activity-dependent neuroprotector (ADNP). Interestingly, the breast cancer amplified sequence 4 (BCAS4) was modestly but significantly regulated by R1881. Finally, we also describe CEBPB (encoding C/EBPβ) as an AR-regulated target. Zhang and colleagues recently demonstrated that C/EBPβ can effectively transactivate the prostate-specific antigen (PSA) promoter, which is regulated by androgen response elements in the absence of androgen. However, increased C/EBPβ expression results in a decreased transactivation of the PSA promoter in the presence of androgen. In contrast, AR activation results in an increased C/EBPβ transcriptional activity on CCAAT enhancer binding protein elements (20). This interesting cross-talk, together with the fact that AR-regulation of the 20q13 CAR is accompanied by AR coamplification, supports a complex
transcriptional rewiring in a significant subset of metastatic tumors.

In summary, we report the frequent amplification of the 20q13 chromosomal region in metastatic prostate cancer, a region that had previously been identified as a hereditary prostate cancer susceptibility locus. The extensive regulation of genes within the 20q13 CAR by AR, some of which are already associated with oncogenic functions, suggests that...
the highly specific coamplification of this chromosomal region with the AR might synergize and contribute to increased tumor aggressiveness and to the development of metastatic disease. These findings reflect the fact that AR amplification is not frequently seen at diagnosis but is very common in advanced therapy-resistant diseases. Thus, like AR amplification status, AR–CAR amplification status should be most informative as marker for disease progression after therapeutic intervention. These results may also reflect a potential example of the amplification of a locus after enhanced transcriptional activity at that locus. Finally, these results justify further investigation to address the respective roles of the different genes within the 20q13 CAR.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: D.P. Labbé, L. Lessard, V. Giguère, L.C. Trotman, M.L. Tremblay
Development of methodology: D.P. Labbé, D.G. Nowak, L.C. Trotman
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): D.P. Labbé, G. Deblois
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D.P. Labbé, D.G. Nowak, G. Deblois, L.C. Trotman

References

Writing, review, and/or revision of the manuscript: D.P. Labbé, D.G. Nowak, L. Lessard, V. Giguère, L.C. Trotman, M.L. Tremblay
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.P. Labbé, G. Deblois, L.C. Trotman
Study supervision: V. Giguère, L.C. Trotman, M.L. Tremblay

Acknowledgments
The authors thank Serge Hardy and Kelly-Anne Pike for helpful discussions and Noriko Uetani for technical assistance with figure design and drawing.

Grant Support
D.P. Labbé is a recipient of a Canadian Institute of Health Research (CIHR) Frederick Banting and Charles Best Doctoral Research Award and a CIHR/Fonds de recherche du Québec–Santé Training Grant in Cancer Research FRN53888 of the McGill Integrated Cancer Research Training Program. G. Deblois is supported by a Predoctoral Traineeship Award (W81XWH-10-1-0489) from the U.S. Department of Defense Breast Cancer Research Program. This work was supported by CIHR operating grants to V. Giguère (MOP64275) and M.L. Tremblay (MOP 62887) as well as a U.S. Army Department of Defense Award (W81XWH-09-1-0259), a Prostate Cancer Canada Grant (#02013-33) and a Jeanne and Jean-Louis Levesque Chair in Cancer Research to M.L. Tremblay, and by grants to L.C. Trotman from the NIH (CA137050), the Department of the Army (W81XWH-09-1-0557), and the Robertson Research Fund of Cold Spring Harbor Laboratory.

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Received September 9, 2013; revised November 20, 2013; accepted December 3, 2013; published OnlineFirst December 30, 2013.
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Access the most recent version of this article at: doi:10.1158/1541-7786.MCR-13-0477

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