Aneuploidy drives lethal progression in prostate cancer

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Aneuploidy, defined as chromosome gains and losses, is a hallmark of cancer. However, compared with other tumor types, extensive aneuploidy is relatively rare in prostate cancer. Thus, whether numerical chromosome aberrations dictate disease progression in prostate cancer patients is not known. Here, we report the development of a method based on whole-transcriptome profiling that allowed us to identify chromosome-arm gains and losses in 333 primary prostate tumors. In two independent cohorts (n = 404) followed prospectively for metastases and prostate cancer-specific death for a median of 15 years, increasing extent of tumor aneuploidy as predicted from the tumor transcriptome was strongly associated with higher risk of lethal disease. The 23% of patients whose tumors had five or more predicted chromosome-arm alterations had 5.3 times higher odds of lethal cancer (95% confidence interval, 2.2 to 13.1) than those with the same Gleason score and no predicted aneuploidy. Aneuploidy was associated with lethality even among men with high-risk Gleason score 8-to-10 tumors. These results point to a key role of aneuploidy in driving aggressive disease in primary prostate cancer.

aneuploidy | prostate cancer | transcriptome | lethal disease

Aneuploidy, defined as a chromosome number that is not a multiple of the haploid complement, has long been proposed to drive the progression of cancer (1). Rare in normal cells (2), aneuploidy is highly prevalent in human cancer cells (3, 4). Losses of tumor suppressors and gains of oncogenes may confer a selective advantage to aneuploid tumor cells (5). Beyond individual genes, complex genetic interactions caused by the loss or gain of entire chromosome arms may contribute to cancer aggressiveness (6, 7). However, how aneuploidy influences cancer progression and whether degree of aneuploidy can be implemented clinically to inform the care of patients with cancer are still unclear.

Prostate cancer is an ideal cancer to study the impact of aneuploidy on disease progression because aneuploidy is relatively rare in this tumor type (3, 4) and the clinical course is highly variable, as highlighted by a ratio of ∼7:1 between new diagnoses and deaths from prostate cancer in the United States in 2016 (8). Previous studies have investigated overall copy-number alterations (CNAs), which include focal amplifications and deletions as well as larger copy-number gains and losses. Patients who had tumors with higher proportions of CNAs were modestly more likely to experience the surrogate outcome biochemical recurrence (9) and, at least if tumors were left untreated, to develop metastases (10). How whole-chromosome or arm-level gains and losses affect prostate cancer prognosis has, however, not been studied in detail. This is an important question, because large-scale copy-number gains and losses cause significant fitness defects in primary cells (2, 11) and possibly even in tumor cells (12). These observations would suggest that CNAs affecting whole chromosomes or chromosome arms would be associated with better prognosis. The opposite result would suggest that specific genes within large regions of CNAs confer a significant selective advantage to prostate tumors. We set out to address this question by examining chromosome arm-level aneuploidy in two independent cohorts followed prospectively for metastases and prostate cancer-specific death. Given the long natural history in prostate cancer, we leveraged a prostate tumor repository with a median of 15 y of follow-up and devised a transcriptional method to detect aneuploidy in archival specimens. We found that higher extent of prostate tumor aneuploidy at diagnosis is strongly associated with lethal disease. Our results indicate that chromosome arm-level CNAs occur early during tumorigenesis and harbor genes that contribute to aggressive disease.

Results

The Landscape of Chromosome Arm-Level Aneuploidy in Prostate Cancer. Today's next-generation sequencing studies tend to enroll highly selected patient populations from academic medical centers and lack longitudinal follow-up data. Such long-term follow-up to study clinically relevant outcomes of metastases and cancer-specific mortality often necessitates access to archival biorepositories of formalin-fixed, paraffin-embedded tumors. To overcome these limitations, we developed a method, summarized in Fig. 1, to quantify chromosome arm-level CNAs using chromosome arm-level transcriptome data available from archival tissue.

Significance

Aneuploidy is usually quantified by measuring intracellular DNA content or chromosome structure and number. We show that the number of altered chromosome arms can be estimated from transcriptome profiling, which allows for assessing aneuploidy within repositories of archival, formalin-fixed, paraffin-embedded tumors. While aneuploidy impedes proliferation in primary cells, we show that it is a feature of aggressiveness in primary prostate cancers that are more likely to become lethal. Our data suggest that losses or gains of entire chromosome arms confers aggressiveness beyond affecting copy numbers of tumor suppressors or oncogenes on those arms. Beyond helping understand the etiology of aggressive prostate cancer, we propose that extent of aneuploidy could also be employed clinically to inform risk stratification and treatment.

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We first employed The Cancer Genome Atlas (TCGA) primary prostate cancer cohort (n = 333; *SI Appendix*, Table S1) (13) to visualize the landscape of chromosome-arm losses or gains in primary prostate cancer, using DNA-sequencing data. To facilitate later comparison with the tumor transcriptome, we used a simplified definition of chromosome-arm aneuploidy. We defined a chromosome arm as gained or lost when the number of gene CNAs matched or exceeded the number of genes encoded on the chromosome arm (Fig. 2*A*). For example, chromosome 3q harbors 517 genes sequenced in tumors from TCGA. We considered this arm gained when at least an additional 517 copies of 3q-encoded genes were detected by sequencing.

Chromosome-arm alterations in tumors from TCGA were not randomly distributed across chromosomes (Fig. 2*B*). In line with studies of focal CNA in prostate cancer (4, 14–16), we observed the most frequent losses at chromosome arm 8p (36% of all tumors) and the most frequent gains at 8q (24%). Of 41 chromosome arms with a sufficient number of genes quantified (*SI Appendix*, Fig. S1), 16 had alterations that occurred in 5% or more of tumors (Fig. 2*B*). Twenty-three percent of primary tumors had five or more chromosome arms that were altered (lost or gained; Fig. 3*A*).

The Transcriptome as a Measure of Aneuploidy. We applied the information obtained through the analysis of tumor DNA from TCGA to the tumor transcriptome from TCGA. Here, we exploited the observation that the transcriptome reflects DNA CNAs, particularly when considering large CNAs (11, 17–19). We defined chromosome arm-level gains and losses on the transcriptome level a priori, using the same algorithm as for the DNA copy-number analysis. We summed normalized gene expression levels for all genes on a chromosome arm for each tumor (Fig. 2*C*). We defined chromosome arms as lost or gained if the sum of expression levels per chromosome arm was more extreme than the chromosome arm-specific quantile cutoff that corresponds to the quantile that encompasses tumors which were identified as harboring an arm-level gain or loss in the DNA copy-number analysis. Because of normalization, all genes had equal weights in this analysis, ensuring that results were not driven by a few highly expressed genes.

We evaluated how well aneuploidy predicted from the transcriptome corresponded to measured aneuploidy based on CNA.

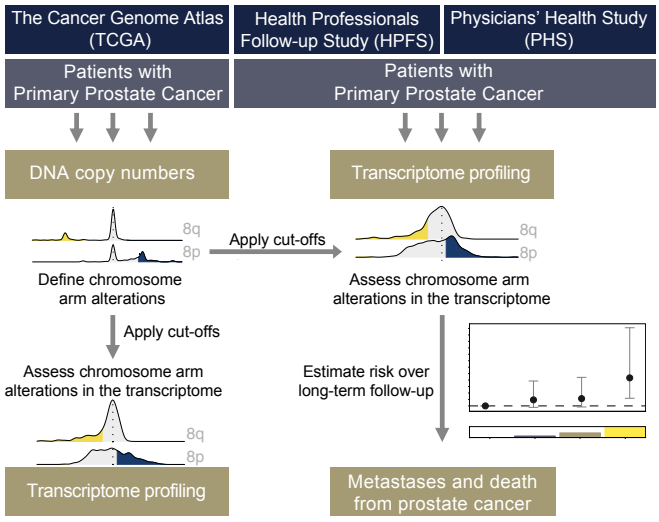

Fig. 1. Methods overview. Aneuploidy was assessed based on copy-number data from The Cancer Genome Atlas, measuring its frequency per chromosome arm. A nearly identical assessment of aneuploidy using the transcriptome was evaluated against DNA copy number-defined aneuploidy and then applied to whole-transcriptome profiling obtained from tumors of prostate cancer patients from the Health Professionals Follow-Up Study and Physicians' Health Study as a predictor of long-term clinical outcomes.

As anticipated, the visual separation of tumors that had gains or losses of specific chromosome arms from those without was not as distinct on the transcriptome level (Fig. 2*C*) as on the DNA copy-number level (Fig. 2*A*). Nevertheless, evaluating the area under the receiver operating curve (AUC), where 0.5 indicates random chance and 1.0 indicates perfect discrimination, the predicted number of chromosome alterations based on the transcriptomic algorithm had an AUC of 0.83 [95% confidence interval (CI), 0.78 to 0.87] for detecting any aneuploidy based on copy-number data (*SI Appendix*, Fig. S2*A*) and 0.87 (95% CI, 0.83 to 0.91) for detecting five or more chromosome-arm alterations (Fig. 3*B*). Five or more altered chromosome arms predicted from the transcriptome had a sensitivity of 67% (95% CI, 55 to 78%) and a specificity of 83% (95% CI, 78 to 87%) to correctly classify this extent of aneuploidy at the DNA copy-number level. As expected, absolute differences between the two measures increased for higher numbers of altered chromosome arms, yet mRNA-based predictions neither systematically overestimated nor underestimated measured aneuploidy scores (*SI Appendix*, Fig. S2*B*).

Having established a method to detect chromosome arm-level gains and losses in gene expression data, we applied the algorithm to quantify chromosome arm-level aneuploidies in 404 patients diagnosed with primary prostate cancer who were participants in the Health Professionals Follow-Up Study (HPFS) and the Physicians' Health Study (PHS) (Table 1) (20–22). Patterns of chromosome-arm alterations in the HPFS and PHS (*SI Appendix*, Fig. S3) were similar to TCGA (Fig. 2*C*).

Features of Aneuploid Prostate Cancers. To understand whether subsets of prostate cancers contain greater aneuploidy, we assessed the histologic and molecular features associated with aneuploidy. Much of prostate cancer risk classification and biology builds on the Gleason score, the standard histological measure of dedifferentiation in prostate cancer. Gleason scores generally range from the least aggressive tumors with score 6 (pattern 3 + 3) to highly aggressive tumors with scores up to 10 (pattern 5 + 5) and are a strong predictor of prostate cancer mortality, particularly when centrally reviewed as in all our cohorts (23). Tumors with higher Gleason scores had markedly higher aneuploidy scores in all three cohorts (Fig. 3*A* and *SI Appendix*, Fig. S4).

Nearly half of all prostate cancers harbor a gene fusion between the androgen-regulated gene *TMPRSS2* and the *ERG* oncogene, an early genetic event with a distinct etiology that shapes how biological and lifestyle factors influence prostate carcinogenesis (24, 25). Consistent with observations in other studies (14, 15), patterns of chromosome alterations were overall similar between fusion-positive and fusion-negative tumors (*SI Appendix*, Figs. S5 and S6). *ERG* status-specific quantile cutoffs were not necessary to improve prediction of aneuploidy from the transcriptome (*SI Appendix*, Fig. S7).

We assessed potential consequences of aneuploidy in prostate tumors. We observed a moderate linear correlation between aneuploidy scores and the proliferation marker Ki-67 in tumors from the HPFS and PHS (Fig. 3*C*; r = 0.15; 95% CI, 0.03 to 0.26) and with *MKI67* mRNA (coding for the Ki-67 protein) in tumors from TCGA (*SI Appendix*, Fig. S8*A*; r = 0.27; 95% CI, 0.17 to 0.37). Apoptosis as measured by the TUNEL index was not decreased in tumors with high levels of aneuploidy (*SI Appendix*, Fig. S8*B*; r = −0.01; 95% CI, −0.13 to 0.12). Together, our observations suggest that although aneuploidy impedes cell proliferation in primary cells, once prostate cells reach a neoplastic state, aneuploidy is associated with modestly increased proliferative potential.

Aneuploidy and Lethal Prostate Cancer over Long-Term Follow-Up. Over a median follow-up of 15.3 y, increasing tumor aneuploidy was strongly associated with an increasing risk of lethal prostate cancer in both the HPFS and PHS (Table 2). Even when adjusting for baseline covariates, the risk of lethal disease increased by 10% (95% CI, 2 to 18%) for each additional chromosome arm lost or gained. Compared with tumors without predicted aneuploidy but the same Gleason score, those 23% of

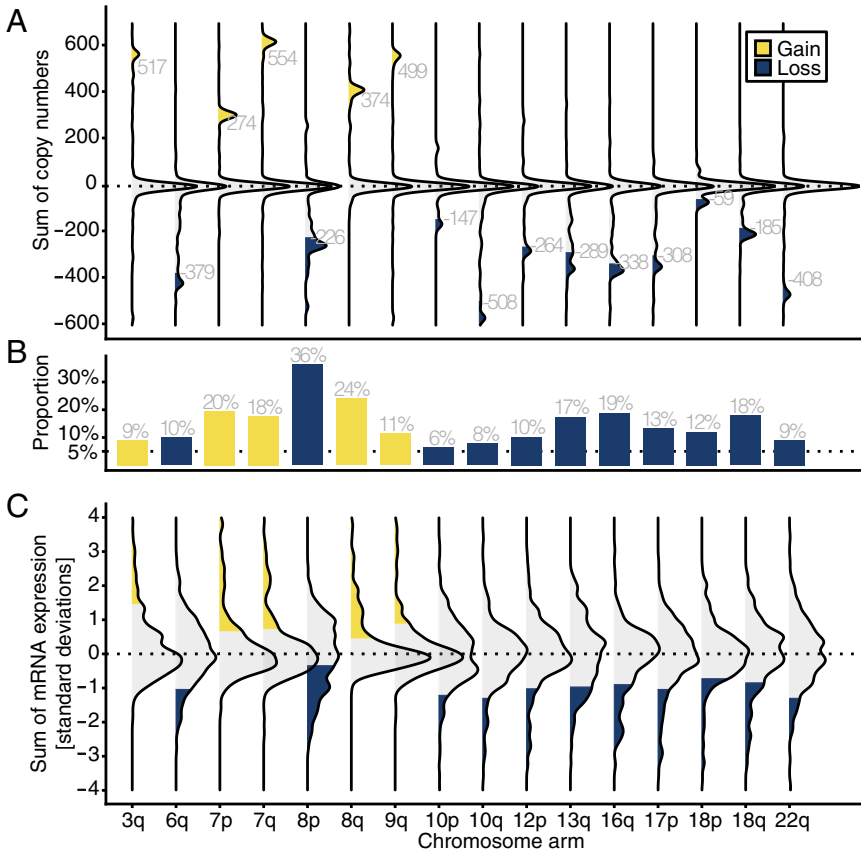

Fig. 2. Occurrence of aneuploidy in primary prostate cancer. (*A*) Aneuploidy measured by copy-number alterations. Gene copy-number alterations [from −2, indicating homozygous deletion (or two-copy loss); to 0, indicating diploidy/euploidy; to +2, indicating amplification (or two-copy gain)] were summed for each tumor and chromosome arm from TCGA. Plotted are distributions of these sums for each chromosome arm and tumor. The labels indicate the number of genes per chromosome arm. Copy-number sums that were more extreme than the number of genes were defined as chromosome-arm gains (yellow) and losses (blue). (*B*) Proportions of tumors with gained (yellow) or lost (blue) chromosome arms. Chromosome arms with more than 5% alterations are plotted. See *SI Appendix*, Fig. S1 for copy-number alterations in all chromosome arms. (*C*) Aneuploidy as predicted from the tumor transcriptome (TCGA). Shown is the distribution of each tumor's sum of mRNA expression levels, normalized in SDs, per chromosome arm. Predicted chromosome-arm gains (yellow) and losses (blue) are highlighted.

patients with five or more altered chromosome arms in their tumors had fivefold higher odds of lethal disease compared with those who had no aneuploidy [odds ratio (OR), 5.34; 95% CI, 2.18 to 13.1; Fig. 4 and Table 2]. Adjusting for prostate-specific antigen (PSA) levels at time of diagnosis, clinical stage, and treatment modality did not considerably attenuate associations (Table 2), nor did adjustment for pathological stage among patients treated with prostatectomy (results not shown). Even among patients with high-risk Gleason 8-to-10 tumors, the degree of tumor aneuploidy predicted future lethal disease (Table 2). Interestingly, there was a suggestion that aneuploidy is associated with lethality among patients who had tumors with low Gleason scores (≤3 + 4); however, as expected due to the limited number of events, these estimates were imprecise (Table 2). It is noteworthy that 58% of tumors with Gleason score 6 had some degree of aneuploidy (*SI Appendix*, Table S1). Tumors with low Gleason scores infrequently metastasize (23), suggesting that changes in copy number of genes located on aneuploid chromosomes drive lethal disease in other ways. Our study was not large enough to assess how the association of aneuploidy and lethal prostate cancer differed between tumors with *TMPRSS2:ERG* and those without.

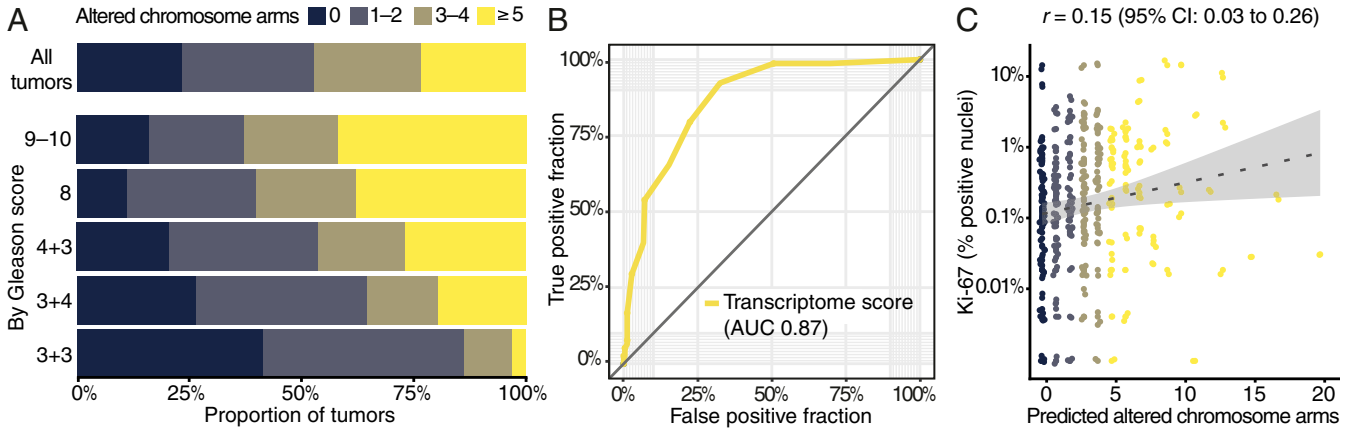

Fig. 3. Features of aneuploid tumors. (*A*) Numbers of altered chromosome arms in primary prostate cancer from TCGA, measured using copy numbers, overall, and by Gleason score. (*B*) Discrimination analysis for predicting aneuploidy (five or more altered chromosome arms) using the transcriptome (yellow line) compared with random chance (gray line). A larger area under the curve indicates better performance. (*C*) Number of predicted altered chromosome arms and expression of the cell-proliferation marker Ki-67 in tumors from the HPFS and PHS.

Table 1. Characteristics of prostate cancer patients from the Health Professionals Follow-Up Study and the Physicians' Health Study

Predicted altered chromosome arms	0	1–2	3–4	≥5
Median (interquartile range)	0 (0)	1 (1–2)	3 (3–4)	7 (5–9)
Patients, *n*	95	118	97	94
Year of diagnosis, *n* (%)				
1982–1989 (pre-PSA era)*	10 (11)	14 (12)	10 (10)	11 (12)
1990–1993 (peri-PSA era)*	21 (22)	30 (25)	22 (23)	40 (43)
1994–2005 (PSA era)*	64 (67)	74 (63)	65 (67)	43 (46)
Age at diagnosis, y, median (range)	64 (49–81)	66 (47–80)	66 (52–80)	67 (49–80)
Gleason grade, *n* (%)				
5–6	22 (23)	20 (17)	12 (12)	3 (3)
3 + 4	43 (45)	47 (40)	27 (28)	22 (23)
4 + 3	20 (21)	25 (21)	28 (29)	29 (31)
8	5 (5)	12 (10)	10 (10)	16 (17)
9–10	5 (5)	14 (12)	20 (21)	24 (26)
Clinical stage, *n* (%)†				
T1/T2	91 (97)	110 (93)	79 (83)	70 (78)
T3	3 (3)	5 (4)	11 (12)	8 (9)
T4/N1/M1	0 (0)	3 (3)	5 (5)	12 (13)
PSA at diagnosis, ng/mL, *n* (%)*,†				
<4	15 (17)	10 (10)	8 (10)	8 (11)
4–10	50 (58)	63 (63)	43 (53)	40 (54)
10–20	13 (15)	19 (19)	19 (23)	12 (16)
≥20	8 (9)	8 (8)	11 (14)	14 (19)
Tissue source, *n* (%)				
Prostatectomy	91 (96)	112 (95)	85 (88)	81 (86)
TURP/LN‡	4 (4)	6 (5)	12 (12)	13 (14)
TMPRSS2:ERG status, *n* (%)†				
ERG-negative	44 (51)	57 (52)	46 (53)	36 (43)
ERG-positive	42 (49)	52 (48)	40 (47)	48 (57)

*Prostate-specific antigen.
†Counts for three variables do not sum to 404 because of missing data (in 7 patients for clinical stage, 63 patients for PSA, and 39 patients for *TMPRSS2:ERG* status).
‡Tissue from transurethral resection of the prostate or a lymph node.

To evaluate whether whole-transcriptome expression profiling is necessary or whether prognostication based on aneuploidy could also be performed with a limited panel of genes, we evaluated the difference in median expressions between the most frequently gained or deleted chromosome arms, 8q and 8p. This measure was associated with lethal disease (*SI Appendix*, Table S2), although less strongly than the overall aneuploidy score. The aneuploidy score was associated with lethal disease beyond the difference between 8q and 8p medians (*SI Appendix*, Table S2), indicating that complete assessment of aneuploidy across all chromosomes is more informative.

Finally, we asked whether the association of aneuploidy and lethal prostate cancer was chiefly driven by a limited number of focal genetic events affecting specific tumor suppressors or oncogenes. As an example, we assessed loss of the tumor suppressor PTEN in comparison with loss of chromosome arm 10q, which contains the *PTEN* locus. PTEN loss is known to be associated with worse prostate cancer prognosis, including in our cohorts (24). Predicted loss of 10q and loss of PTEN tended to co-occur, though not in a deterministic fashion (*SI Appendix*, Table S3). Both PTEN protein loss and loss of 10q were individually associated with lethal disease, and the associations only changed modestly when mutually adjusting for 10q loss and PTEN loss (OR for 10q loss, 3.14; 95% CI, 1.13 to 8.75; OR for PTEN loss, 1.97; 95% CI, 1.04 to 3.73). Similarly, the association of 8q gain and lethal disease was slightly attenuated when adjusting for the 8q genes *MYC* or *SQLE* (*SI Appendix*, Table S3), although neither MYC protein nor *MYC* mRNA expression was associated with lethal disease in our cohorts (26). This indicates that single genes frequently altered in cancer cannot explain the association of chromosome-arm alterations with lethal disease. Instead, our data suggest that multiple genes located on the aneuploid chromosome arms drive lethal disease. Identifying them will be critical to understand prostate cancer evolution and provide new targets for therapeutic intervention.

Discussion

We developed and applied a method to infer aneuploidy from the transcriptome of archival tumor samples. It is important to note that our analysis only captures chromosome-arm alterations that occurred in tumors collected by TCGA. For application to tumors with different prevalence of chromosome-arm alterations, absolute cutoffs of gene expression levels or a direct definition of chromosome arm-level aneuploidy through DNA copy numbers would be necessary. Complementary to our approach, feasibility of DNA copy-number determination from archival prostate biopsies has been demonstrated (10). Both approaches open the door to leveraging archival tumor specimens from prospective cohort studies to assess the impact of aneuploidy on cancer, decreasing the risk of introducing selection bias and allowing researchers to harness decades of follow-up for clinically relevant outcomes. The two methods may also help overcome limitations of previous studies of CNAs in prostate cancer, which could only enroll patients from whom fresh-frozen biopsy samples could be obtained for DNA copy-number analysis (9, 14, 27, 28).

Previous prostate cancer studies with data on clinical outcomes employed a binary indicator of whole-genome doubling (or tetraploidy) (29–31) and found weak associations with prognosis. Hieronymus et al. (10) demonstrated an association between the proportion of the genome with CNAs (irrespective of chromosomal location) and cancer-specific prognosis. Our study expands on this work to show that extent of chromosome arm-level gains and losses is a strong predictor of disease outcome in prostate

Table 2. Aneuploidy predicted from the tumor transcriptome and risk of lethal prostate cancer over long-term follow-up in the Health Professionals Follow-Up Study and the Physicians' Health Study

		By number of altered chromosome arms in categories				
		0	1–2	3–4	≥5	Per additional chromosome arm*
HPFS and PHS cohorts combined						
Lethal: nonlethal cases, *n*		9: 86	25: 93	28: 69	51: 43	
OR for lethality (95% CI)						
A. Age, year-adjusted		1 (ref.)	2.33 (1.01–5.54)	3.57 (1.55–8.24)	9.81 (4.34–22.2)	1.15 (1.07–1.22)
B. Model A + Gleason		1 (ref.)	1.94 (0.77–4.86)	2.13 (0.84–5.42)	5.34 (2.18–13.1)	1.10 (1.02–1.18)
C. Model B + PSA		1 (ref.)	1.98 (0.73–5.34)	2.38 (0.87–6.50)	6.49 (2.46–17.1)	1.11 (1.03–1.19)
D. Model C + stage, Rx[†]		1 (ref.)	2.03 (0.75–5.47)	2.08 (0.75–5.81)	5.78 (2.15–15.6)	1.09 (1.01–1.17)
By cohort						
HPFS	Lethal: nonlethal cases, *n*	5: 50	21: 57	18: 40	39: 24	
	OR for lethality (95% CI)[‡]	1 (ref.)	3.68 (1.29–10.5)	4.50 (1.54–13.2)	16.25 (5.68–46.4)	1.20 (1.11–1.32)
PHS	Lethal: nonlethal cases, *n*	4: 36	4: 36	10: 29	12: 19	
	OR for lethality (95% CI)[‡]	1 (ref.)	1.00 (0.23–4.31)	3.10 (0.88–10.9)	5.68 (1.61–20.1)	1.10 (1.01–1.20)
By Gleason grade						
≤3 + 4	Lethal: nonlethal cases, *n*	2: 63	6: 61	3: 36	3: 22	
	OR for lethality (95% CI)[‡]	1 (ref.)	3.01 (0.58–15.6)	2.57 (0.40–16.3)	3.46 (0.53–22.4)	1.05 (0.91–1.21)
4 + 3	Lethal: nonlethal cases, *n*	4: 16	8: 17	6: 22	17: 12	
	OR for lethality (95% CI)[‡]	1 (ref.)	1.88 (0.47–7.49)	1.09 (0.26–4.51)	5.67 (1.51–21.2)	1.08 (0.97–1.20)
8–10	Lethal: nonlethal cases, *n*	3: 7	11: 15	19: 11	31: 9	
	OR for lethality (95% CI)[‡]	1 (ref.)	1.72 (0.33–8.86)	3.69 (0.73–18.7)	8.78 (1.72–44.8)	1.20 (1.04–1.40)

CI, confidence interval; OR, odds ratio; ref., reference category.

*Number of predicted chromosome-arm alterations modeled linearly to estimate the increase in risk with each additional altered chromosome arm.

[†]Additionally adjusted for clinical stage and primary treatment.

[‡]Analyses stratified by cohort and by Gleason grade are unadjusted, except for adjustment for Gleason grade 3 + 3 vs. 3 + 4 in the ≤3 + 4 group and Gleason grade 8 vs. 9 to 10 in the 8-to-10 group. Analyses stratified by Gleason grade combine the HPFS and PHS.

cancer patients. In fact, aneuploidy was associated with prognosis beyond strong predictors such as Gleason score. Each gene contributed to the aneuploidy score not just by its copy number or mRNA expression but also by its location in the genome, together with its neighboring genes. This integrative measure of aneuploidy might explain why aneuploidy was so strongly associated with risk of lethal disease. Additional studies are needed to determine whether assessing aneuploidy at cancer diagnosis (i.e., at prostate biopsy) has sufficient utility as a prognostic marker for a more accurate discrimination between patients with lethal and nonlethal disease, as well as more generally to provide information on the biological features of the entire tumor.

The observation that aneuploidy is strongly associated with lethal prostate cancer begs the question of how aneuploidy determines patient outcome. The relatively weak association between aneuploidy and proliferation demonstrated here, and of proliferative indices with prostate cancer prognosis demonstrated previously (29), suggests that increased proliferation is not the main mechanism through which aneuploid tumors become lethal. In line with this conclusion is our observation that recurrent aneuploidies do not appear to be solely driven by oncogenes and tumor suppressor genes known to drive and inhibit proliferation, respectively. We propose that other genes located on recurrent aneuploid chromosome arms do not necessarily drive proliferation but contribute to other aspects of lethal disease such as invasion, growth at metastatic sites, and/or promotion of genomic instability, thereby accelerating tumor evolution (32).

Our analyses lead to the interesting conclusion that recurrent chromosome-arm gains and losses are not solely driven by single oncogenes and tumor suppressor genes on these chromosome arms. Specifically, the risk of lethal disease associated with 10q loss and 8q gain could not simply be explained by the loss of *PTEN* and gain of *MYC* or *SQLE*, respectively. This conclusion is in line with the observation that genes are more than twice as likely to be affected by CNA on a chromosome-arm level than by a focal event (33). Identifying the genes that drive recurrent aneuploidies in prostate cancer will be critical. What causes aneuploidy in some patients but not others in the first place also remains to be determined.

An important question posed by our findings is whether aneuploidy can also inform treatment decisions. Considering that the vast majority of patients (92%) in our study underwent curative-intent prostatectomy, aneuploidy may be a category C prostate biomarker that identifies patients at risk for lethality despite surgical treatment (34). In this regard, we note a strong association between aneuploidy and lethality even among high-risk patients with tumors of Gleason scores 8 to 10, some of whom might be candidates for adjuvant therapy. Taxane sensitivity has been suggested to be associated with a transcriptional measure that could

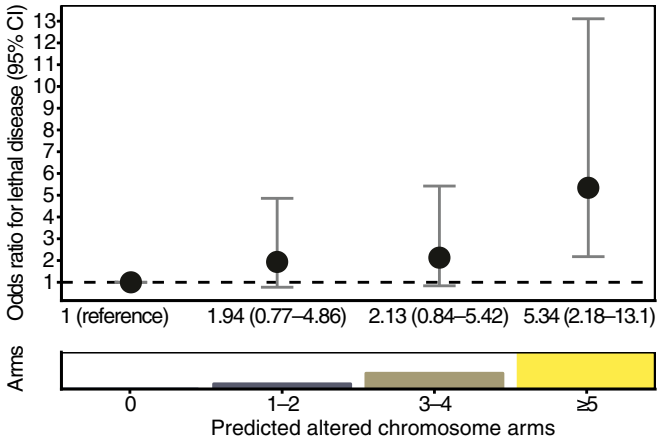

Fig. 4. Aneuploidy and lethal disease. Aneuploidy predicted from the tumor transcriptome at cancer diagnosis (in categories) and odds ratios (with 95% CIs) for lethal disease (metastases and death from prostate cancer) over long-term follow-up, adjusted for age at cancer diagnosis, calendar year of cancer diagnosis, and Gleason score.

reflect chromosomal instability (35) or proliferation (36). Thus, aneuploidy deserves further study as a predictive biomarker for benefit from adjuvant docetaxel, which is currently used in prostate cancer only once metastases have been detected (37).

In summary, a wide spectrum of genomic alterations, ranging from genome duplications to focal CNA, is characteristic of cancer. Our results suggest that important clues to the progression of primary prostate cancer lie in the middle ground, on a chromosome-arm level.

Methods

Patient Cohorts. We studied men with clinically localized prostate cancer included in three studies: TCGA, HPFS, and PHS. TCGA included 333 patients with localized prostate cancer from clinical research centers from whom fresh-frozen prostatectomy specimens were available (13). No information on patients before cancer diagnosis or follow-up for clinically relevant outcomes was available.

HPFS is an ongoing cohort of male health professionals ages 40 to 75 y at baseline in 1986 (n = 51,529) (20). PHS started as randomized controlled trials of aspirin and multivitamins in male physicians ages 40 to 84 y at baseline in 1982 (n = 29,067) (21, 22). All prostate cancer patients were followed prospectively from diagnosis for lethal disease (metastasis or cancer-specific death). For gene expression profiling, we undertook an extreme case–control design (38) of 404 men with clinically localized prostate cancer: "Cases" included men who developed distant metastatic disease or died of their cancer at any time during follow-up, and "controls" were prostate cancer cases without any evidence of metastatic disease after at least 8 y of follow-up.

Measures of Aneuploidy. Using the copy-number data in TCGA, scores of copy-number alterations per chromosome arm were generated by summing gene-level copy-number changes (coded on an ordinal scale from homozygous deletion, −2, to amplification, +2), assigning equal weights to all genes. Chromosome arms were defined as altered (lost or gained) if they had a score above a cutoff that corresponded to the number of transcribed genes on that chromosome arm, namely the score they would have had if all genes had either a gene copy loss or a gain (+1 or −1); the quantile of this value was later used as the arm-specific quantile cutoff. Individual chromosome arms were defined as lost or gained based on whether the 10th or 90th percentile of the copy-number sum was farther from 0.

In the transcriptome, mRNA expression from RNA sequencing and microarrays was normalized to a mean of 0 and SD of 1 (z scores), again assigning equal weights to all genes, and summed z scores for all genes per chromosome arm. Tumors were defined as aneuploid for a chromosome arm if these sums were higher than the chromosome-arm quantile cutoff defined in the DNA copy-number analysis.

The number of altered chromosome arms per tumor had a scale from 0 to 41, because chromosome arms 13p, 14p, 15p, 21p, and 22p and the Y chromosome did not have sufficient numbers of genes measured. Please see *SI Appendix, Supplementary Methods* for details regarding genomic and histologic data and statistical analysis.

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