MOLECULAR CANCER BIOLOGY

Chromosome 5 harbors two independent deletion hotspots at 5q13 and 5q21 that characterize biologically different subsets of aggressive prostate cancer

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Abstract
Deletion of chromosome 5q is common in prostate cancer and is linked to aggressive disease. Most previous studies focused on 5q21 where CHD1 is located, but deletion of mapping studies has identified a second deletion hotspot at 5q13. To clarify the prevalence and clinical relevance of 5q13 deletions and to determine the relative importance of 5q13 and 5q21 abnormalities, a tissue microarray containing samples from 12,427 prostate cancers was analyzed by fluorescence in situ hybridization. Deletion of 5q13 and 5q21 was found in 13.5% and 10%, respectively, of 7932 successfully analyzed cancers. Deletion was restricted to 5q13 in 49.4% and to 5q21 in 32.0% of cancers with a 5q deletion. Only 18.6% of 5q-deleted cancers had deletions of both loci. Both 5q13 and 5q21 deletions were significantly linked to advanced tumor stage, high Gleason grade, nodal metastasis and early biochemical recurrence (P < .005 each). Cancers with co-deletion of 5q13 and 5q21 had a worse prognosis than cancers with isolated 5q13 or 5q21 deletion (P = .0080). Comparison with TMPRSS2:ERG fusion status revealed that 5q21 deletions were tightly linked to ERG negativity (P < .0001) while 5q13 deletions were unrelated to the ERG status. In summary, 5q13 deletion and 5q21 deletion are common, but independent genomic alterations with different functional effects lead to aggressive prostate cancer.

KEYWORDS
5q deletion, prognosis, prostate cancer, tissue microarray

Abbreviations: aCGH, array comparative genomic hybridization; AR, androgen receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; PSA, prostate-specific antigen; SNP, single-nucleotide polymorphism; TMA, tissue microarray.

Shared last authorship: David Dum and Ronald Simon contributed equally to this work.

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1 | INTRODUCTION

Prostate cancer is the most prevalent cancer in men in Western societies. Although the majority of prostate cancers behave in an indolent manner, a small subset is highly aggressive and requires extensive treatment. Established preoperative prognostic parameters are limited to Gleason score, tumor extent on biopsies, prostate-specific antigen (PSA) level and clinical stage. These data are statistically powerful but often not sufficient for the optimization of individual treatment decisions. The hope is that a better understanding of disease biology will eventually lead to the identification of clinically applicable molecular markers, which enable a more reliable prediction of prostate cancer aggressiveness.

Chromosomal deletions are a hallmark of prostate cancer genetics. The most commonly deleted chromosomal regions include 8p, 16q, 10q23 (PTEN), 6q and 5q. These occur in up to 40% of tumors. Deletions in 5q have been reported to occur in 6% to 19% of prostate cancers. The region 5q21 has been most extensively studied. It contains the chromodomain helicase DNA-binding protein 1 (CHD1), representing an important target gene of 5q deletions. Proper CHD1 function is required for maintenance of androgen receptor (AR) signaling and, consequently, development of AR-dependent TMPRSS2:ERG fusion. This provides a mechanistic explanation for the strong association of 5q deletion with TMPRSS2:ERG fusion-negative cancers. Deletions of 5q21 are tightly linked to poor prognosis in prostate cancer.

Although most previous work has focused on the CHD1 locus, other regions of chromosome 5q are also involved in prostate cancer biology. Copy number profiling studies suggest that 5q deletions are typically large, often extending from centromeric (5q11) to telomeric (5q35) regions, with remarkable variation in size. In addition, apart from the CHD1 locus exists another deletion hot spot located at 5q11-q14. It is currently unknown whether this hot spot characterizes a distinct subset of prostate cancers with molecular and/or clinical features that are different from 5q21-deleted cancers.

To learn more about the role of 5q13 deletions, we expanded our previous 5q21 deletion analysis through additional fluorescence in situ hybridization (FISH) analysis of 5q13 and 5q21 to study different 5q deletion patterns and their clinical relevance in prostate cancers. We made use of our large prostate cancer tissue microarray (TMA), consisting of more than 12,000 prostate cancers linked with a corresponding clinical database.

2 | MATERIALS AND METHODS

2.1 | Patients

Radical prostatectomy specimens were available from 12,427 patients undergoing surgery between 1992 and 2012 at the Department of Urology and the Martini Clinic at the University Medical Center, Hamburg-Eppendorf. Histopathologic data were retrieved from patient files, including tumor stage, nodal stage and status of the resection margin. In addition to the traditional Gleason categories, “quantitative” Gleason grading was performed as previously described. Briefly, for every prostatectomy specimen, the percentage of Gleason 4 patterns was estimated throughout the cancerous tissue during the routine histologic evaluation. This allows the subdivision of Gleason 3 + 4 and 4 + 3 cancers according to their percentage of Gleason 4. For practical use, we subdivided the 3 + 4 and 4 + 3 cancers into eight subgroups: 3 + 4 with ≤5% Gleason 4, 3 + 4 6% to 10%, 3 + 4 11% to 20%, 3 + 4 21% to 30%, 3 + 4 31% to 49%, 4 + 3 50% to 60%, 4 + 3 61% to 80% and 4 + 3 >80% Gleason 4. Additional groups were defined by the presence of a tertiary Gleason 5 pattern, namely 3 + 4 Tert. 5 and 4 + 3 Tert. 5. Follow-up data were available for a total of 11,665 patients with a median follow-up of 36 months (range: 1-241 months; Supplementary Table 1). PSA values were measured following surgery and PSA recurrence was defined as the time point when postoperative PSA was at least 0.2 ng/mL and increasing at subsequent measurements. All prostate specimens were diagnosed according to a standard procedure, including complete embedding of the entire prostate for histologic analysis. The TMA manufacturing process was described previously in detail. In short, one 0.6 mm core was taken from a tumor-containing tissue block from each patient. The molecular database attached to this TMA includes data on ERG expression by immunohistochemistry in 10,678 (extended from References 19 and 20), PSA rearrangement by FISH analysis in 7,932 (extended from References 19 and 20) and 5q21 (CHD1) deletion by FISH in 7,099 cancers (extended from Reference 5).

2.2 | Fluorescence in situ hybridization

Four micrometer TMA sections were used for FISH. TMA sections were deparaffinized, air-dried and dehydrated in 70%, 85% and 100% ethanol. Slides were pretreated in VP 2000 Pretreatment Reagent (Abbott, Des Plaines) for 15 minutes at 80°C, followed by a 150 minute incubation at 37°C in 0.5% protease 1 solution (Abbott, Des Plaines).
Des Plaines). 4 μL of FISH probe mix in 70% formamide 2x SSC solution was applied to the slides and co-denatured with the cellular DNA in a Hybrite hybridization oven for 10 minutes at 72°C, followed by overnight hybridization at 37°C in a humidified chamber. The FISH probe mix consisted of a spectrum-orange labeled 5q13 (CDK7 locus) probe (made from BACs RP11-815B01 and RP11-443M17) and a spectrum green-labeled commercial centromere 10 probe (#06J36-090; Abbott, Wiesbaden, Germany) as a reference—a specific centromere 5 probe is not available. It is unlikely that using the centromere 10 probe caused a significant fraction of false deletion calls, because in our previous FISH studies about 80% of all tumors showed two copies of the respective chromosome.6-8,10,14,24-26 Analysis of 5q21 deletion was performed as previously described.5 After hybridization, slides were subjected to serial stringent washings (2x SSC solution with 0.3% NP40 at 72°C for 2 minutes) and counterstained with 0.2 μmol/L 4′,6-diamidino-2-phenylindole (DAPI) in antifade solution. Stained slides were manually interpreted under an epifluorescence microscope, and the predominant green and orange FISH signal numbers were recorded in each tissue spot. Homozygous deletion of 5q13 was defined as complete lack of 5q13 FISH signals in the tumor nuclei, but presence of 5q13 FISH signals in adjacent normal cells. Tissue spots lacking 5q13 signals in all (tumor and normal) cells or lacking any normal cells to serve as an internal control for successful hybridization of the 5q13 probe were excluded from analysis. Heterozygous deletion of 5q13 was defined as the presence of fewer 5q13 signals than centromere 10 probe signals in ≥60% of tumor nuclei. These thresholds were based on a previous study analyzing PTEN deletions in a subset of slides of the TMA set. Representative FISH images are shown in Supplementary Figure 1.

2.3 | 5q copy number data sources and analysis

Raw data were obtained from four large studies employing array comparative genomic hybridization (aCGH) or single-nucleotide polymorphism (SNP) array analysis in a total of 442 prostate cancers.13-16 Data were imported into the FISH Oracle browser27,28 and visualized in different tracks corresponding to each study. A global threshold of −0.3 was applied to all four data sets to display deletions.

2.4 | Statistics

For statistical analysis, the JMP 14.0 software (SAS Institute Inc., NC) was used. Contingency tables were calculated to study the association between 5q deletion and clinicopathologic variables, and the chi-square (likelihood) test was used to find significant relationships. Kaplan-Meier curves were generated for PSA recurrence free survival. The log-rank test was applied to test the significance of differences between stratified survival functions. Cox proportional hazards regression analysis was performed to test the statistical independence and significance between pathological, molecular and clinical variables.

3 | RESULTS

3.1 | Architecture of 5q deletions

A re-analysis of own Reference 14 and published References 13, 15, and 16 microarray-based 5q copy number data from 442 prostate cancers using the FISH-Oracle browser27,28 is shown in Figure 1. This analysis suggests the existence of two deletion hotspots located at 5q13 and 5q21, both of which occurred at comparable frequency (about 10% to 12% of the 442 cancers). Only about 3% of these tumors showed unequivocal large deletions including both 5q13 and 5q21.

3.2 | Prevalence of 5q13 and 5q21 deletion

FISH analysis was successful in 6866 of 12 247 (56.1%) arrayed cancers for 5q13 and in 7932 of 12 247 (64.8%) cancers for 5q21 (including 2093 cancers from our previous analysis). Analysis was not informative in the remaining 5381 (5q13) and 4315 (5q21) tumors because of the lack of tumor cells in the tissue spots, faint or absent FISH signals, or missing tissue spots on the TMA section. Heterozygous deletions were found for 5q13 in 13.5% (926/6866) and for 5q21 in 10.0% (793/7932) of prostate cancers. Homozygous deletions were only found for 5q21 (2.5%) but not for 5q13.

3.3 | 5q13 and 5q21 deletion and prostate cancer phenotype

Deletions of 5q13 (Table 1) and 5q21 (Supplementary Table 2) were strongly associated with an adverse tumor phenotype, including advanced tumor stage, high Gleason grade, high quantitative Gleason score and early PSA recurrence (P < .0050 each) if all cancers were jointly analyzed (Figure 2). These associations—with the exception of the lacking prognostic role of 5q21 deletion in ERG-positive cancers—also held true if the subsets of ERG-negative and ERG-positive cancers were analyzed separately (Table 1, Figure 2, Supplementary Table 1). However, neither 5q13 deletions nor 5q21 deletions were prognostically relevant in subgroups of tumors with similar "traditional" or quantitative Gleason score (Supplementary Figure 2 and Supplementary Figure 3).

3.4 | Patterns of 5q13 and 5q21 co-deletion

Data on both 5q loci were available for 5510 cancers, including 1137 (20.6%) cancers harboring deletions of 5q13 and/or 5q21. Deletion patterns fit well to the copy number profiling results, suggesting that 5q13 and 5q21 are distinct deletion regions: Only 20% (211/1137) of the 5q-deleted cancers had co-deletions, while 81% (926/1137) harbored only one deletion, either at 5q13 (49.4%) or 5q21 (32.0%).
If 5q13 and 5q21 deletions were considered separately (irrespective of co-deletions), there was a link for both 5q13 and 5q21 deletions to ERG-negative cancers, which was strong for 5q21 ($P < .0001$ for ERG-immunohistochemistry [IHC] and ERG-FISH analysis) but only marginal for 5q13 ($P = .0018$ for ERG-IHC and $P = .0026$ for ERG-FISH analysis), regardless of the method of ERG analysis. For example, deletions of 5q13 were found in 14.9% and 15.0% ERG-negative cancers (according to ERG IHC and FISH analysis) and in 12.2% (IHC) and 12.2% (FISH) ERG-positive cancers (Figure 3A), but in 16.2% and 15.5% ERG-negative cancers (according to ERG IHC and FISH analysis) and in 4.1% (IHC) and 4.3% (FISH) ERG-positive cancers for 5q21 (Figure 3B). To better understand the impact of 5q21 deletion on the association between 5q13 deletion and ERG status, we performed additional subset analysis. It showed that the overall weak association between 5q13-deleted and ERG-negative cancers was driven solely by the strong link between deletions of 5q21 and absence of
5q13 deletion and cancer phenotype

|                      | All cancers | ERG-negative cancers | ERG-positive cancers |
|----------------------|-------------|----------------------|---------------------|
|                      | n           | 5q13 deletion (%)    | P value             | n           | 5q13 deletion (%)    | P value             | n           | 5q13 deletion (%) | P value             |
| Tumor stage          |             |                      |                     |             |                      |                     |             |                      |                     |
| pT2                  | 4312        | 11.3%                | <.0001              | 2298        | 12.5%                | <.0001              | 1735        | 10.1%               | .0002              |
| pT3a                 | 1561        | 15.3%                | 679                  | 17.2%        | 808                  | 14.1%               |
| pT3b-pT4             | 967         | 20.0%                | 464                  | 22.8%        | 447                  | 17.0%               |
| Gleason grade        |             |                      |                     |             |                      |                     |             |                      |                     |
| ≤3 + 3               | 1494        | 7.0%                 | <.0001              | 746         | 7.6%                 | <.0001              | 622         | 6.4%                | <.0001              |
| 3 + 4                | 3455        | 12.7%                | 1704                 | 13.3%        | 1582                 | 12.1%               |
| 3 + 4 Tert. 5        | 235         | 14.9%                | 132                  | 16.7%        | 87                   | 11.5%               |
| 4 + 3                | 640         | 23.4%                | 343                  | 26.5%        | 269                  | 20.1%               |
| 4 + 3 Tert. 5        | 381         | 20.7%                | 199                  | 23.1%        | 164                  | 18.3%               |
| ≥4 + 4               | 298         | 23.8%                | 172                  | 25.6%        | 106                  | 22.6%               |
| Quantitative         |             |                      |                     |             |                      |                     |             |                      |                     |
| Gleason grade        |             |                      |                     |             |                      |                     |             |                      |                     |
| ≤3 + 3               | 1494        | 7.0%                 | <.0001              | 746         | 7.6%                 | <.0001              | 622         | 6.4%                | <.0001              |
| 3 + 4 ≤ 5%           | 937         | 11.1%                | 462                  | 9.7%         | 417                  | 13.2%               |
| 3 + 4 6-10%          | 935         | 10.3%                | 458                  | 10.7%        | 436                  | 9.2%                |
| 3 + 4 11%-20%        | 794         | 13.2%                | 402                  | 13.7%        | 353                  | 13.0%               |
| 3 + 4 21%-30%        | 445         | 13.9%                | 206                  | 15.5%        | 223                  | 13.5%               |
| 3 + 4 31%-49%        | 344         | 20.6%                | 176                  | 26.1%        | 153                  | 13.7%               |
| 3 + 4 Tert. 5        | 235         | 14.9%                | 132                  | 16.7%        | 87                   | 11.5%               |
| 4 + 3 50%-60%        | 300         | 22.7%                | 161                  | 23.6%        | 130                  | 20.0%               |
| 4 + 3 61%-80%        | 278         | 23.0%                | 144                  | 27.1%        | 122                  | 19.7%               |
| 4 + 3 > 80%          | 62          | 29.0%                | 38                   | 36.8%        | 17                   | 23.5%               |
| 4 + 3 Tert. 5        | 381         | 20.7%                | 199                  | 23.1%        | 164                  | 18.3%               |
| ≥4 + 4               | 298         | 23.8%                | 172                  | 25.6%        | 106                  | 22.6%               |
| Lymph node metastasis|             |                      |                     |             |                      |                     |             |                      |                     |
| N0                   | 3914        | 14.3%                | <.0001              | 1967        | 16.0%                | .0047               | 1723        | 12.9%               | .0024              |
| N+                   | 425         | 22.8%                | 193                  | 24.4%        | 209                  | 21.1%               |
| PSA level (ng/μL)    |             |                      |                     |             |                      |                     |             |                      |                     |
| <4                   | 827         | 13.8%                | .0834                | 359         | 15.3%                | .6201               | 401         | 13.0%               | .1846              |
| 4–10                 | 4045        | 12.7%                | 2027                 | 14.3%        | 1781                 | 11.2%               |
| 10–20                | 1398        | 15.2%                | 759                  | 16.2%        | 568                  | 14.1%               |
| >20                  | 511         | 15.1%                | 272                  | 15.4%        | 210                  | 14.8%               |
| Surgical margin      |             |                      |                     |             |                      |                     |             |                      |                     |
| Negative             | 5412        | 13.1%                | .3012                | 2743        | 14.6%                | .4769               | 2330        | 11.8%               | .5258              |
| Positive             | 1329        | 14.2%                | 655                  | 15.7%        | 612                  | 12.8%               |

**ERG fusion:** deletion of 5q13 alone (ie, no 5q21 co-deletion) occurred in 49.9% of 256 ERG-negative and 50.1% of 287 ERG-positive cancers, whereas co-deletion of 5q13 and 5q21 was strongly linked to ERG-negative cancers. All data are summarized in Figure 3C.

**3.6 | 5q13 and 5q21 co-deletion and PSA recurrence**

The combined analysis of both deletions revealed that co-deletions of 5q13 and 5q21 had a higher risk of early biochemical recurrence than...
deletion of 5q13 or 5q21 alone in all cancers (P ≤ .0090, Figure 4A) as well as in the subset of ERG-negative cancers (P ≤ .0090, Figure 4B). In ERG-positive cancers, no significant additional prognostic impacts over deletions of only one locus were found (P ≥ .4, Figure 4C).

### 3.7 Multivariate analyses

The prognostic relevance of 5q13 deletion, both with and without 5q21 co-deletion, was further assessed in four different multivariate analyses based on established preoperative and postoperative prognostic parameters. Scenario 1 investigated the postoperatively available prognostic parameters pT, pN, surgical margin status, preoperative PSA value and prostatectomy Gleason grade. In Scenario 2, nodal metastasis was excluded from the postoperatively available set of data, because lymph node dissection is not standardized and is preferentially performed in high-risk cancers, which may introduce a statistical bias. The other two scenarios model the preoperative situation to the best possible extent. Scenario 3 included 5q deletion status, preoperative PSA value, clinical tumor stage (cT) and Gleason...
grade obtained in the prostatectomy specimen. It is of note that the postoperative determination of Gleason grade is “better” than the preoperatively determined Gleason grade (subject to sampling error and consequent undergrading in more than one third of cases). Finally, in Scenario 4, the preoperative Gleason grade obtained on the original biopsy was combined with preoperative PSA value, cT and 5q deletion status. In these analyses, 5q13 deletion (either alone or in combination with 5q21 deletion) predicted PSA recurrence independent of the preoperative parameters (Scenario 4, \( P \leq .0020 \), Table 2). In addition, in ERG-negative cancers, 5q21 deletion alone or in combination with 5q13 deletion predicted PSA recurrence independent of the preoperative and postoperative parameters (Scenarios 1-4, \( P < .05 \)).
DISCUSSION

The results of our study identify 5q13 as a deletion hotspot that develops independent of 5q21 deletion in a relevant subset of prostate cancers.

Successful FISH analysis of more than 6800 prostate cancers in our study revealed 5q13 deletion in 13.5% and 5q21 deletion in 10.0% of tumors. These frequencies are in the range of data from earlier studies employing aCGH for the analysis of copy number changes. These studies on 72 to 504 primary prostate cancers reported 5q13 and/or 5q21 deletions in 6% to 19% (www.cbioportal.org11-16). In a previous FISH study on a subset of our prostate cancer TMA, we found 5q21 deletions in 8.7% of 2093 cancers. FISH is the most precise method for deletion analysis as it allows to determine exact gene copy numbers on a single cell level and is not disturbed by contaminating non-neoplastic cells that are inevitably present in cancer tissues. Scoring criteria used for our deletion analyses in tissue sections had earlier been validated by comparison of FISH and aCGH data.8 The choice of our FISH probes was based on the known role of CHD1 (5q21) in prostate cancer biology30 and because of the position of CDK7 in the center of the 5q13 core deletion region, rather than because of a possible biological role of CDK7.

Our study demonstrates that both 5q13 and 5q21 deletions are strongly linked to adverse tumor phenotype and early poor patient outcome. These findings are in line with previous studies from others and us showing a link between 5q deletion and unfavorable tumor parameters5,9 and poor patient prognosis.5,16 In contrast, one study identified a link between 5q21 deletion and a favorable prognosis in a cohort of 55 patients31 and one other study failed to find associations of CHD1 (5q21) deletions and expression with relevant clinicopathologic features in 86 cancers.13 The fact that deletions of 5q13 and of 5q21 are linked to poor patient outcome is not surprising. Using our TMA resource, our group had earlier studied deletions of 3p13,14 6q15,6 8p21,10 PTEN8, 12p13,34 13q14,32 16q23,7 TP5326 and 18q2425 and found that all of these deletions were linked to an adverse tumor phenotype and poor prognosis.

Earlier studies analyzing 5q deletions in prostate cancer consistently focused on the 5q21 deletion hotspot harboring the CHD1 tumor suppressor gene.5,16,18,31,33 The results of our deletion mapping and the observation that about 80% of the 5q deletions identified in our cohort included either 5q13 or 5q21 but not both loci demonstrate that these deletions constitute two separate, unrelated recurrent genomic alterations in prostate cancer. Despite minor overlap, 5q13 and 5q21 deletions include largely different sets of genes with a potential role in cancer. 5q13 and 5q21 deletions will thus induce different biologic changes in affected cells. The variance in their relationship to ERG status represents an example of different functional effects of these two deletions. TMPRSS2:ERG fusions occur in about 50% of prostate cancer samples.19 TMPRSS2:ERG fusions are inversely linked to 5q21 deletions because inactivation of the CHD1 tumor suppressor gene at 5q21 hampers the formation of TMPRSS2:ERG fusion by abrogation of AR signaling.5 That isolated 5q13 deletions were equally frequent in ERG-negative and ERG-positive cancers, while 5q21 deletions were strongly linked to absence of TMPRSS2:ERG fusions, which suggests that CHD1 is the primary driver on 5q preventing these fusions.

Tumors with large deletions covering both 5q13 and 5q21 had a worse prognosis than those with only one of these deletions. That the quantity of lost chromosomal material is linked to cancer aggressiveness is consistent with data from earlier studies. For prostate cancer, our group had earlier shown that the prognostic impact of 6q and 16q deletion depends on the length of the deleted segment.34 The combination of deletions involving different chromosomes was also found to correlate with patient prognosis in prostate cancers.16,31,34 In
| Tumor subset   | Scenario | Analyzable (n) | Preop. PSA-level | pT-Stage | cT-Stage | Gleason grade prostatectomy | Gleason grade biops | pN-Stage | R-Status | Sq13 deletion alone | Sq21 deletion alone | Sq13/Sq21 co-deletion |
|---------------|----------|----------------|-------------------|----------|----------|----------------------------|---------------------|----------|----------|---------------------|---------------------|-----------------------|
| All cancers   | 1        | 3842           | <.0001            | <.0001   | <.0001   | <.0001                     | <.0001              | .0354    | .5416    | .2157               | .5448               |
|               | 2        | 6102           | <.0001            | <.0001   | <.0001   | <.0001                     | <.0001              | .3617    | .0414    | .1039               |
|               | 3        | 5995           | <.0001            | <.001    | <.0001   | <.0001                     | <.0001              | .1563    | .0656    | .2181               |
|               | 4        | 5907           | <.0001            | <.0007   | <.0001   | <.0001                     | <.0001              | .0013    | .0013    | .0013               |
| ERG-negative  | 1        | 1914           | <.0001            | <.0001   | <.0001   | <.0001                     | <.0001              | .0010    | .1463    | .0010               | .0036               |
| cancers       | 2        | 3069           | <.0001            | <.0001   | <.0001   | <.0001                     | <.0001              | .0015    | .0156    | .0417               |
|               | 3        | 3030           | <.0001            | <.0001   | <.0001   | <.0001                     | <.0001              | .0051    | .0003    | .0011               |
| ERG-positive  | 1        | 1715           | .0005             | <.0001   | <.0001   | <.0001                     | <.0295              | .6298    | .5554    | .0499               | .0542               |
| cancers       | 2        | 2673           | <.0001            | <.0001   | <.0001   | <.0001                     | <.0594              | .5063    | .0555    | .0919               |
|               | 3        | 2608           | <.0001            | <.0001   | <.0001   | <.0001                     | .9486               | .3125    | .6255    |
|               | 4        | 2566           | <.0001            | <.0001   | <.0001   | <.0001                     | .2719               | .8290    | .8369    |
chronic lymphocytic leukemia, cancers with focal heterozygous deletion of the DLEU2 gene at 13q14 showed a more favorable prognosis than cancers with a larger deletion, which includes the RB1 tumor suppressor gene locus. Compound haplo-insufficiency is the most likely cause for the link between deletion size and patient outcome. For many tumor suppressor genes such as p27Kip1, TP53, DMP1, NF1 and PTEN, it has been shown that the lack of one allele results in the inability of the cell to execute normal cellular functions and contributes to tumor development (reviewed in Reference 36). It is suspected that the proper function of many more genes is dependent on the presence of two functional gene copies. The larger the combined size of all deletions of a cancer cell, the higher the number of possible haplo-insufficient genes with a potential tumor suppressive role. Several studies have suggested a tumor-promoting collaboration of multiple haplo-insufficient genes. Genes on 5q with a putative tumor suppressive role, for example, include OCLN (5q13), RAD17 (5q13), NSA2 (5q13), MCC (5q21), CHD1 (5q21), APC (5q22), LOX (5q23), DIAPH1 (5q31) and RPS14 (5q33).

In our multivariate analyses, deletions of 5q13 and 5q21 were significant independent of the less precise Gleason grade obtained from presurgical biopsies, but not the definite Gleason grade determined in the prostatectomy specimens. This suggests that the potential clinical utility of 5q deletion assessment is restricted to the context of prostate biopsies, where strong histologic prognostic parameters are lacking. The fact that both deletions had no prognostic impact in prostatectomy specimens defined by identical traditional and quantitative Gleason grade in the postsurgical prostatectomy specimen demonstrates the power of the Gleason grading system and how difficult it is for molecular prognostic parameters to outperform conventional morphology. Future prognostic biomarkers for prostate cancer should not only be independent of currently established factors but also better reproducible and thus more reliable. In principle, FISH analysis is optimally suited for diagnostic testing as it provides unequivocal yes/no answers.

There are two major limitations pertaining to our study. First, we did not perform functional analysis to identify the gene(s) responsible for the observed prognostic differences. Second, it cannot be excluded that the adverse prognostic impact of large deletions is related to the tumor’s overall burden of copy number changes, which might generally be higher in tumors with larger deletions. Further studies are required to clarify these issues.

In summary, our study demonstrates that chromosome 5 harbors two distinct deletion hotspots at 5q13 and 5q21, which typically occur independently from one another. Although these deletions have different biologic implications, both are associated with aggressive disease.

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CONFLICT OF INTEREST
The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT
Analysis of patient and corresponding histopathologic data for research purposes, as well as construction of tissue microarrays from remnants of archived diagnostic tissues, was approved by local laws (HmbKHG, §12,1) and by the local ethics committee (Ethics Commission Hamburg, WF-049/09 and PV3652). All work was carried out in compliance with the Helsinki Declaration. An informed consent of the patients is not required.

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REFERENCES
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Bill-Axelson A, Holmberg L, Ruutu M, et al. Radical prostatectomy versus watchful waiting in early prostate cancer. N Engl J Med. 2011;364:1708-1717.
3. Klötz L, Vespriini D, Sethukavalan P, et al. Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. J Clin Oncol. 2015;33:272-277.
4. Witt T, Jones KM, Barry MJ, et al. Follow-up of prostatectomy versus observation for early prostate cancer. N Engl J Med. 2017;377:132-142.
5. Burkhardt L, Fuchs S, Krohn A, et al. CHD1 is a 5q21 tumor suppressor required for ERG rearrangement in prostate cancer. Cancer Res. 2013;73:2795-2805.
6. Kluth M, Hesse J, Heinl A, et al. Genomic deletion of MAP3K7 at 6q12-22 is associated with early PSA recurrence in prostate cancer and absence of TMPRSS2:ERG fusions. Mod Pathol. 2013;26:975-983.
7. Kluth M, Runte F, Barow P, et al. Concurrent deletion of 16q23 and PTEN is an independent prognostic feature in prostate cancer. Int J Cancer. 2015;137:2354-2363.
8. Krohn A, Diedler T, Burkhardt L, et al. Genomic deletion of PTEN is associated with tumor progression and early PSA recurrence in ERG fusion-positive and fusion-negative prostate cancer. Am J Pathol. 2012;181:401-412.
9. Sun J, Liu W, Adams TS, et al. DNA copy number alterations in prostate cancers: a combined analysis of published CGH studies. Prostate. 2007;67:692-700.
10. Kluth M, Amschler NN, Galal R, et al. Deletion of 8p is an independent prognostic parameter in prostate cancer. Oncotarget. 2017;8:379-392.
11. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401-404.
12. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.
13. Huang S, Gulzar ZG, Salari K, Lapointe J, Brooks JD, Pollack JR. Recurrent deletion of CHD1 in prostate cancer with relevance to cell invasiveness. Oncogene. 2011;31:4164-4170.

14. Krohn A, Seidel A, Burkhardt L, et al. Recurrent deletion of 3p13 targets multiple tumour suppressor genes and defines a distinct subgroup of aggressive ERG fusion-positive prostate cancers. J Pathol. 2013;231:130-141.

15. Mao X, Boyd LK, Yanez-Munoz RJ, et al. Lu YJ. Recipient block TMA technique.

16. Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;18:11-22.

17. Metzger E, Willmann D, McMillan J, et al. Assembly of methylated KDM1A and CHD1 drives androgen receptor-dependent transcription and translation. Nat Struct Mol Biol. 2016;23:132-139.

18. Liu W, Lindberg J, Sui G, et al. Identification of novel CHD1-associated collaborative alterations of genomic structure and functional assessment of CHD1 in prostate cancer. Oncogene. 2012;31:3939-3948.

19. Minner S, Endien M, Sirha H, et al. ERG status is unrelated to PSA recurrence in radically operated prostate cancer in the absence of antihormonal therapy. Clin Cancer Res. 2011;17:5878-5888.

20. Weischenfeldt J, Simon R, Feuerbach L, et al. Genomic deletion of chromosome 13q14 deletion size and number of deleted cells both influence prognosis in chronic lymphocytic leukemia. Genes Chromosomes Cancer. 2011;50:633-643.

21. Sauter G, Steurer S, Clauditz TS, et al. Clinical utility of quantitative Gleason grading in prostate biopsies and prostatectomy specimens. Eur Urol. 2016;69:592-598.

22. Demichelis F, Setlur SR, Beroukhim R, et al. Distinct genomic aberrations associated with ERG rearranged prostate cancer. Genes Chromosomes Cancer. 2009;48:366-380.

23. Mirlacher M, Simon R. Recipient block TMA technique. Methods Mol Biol. 2010;664:37-44.

24. Kluth M, Ahrary R, Hube-Magg C, et al. Genomic deletion of chromosome 12p is an independent prognostic marker in prostate cancer. Oncotarget. 2015;6:27966-27979.

25. Kluth M, Graunke M, Moller-Koop C, et al. Deletion of 18q is a strong and independent prognostic feature in prostate cancer. Oncotarget. 2016;7:86339-86349.

26. Kluth M, Harasimowicz S, Burkhardt L, et al. Clinical significance of different types of p53 gene alteration in surgically treated prostate cancer. Int J Cancer. 2014;135:1369-1380.

27. Mader M, Simon R, Kurtz S, FISH Oracle 2: a web server for integrative visualization of genomic data in cancer research. J Clin Bioinformat. 2014;4:5.

28. Mader M, Simon R, Steinbiss S, Kurtz S, FISH Oracle 2: a web server for flexible visualization of DNA copy number data in a genomic context. J Clin Bioinformat. 2011;1:20.

29. Epstein JI, Feng Z, Trock BJ, Pierorazio PM. Upgrading and downgrading of prostate cancer from biopsy to radical prostatectomy: incidence and predictive factors using the modified Gleason grading system and factoring in tertiary grades. Eur Urol. 2012;61:1019-1024.

30. Augello MA, Liu D, Deonarine LD, et al. CHD1 loss alters AR binding at lineage-specific enhancers and modulates distinct transcriptional programs to drive prostate tumorigenesis. Cancer Cell. 2019;35:603-17 e8.

31. Lapointe J, Li C, Giacomini CP, et al. Genomic profiling reveals alternative genetic pathways of prostate tumorigenesis. Cancer Res. 2007;67:8504-8510.

32. Kluth M, Scherzai S, Buschek F, et al. 13q deletion is linked to an adverse phenotype and poor prognosis in prostate cancer. Genes Chromosomes Cancer. 2018;57:504-512.

33. Cunningham JM, Shan A, Wick MJ, et al. Allelic imbalance and microsatellite instability in prostatic adenocarcinoma. Cancer Res. 1996;56:4475-4482.

34. Kluth M, Jung S, Habib O, et al. Deletion lengthening at chromosomes 6q and 16q targets multiple tumor suppressor genes and is associated with an increasingly poor prognosis in prostate cancer. Oncotarget. 2017;8:108923-108935.

35. Dal Bo M, Rossi FM, Rossi D, et al. 13q14 deletion size and number of deleted cells both influence prognosis in chronic lymphocytic leukemia. Genes Chromosomes Cancer. 2011;50:633-643.

36. Inoue K, Fry EA. Haploinsufficient tumor suppressor genes. Adv Med Biol. 2017;118:83-122.

37. Berger AH, Pandolfi PP. Haplo-insufficiency: a driving force in cancer. J Pathol. 2011;223:137-146.

38. Xue W, Kitzing T, Roessler S, et al. A cluster of cooperating tumor-suppressor gene candidates in chromosomal deletions. Proc Natl Acad Sci U S A. 2012;109:8212-8217.

39. Inoue K, Fry EA, Taneja P. Recent progress in mouse models for tumor suppressor genes and its implications in human cancer. Clin Med Insights Oncol. 2013;7:103-122.

40. Ebert BL. Molecular dissection of the 5q deletion in myelodysplastic syndrome. Semin Oncol. 2011;38:621-626.

41. Dyrsø T, Li J, Wang K, et al. Identification of chromosome aberrations in sporadic microsatellite stable and unstable colorectal cancers using array comparative genomic hybridization. Cancer Genet. 2011;204:84-95.

42. Wu X, Ivanova G, Merup M, et al. Molecular analysis of the human chromosome 5q13.3 region in patients with hairy cell leukemia and identification of tumor suppressor gene candidates. Genomics. 1999;60:161-171.

43. Fukuyama R, Niculaita R, Ng KP, et al. Mutated in colorectal cancer, a putative tumor suppressor for serrated colorectal cancer, selectively represses beta-catenin-dependent transcription. Oncogene. 2008;27:6044-6055.

44. Xu W, Wang B, Xu Y. Expression of lysyl oxidase in human osteosarcoma and its clinical significance: a tumor suppressive role of LOX in human osteosarcoma cells. Int J Oncol. 2013;43:1578-1586.

45. Boulwood J, Pellagatti A, McKenzie AN, Wainscoat JS. Advances in the 5q- syndrome. Blood. 2010;116:5803-5811.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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