Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

Genomics

Molecular Cancer Research

Genomic Gain of 16p13.3 in Prostate Cancer Predicts Poor Clinical Outcome after Surgical Intervention

Yogesh M. Bramhecha[1,2], Karl-Philippe Guérard[1], Shaghayegh Rouzbeh[1], Eleonora Scarlata[1], Fadi Brimo[3], Simone Chevalier[1,2], Lucie Hamel[1], Alice Dragomir[1], Armen G. Aprikian[1], and Jacques Lapointe[1,2]

Abstract

Identifying tumors with high metastatic potential is key to improving the clinical management of prostate cancer. Recently, we characterized a chromosome 16p13.3 gain frequently observed in prostate cancer metastases and now demonstrate the prognostic value of this genomic alteration in surgically treated prostate cancer. Dual-color FISH was used to detect 16p13.3 gain on a human tissue microarray representing 304 primary radical prostatectomy (RP) cases with clinical follow-up data. The results were validated in an external dataset. The 16p13.3 gain was detected in 42% (113/267) of the specimens scorable by FISH and was significantly associated with clinicopathologic features of aggressive prostate cancer, including high preoperative PSA ($P = 0.03$) levels, high Gleason score (GS, $P < 0.0001$), advanced pathologic tumor stage ($P < 0.0001$), and positive surgical margins ($P = 0.009$). The 16p13.3 gain predicted biochemical recurrence (BCR) in the overall cohort (log-rank $P = 0.0005$), and in subsets of patients with PSA ≤ 10 or GS ≤ 7 (log-rank $P = 0.02$ and $P = 0.006$, respectively). Moreover, combining the 16p13.3 gain status with standard prognostic markers improved BCR risk stratification and identified a subgroup of patients with high probability of recurrence. The 16p13.3 gain status was also associated with an increased risk of developing distant metastases (log-rank $P = 0.03$) further substantiating its role in prostate cancer progression.

Implications: This study demonstrates the prognostic significance of the 16p13.3 genomic gain in primary prostate tumors, suggesting potential utility in the clinical management of the disease by identifying patients at high risk of recurrence who may benefit from adjuvant therapies. *Mol Cancer Res; 16(1); 115–23. ©2017 AACR.*

Introduction

Prostate cancer is the most commonly diagnosed malignancy and one of the leading causes of cancer-related mortality in North American men (1). It is a heterogeneous disease ranging from clinically insignificant tumors that may not require any treatment to lethal forms of prostate cancer. Standard treatment options for localized prostate cancer consist mainly of radical prostatectomy (RP) or radiotherapy. Active surveillance, where the cancer is closely monitored and the definitive treatments are delayed until disease progression, has become a viable option for patients considered at low risk of progression (2). A key issue in the clinical management of prostate cancer is the difficulty to accurately distinguish aggressive from indolent tumors, which inevitably leads to overtreatment of harmless diseases and undertreatment of aggressive cancers with metastatic potential (3). The routinely used prognostic indicators like serum PSA levels, clinical tumor stage (cT-stage), and biopsy Gleason score (GS) have demonstrated limitations at accurately predicting the individual clinical outcome. Precisely assessing GS on biopsies is limited by the fact that partial sampling may result in an underestimation of the final score of cancer in the RP specimen (4). The majority of patients undergoing RP present low–intermediate risk clinical features, and accurate prognosis within this subgroup of patients still remains a clinical challenge. Moreover, although most patients respond well to RP, a significant proportion will experience a disease recurrence, as assessed by a rise in serum PSA that might eventually progress to the metastatic stage. Early identification and more accurate risk stratification may, therefore, allow patients with aggressive tumors to receive appropriate treatment without delay while sparing patients with clinically favorable tumors from treatment side effects (3, 5).

Genomic profiling studies have highlighted disease heterogeneity, suggesting alternative molecular pathways driving prostate cancer development (6–13). Lapointe and colleagues have

[1]Department of Surgery, Division of Urology, McGill University and the Research Institute of the McGill University Health Centre, Montréal, Québec, Canada. [2]Division of Experimental Medicine, McGill University and the Research Institute of the McGill University Health Centre, Montréal, Québec, Canada. [3]Department of Pathology, McGill University and the Research Institute of the McGill University Health Centre, Montréal, Québec, Canada.

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

Corresponding Author: Jacques Lapointe, Department of Surgery, McGill University and the Research Institute of the McGill University Health Centre, Room EM2.2212, 1001 Décarie Blvd, Montréal, Québec H4A3J1, Canada. Phone: 514-934-1934; Fax: 514-934-8297; E-mail: jacques.lapointe@mcgill.ca

doi: 10.1158/1541-7786.MCR-17-0270

Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

reported high frequency of DNA copy number alterations (CNA) in prostate cancer lymph node metastases (LN mets) including a gain of 16p13, 8q24 and deletion of 10q23 and 16q23 (11). These CNAs represent potential prognostic biomarkers that can be assessed in routine formalin-fixed paraffin-embedded (FFPE) tissue sections using specific DNA probes by FISH (14–19). We recently mapped the previously uncharacterized focal 16p13.3 genomic gain in primary prostate tumors and identified *PDPK1* encoding 3-phosphoinositide–dependent protein kinase-1 (PDK1) as a likely driver of the gain with functional impact on prostate cancer cell migration (20). The current study, for the first time, demonstrates that 16p13.3 genomic gain is a predictor of poor clinical outcome in patients treated by RP that can aid to better risk stratify these patients when combined with standard clinicopathologic prognostic markers.

Materials and Methods

Study population and tissue microarray

The study was conducted with the written informed consent of the participants and approval from the Research Ethics Board of McGill University Health Centre (Québec, Canada, BDM-10-115). This biomarker study was done in accordance with REMARK guidelines (21). FFPE RP tissue specimens ($n = 304$) collected between 1993 and 2008 at the McGill University Health Centre were represented on a tissue microarray (TMA) by duplicate 1-mm cores taken from the dominant tumor nodule. The clinical correlates were retrieved from the medical chart and pathologic data were obtained by re-review of all RP cases by a single dedicated genitourinary pathologist (F. Brimo). The recent 2014 International Society of Urological Pathology (ISUP) criteria were used for assigning the final grade (22). The clinicopathologic characteristics of the study subjects are summarized in Table 1. Briefly, the mean preoperative serum PSA level for the cohort was 8.60 (±8.21), and the distribution of GS 6, 7, and ≥8 was 20.4%, 70.4%, and 9.2%, respectively. Sixty-four percent of patients belonged to pT2-stage, while 36% were at stage pT3. Cases for which the serum PSA did not fall to undetectable levels postsurgery were considered as surgical failure and were not included in BCR analyses ($n = 14$). Patients who received neoadjuvant hormone therapy ($n = 5$) and cases with missing serum PSA data postsurgery were also excluded from BCR analyses ($n = 15$). BCR was defined by serum PSA elevation of >0.2 ng/mL after RP (29%), and the recurrence-free interval was defined as the time between the date of surgery and the date of first PSA increase above 0.2 ng/mL. Patients with no BCR were censored at the last follow-up date with a PSA measurement. The median follow-up for the cohort was 118 months (1–253 months, min–max). The metastatic status was confirmed by imaging in patients with clinical signs or symptoms ($n = 16$). The metastasis-free interval was defined as the time between the date of surgery and the date of first metastasis detection. Patients with no signs/symptoms of metastasis were censored at the last follow-up/PSA date. The CAPRA-S (Cancer of the Prostate Risk Assessment Post-Surgical) score was calculated on the basis of the status of six clinicopathologic variables [preoperative PSA, GS, surgical margin (SM), extracapsular extension, seminal vesicle invasion, lymph node invasion], and each patient was assigned to one of the three risk groups: low (0–2), intermediate (3–5), and high (≥6) according to Cooperberg and colleagues (23). Of note, patients who did not undergo a lymph node dissection were deemed to have negative lymph nodes for CAPRA-S score calculation as described previously (24). The prostate cancer DNA CNAs profiling data reported by Taylor and colleagues (10) was used for validation, and the clinical correlates were derived directly from the MSKCC Prostate Cancer Genomics Data Portal.

FISH

Dual-color FISH was performed on TMA sections using as probes, a 16p13.3 specific BAC clone RP11-20I23 (BACPAC Resources Center) and the recombinant DNA clone pHuR-195 (ATCC), mapping to the 16qh centromeric region (25). RP11-20I23 and pHuR-195 DNA were labeled with Spectrum Orange-dUTP and Spectrum Green-dUTP (Enzo Life Sciences) respectively, using Nick Translation Reaction Kit (Abbott Molecular) and were used to perform FISH on 5-μm TMA sections as we described previously (20).

FISH data analysis

To evaluate the 16p13.3 copy number status, fluorescent signals were counted in 100 nonoverlapping interphase nuclei for each case (as identified on corresponding H&E) counterstained with ProLong Gold antifade reagent with DAPI (Life Technologies), to delineate nuclei. The 16p13.3 gain was defined as present at a threshold of ≥15% of tumor nuclei containing three or more 16p13.3 locus signals and two pHuR-195 signals, as we previously reported (20). Images were acquired with an Olympus IX-81 inverted microscope at ×96 magnification, using Image-Pro Plus 7.0 software (Media Cybernetics).

Statistical analyses

Associations between the 16p13.3 gain and clinicopathologic variables were evaluated by Fisher exact test for dichotomous variables and unpaired *t* test for continuous variables. Kaplan–Meier method and the log-rank test were used to generate and compare recurrence-free survival and metastasis-free survival curves, respectively. Cox regression analyses and the Wald test were used to evaluate univariate and multivariate HRs. The C-index was calculated as described by Harrel and colleagues (26). Analyses were performed using SPSS, WinStat, and R (Version 3.3.2).

Results

Association of 16p13.3 gain with adverse clinicopathologic features in RP

We used dual-color FISH to assess CNA at chromosome 16p13.3 on 304 RP specimens represented on a TMA. The clinicopathologic characteristics of the study subjects are summarized in Table 1. A total of 267 primary tumors were scorable by FISH, among which 113 (42%) harbored significant 16p13.3 genomic gain (Fig. 1). The 16p13.3 gain was significantly associated with clinicopathologic features of aggressive prostate cancer (Table 2) including high preoperative serum PSA levels ($P = 0.03$), GS ($P < 0.0001$), advanced pT-stage ($P < 0.0001$), and positive SMs ($P = 0.009$). The level of gain was restricted to single copy gain in most specimens, whereas 20 cases with 16p13.3 gain exhibited more than three copies of the locus in at least 10% of their nuclei. As

Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

Table 1. Clinicopathologic features of RP cases represented on the TMA

Clinicopathologic variables	Category	*n* (%)
Total number of cases	*N*	304
Age (years)	Median	61
	Min–max	43–73
Preoperative PSA (ng/mL)	*n*[a]	298
	Mean (±SD)	8.60 (±8.21)
	PSA < 10	232 (78%)
	PSA ≥ 10	66 (22%)
GS at surgery	GS 6	62 (20.4%)
	GS 7	214 (70.4%)
	GS ≥ 8	28 (9.2%)
Pathologic stage (T-stage)	pT2	195 (64%)
	pT3A	87 (29%)
	pT3B	22 (7%)
Surgical margin status	Positive	91 (30%)
Follow-up (months)	*n*[a]	270
	Median (min–max)	118 (1–253)
BCR	*n*[a]	270
	Positive	78 (29%)
Metastases	Positive	16/293[a] (5.4%)

[a]Values not available for all the 304 cases (*n* noted for each variable).

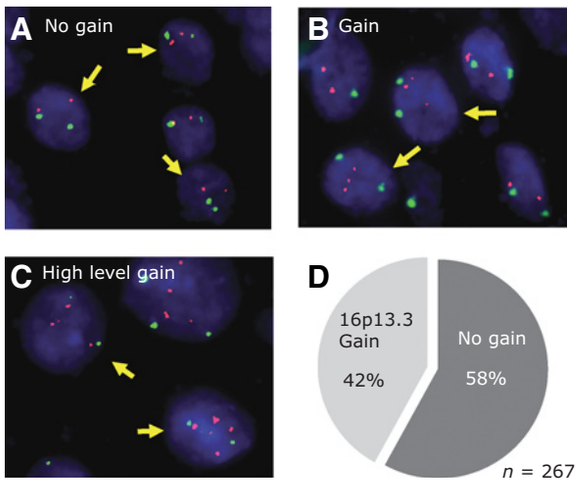


Figure 1.

Dual-color FISH analysis of 16p13.3 gain in FFPE prostate cancer specimens. The arrows indicate, normal interphase nuclei with 2 orange signals (16p13.3 locus) and 2 green signals (centromere 16) in the tumor specimen with no 16p13.3 gain (**A**); nuclei with 3 orange signals (16p13.3 locus) and 2 green signals (centromere 16) per nucleus, indicating a single copy 16p13.3 gain in a prostate tumor (**B**); and nuclei with high level of gain (>3 orange signals and 2 green signals) in a prostate tumor (**C**). All representative pictures were taken at ×96 magnification. **D,** The pie chart represents the proportion of RP specimens in McGill cohort, harboring 16p13.3 gain as assessed by FISH.

compared with the organ-confined stage-T2 tumors, the advanced stage-T3 tumors harbored significantly higher percentage of nuclei with more than three copies (Mann–Whitney U test, $P = 0.01$).

Prognostic significance of 16p13.3 genomic gain in prostate cancer

We assessed the impact of the 16p13.3 genomic gain on clinical outcome using BCR as a surrogate primary endpoint following RP. Of the 238 cases for which both FISH and complete PSA follow-up data were available (median follow-up = 117 months), 65 (26%) experienced BCR. The Kaplan–Meier analysis revealed that the 16p13.3 gain was significantly associated with shorter BCR-free survival following RP (log-rank $P = 0.0005$; Fig. 2A). We then assessed the 16p13.3 gain in terms of its ability to stratify patients with low–intermediate risk, representing a majority of patients encountered in clinical practice. We observed that 16p13.3 gain status could further risk stratify patients for BCR when considering the subgroup of patients with either preoperative PSA ≤10 or GS ≤7 (log-rank $P = 0.02$ and $P = 0.006$, respectively; Fig. 2B and C), but not significantly in the pT-stage ≤T2 subgroup (log-rank $P = 0.24$; Fig. 2D).

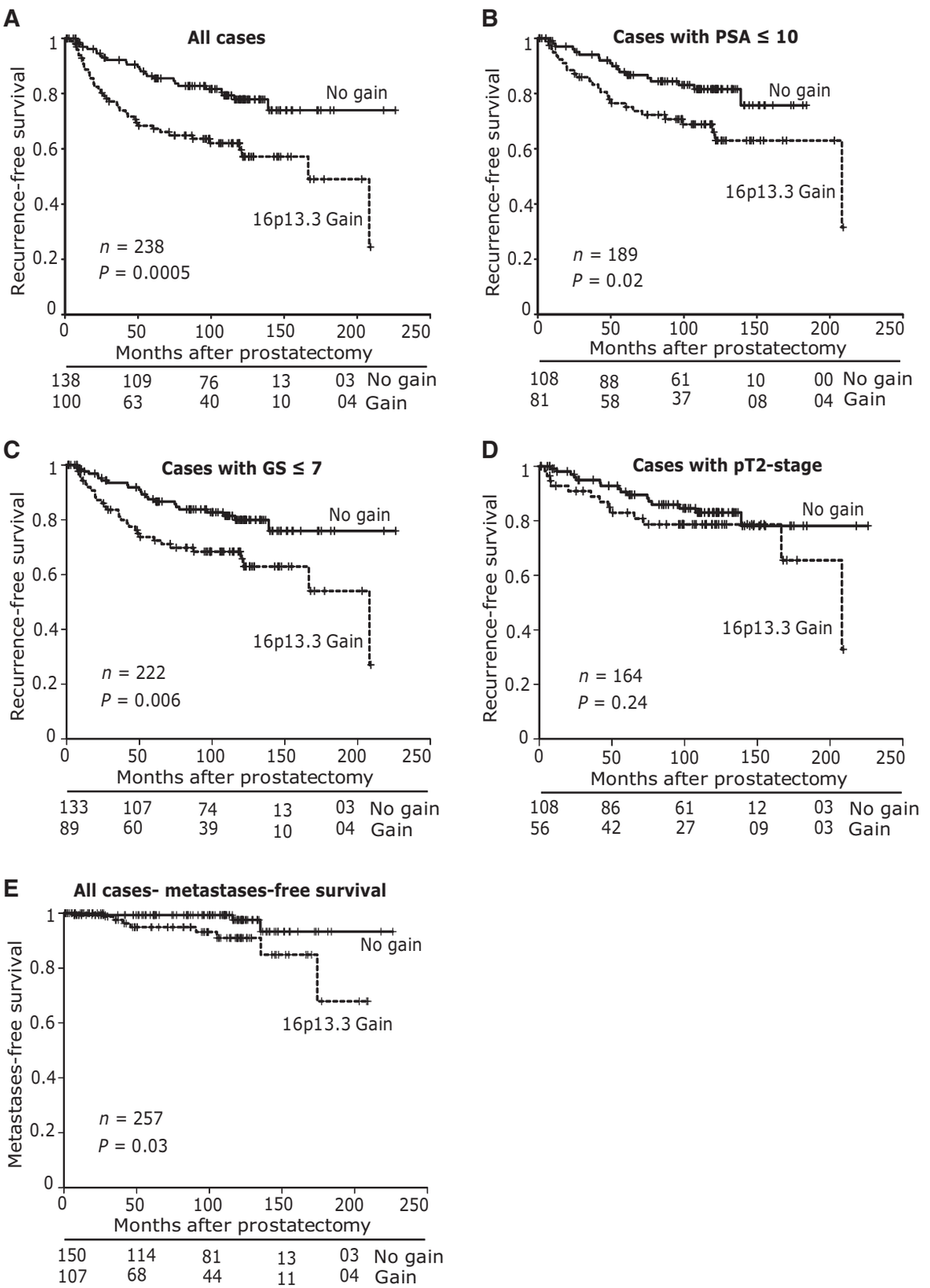
Although the main endpoint of our study was BCR, we also evaluated secondary endpoints, such as bone or soft tissue metastases. The 16p13.3 gain status was significantly associated with an increased risk of metastases (log-rank $P = 0.03$; Fig. 2E), supporting its potential utility as a marker of prostate cancer progression.

Table 2. Association of 16p13.3 gain with clinicopathologic features of aggressive prostate cancer

		16p13.3 Status		
Variables_McGill cohort	Total cases *n* (%)	No gain	Gain	*P*
16p13.3 Status	267	154 (58%)	113 (42%)	
Age (years) mean (±SD)	61.8 (±5.8)	61.0 (±6.12)	60.7 (±5.64)	0.68[a]
Preoperative PSA (mean/±SD)	261[b]	7.6 (±6.88)	9.61 (±8.28)	0.03[a]
GS	267			
GS = 6	59	49 (83%)	10 (7%)	<0.0001[c]
GS = 7	185	98 (53%)	87 (47%)	
GS ≥ 8	23	7 (30%)	16 (70%)	
Pathologic T-stage	267			
pT2	176	118 (67%)	58 (33%)	<0.0001[c]
pT3A	72	31 (43%)	41 (57%)	
pT3B	19	05 (26%)	14 (74%)	
Surgical margins	267			0.009[c]
Negative	189	119 (63%)	70 (37%)	
Positive	78	35 (45%)	43 (55%)	

[a]Unpaired *t* test.
[b]Values not available for all the 267 cases that could be assessed by FISH (*n* noted for each variable).
[c]Two-sided Fisher exact test.

Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270



Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

Table 3. Univariate and multivariate Cox proportional hazards analysis for 16p13.3 gain adjusting for standard clinicopathologic parameters

McGill cohort	Univariate analysis		Multivariate analysis	
Variable	**HR (95% CI)**	***P*[a]**	**HR (95% CI)**	***P*[a]**
Preoperative PSA[b]	1.07 (1.04–1.10)	<0.0001	1.05 (1.02–1.08)	<0.0001
GS (≥8 vs. ≤GS7)	5.69 (3.01–10.76)	<0.0001	3.06 (1.47–6.38)	0.003
pT-stage (T3 vs. T2)	3.46 (2.10–5.69)	<0.0001	1.96 (1.10–3.48)	0.02
16p13.3 status (gain vs. no gain)	2.30 (1.40–3.78)	0.001	1.71 (1.01–2.89)	0.04
Surgical margin (positive vs. negative)	2.14 (1.30–3.51)	0.003	1.53 (0.88–2.64)	0.12

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a]Wald test.
[b]Analyzed as continuous variable.

Improved risk stratification of existing prognostic tools when combined with 16p13.3 gain

The HR for the 16p13.3 gain (HR = 2.30; 95% CI, 1.42–3.84; $P = 0.001$) was estimated by univariate Cox regression analysis along with preoperative PSA levels, GS, pT-stage, and SM, which were also significantly associated with BCR (Table 3). In multivariate analysis, the 16p13.3 gain status remained a significant predictor of BCR after adjusting for the above standard clinicopathologic variables (Table 3). These observations were validated in an independent dataset from Taylor and colleagues (10), who reported DNA CNAs by array-CGH analyses of 194 prostate cancer cases with clinical follow-up (Supplementary Table S1).

We further evaluated whether combining 16p13.3 gain status with preoperative PSA, GS, and pT-stage, respectively, would improve patient stratification. The combination of the 16p13.3 gain status with each of these standard clinicopathologic variables segregated prostate cancer cases into three prognostic subgroups (log-rank $P < 0.0001$, respectively; Fig. 3A–C) stratified by the number of positive markers: (i) worst prognostic group characterized by presence of both markers; (ii) intermediate prognostic group with either of the two positive markers; and (iii) favorable prognostic group, with both markers absent. Furthermore, the BCR risk stratification significantly improved when all the 4 variables were considered to group the patients (log-rank test, $P < 0.0001$; Fig. 3D).

We then explored whether the 16p13.3 gain status could provide further prognostic information to the CAPRA-S score, a recently developed and validated clinicopathologic tool to predict the risk of recurrence post RP (23). In multivariate analysis, the 16p13.3 gain status was a significant predictor of BCR along the CAPRA-S score risk groups (Table 4). As expected, the three risk groups defined by the CAPRA-S score were associated with distinct BCR-free survival probabilities (Fig. 4A). We then assessed whether the 16p13.3 gain status could further stratify each of these risk groups. Although the 16p13.3 gain status did not further stratify the low-risk group, cases in the intermediate-risk group harboring the gain presented a similar risk of BCR as the high CAPRA-S risk group without this genomic alteration, while those belonging to the high CAPRA-S risk group with the gain had the worst outcome (Fig. 4B). By merging groups with the overlapping risk of BCR, we delineated four risk groups (Fig. 4C). The addition of 16p13.3 gain status to the CAPRA-S score further led to the increase of the C-index as compared with the CAPRA-S score alone (0.78 vs. 0.77). Similarly, in Taylor and colleagues' validation dataset, the gain status with CAPRA-S score also identified cases with very high risk of recurrence with a C-index of 0.73 as compared with 0.72 for the CAPRA-S alone (Supplementary Fig. S1).

Discussion

The detection of 16p13.3 gain in primary prostate cancer specimens was in coherence with our previously published report, where Choucair and colleagues detected 16p13.3 gain by FISH in 20% of the 46 RP specimens assessed (20). The difference in the proportion of cases harboring the gain (20% vs. 42%) between the two studies might be attributed to the small sample size of the earlier study ($n = 46$) or reflected real biological differences between these two independent patient sets. Similar to our previous observations (20), the majority of cases with 16p13.3 gain harbored a single extra copy. Single copy gains of loci or entire chromosome are known recurrent events in other types of cancer that effectively contribute to tumor phenotypes (27–30). A few cases harbored more than a single copy gain, and they were more prevalent in the pT3-stage tumors. In our previous FISH study, nuclei with more than three copies of 16p13.3 locus were more common in LN mets and transurethral resections of prostate tissues of advanced castrate-resistant prostate cancer (CRPC) than in RP specimens (20), supporting a potential role for this locus in disease progression.

Consistent with our previous study, the 16p13.3 genomic gain was associated with clinicopathologic features of aggressive prostate cancer, such as high GS and preoperative PSA levels. Patients harboring 16p13.3 gain were more than twice as likely to experience BCR following RP than those without the gain. Combining the 16p13.3 gain status with individual clinicopathologic markers significantly improved BCR risk stratification in our study, wherein the incremental number of positive variables was associated with a higher risk of BCR,

Figure 2.
Prognostic value of the 16p13.3 genomic gain in primary tumors of prostate cancer patients. Kaplan–Meier recurrence-free survival analysis of prostate cancer patients stratified on the basis of 16p13.3 gain status determined by FISH in all RP patients with clinical follow-up for BCR (**A**; $n = 238$), as well as in the subgroup of patients belonging to low–intermediate risk patients with PSA ≤10 (**B**; $n = 189$); patients with GS ≤ 7 (**C**; $n = 222$); and patients with stage T2 (**D**; $n = 164$). **E,** Kaplan–Meier metastases-free survival analysis of all cases stratified on the basis of 16p13.3 gain status ($n = 257$). Number of patients at risk at respective time points and *P* value (log-rank test) are indicated.

Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

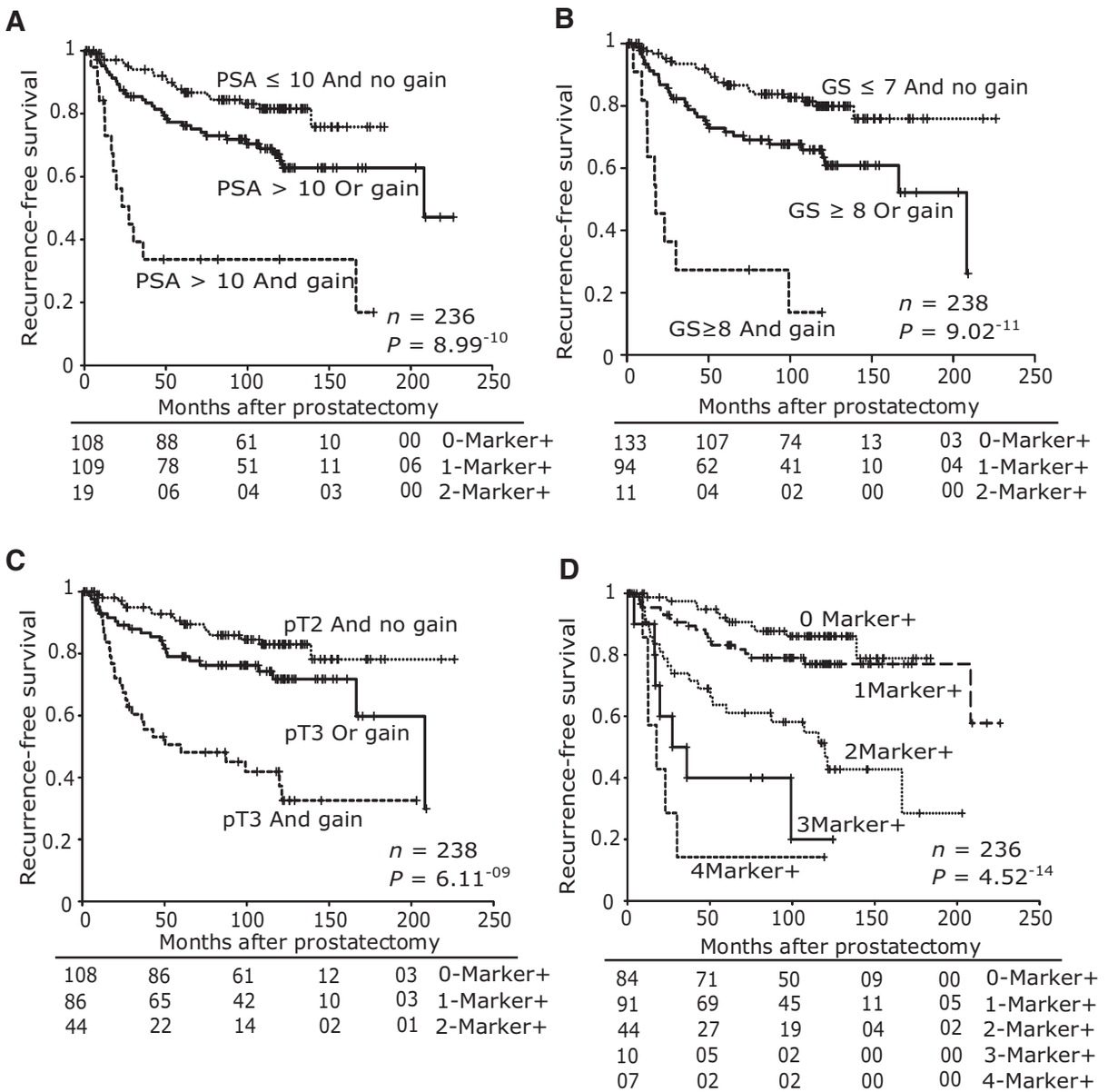


Figure 3.

Improved risk stratification upon combination of 16p13.3 gain and standard prognostic markers. The 16p13.3 gain status was combined with individual standard clinicopathologic variables: PSA (**A**; ≤10 vs. >10 ng/mL); GS (**B**; ≤GS7 vs. ≥8); and pT-stage (**C**; pT2 vs. pT3) and cases with zero, one or two positive markers were compared by Kaplan–Meier analyses. **D,** The 16p13.3 gain status and all the 3 clinicopathologic variables described above were used to stratify patients and cases with zero, one, two, three, or all four positive markers were grouped to compare BCR outcome. Number of patients at risk at respective time points and *P* value (log-rank test) are indicated.

Table 4. Univariate and multivariate Cox proportional hazards analysis for CAPRA-S score risk groups and 16p13.3 gain

McGill cohort	Univariate analysis		Multivariate analysis	
Variable	**HR (95% CI)**	***P*[a]**	**HR (95% CI)**	***P*[a]**
CAPRA-S Risk				
Low (0–2) Ref.		<0.0001		<0.0001
Intermediate (3–5)	3.13 (1.66–5.91)	0.0004	2.91 (1.54–5.51)	0.001
High (≥6)	8.94 (4.49–17.79)	<0.0001	7.82 (3.89–15.73)	<0.0001
16p13.3 Status (gain vs. no gain)	2.30 (1.40–3.78)	0.001	1.81 (1.09–3.00)	0.02

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a]Wald test.

Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

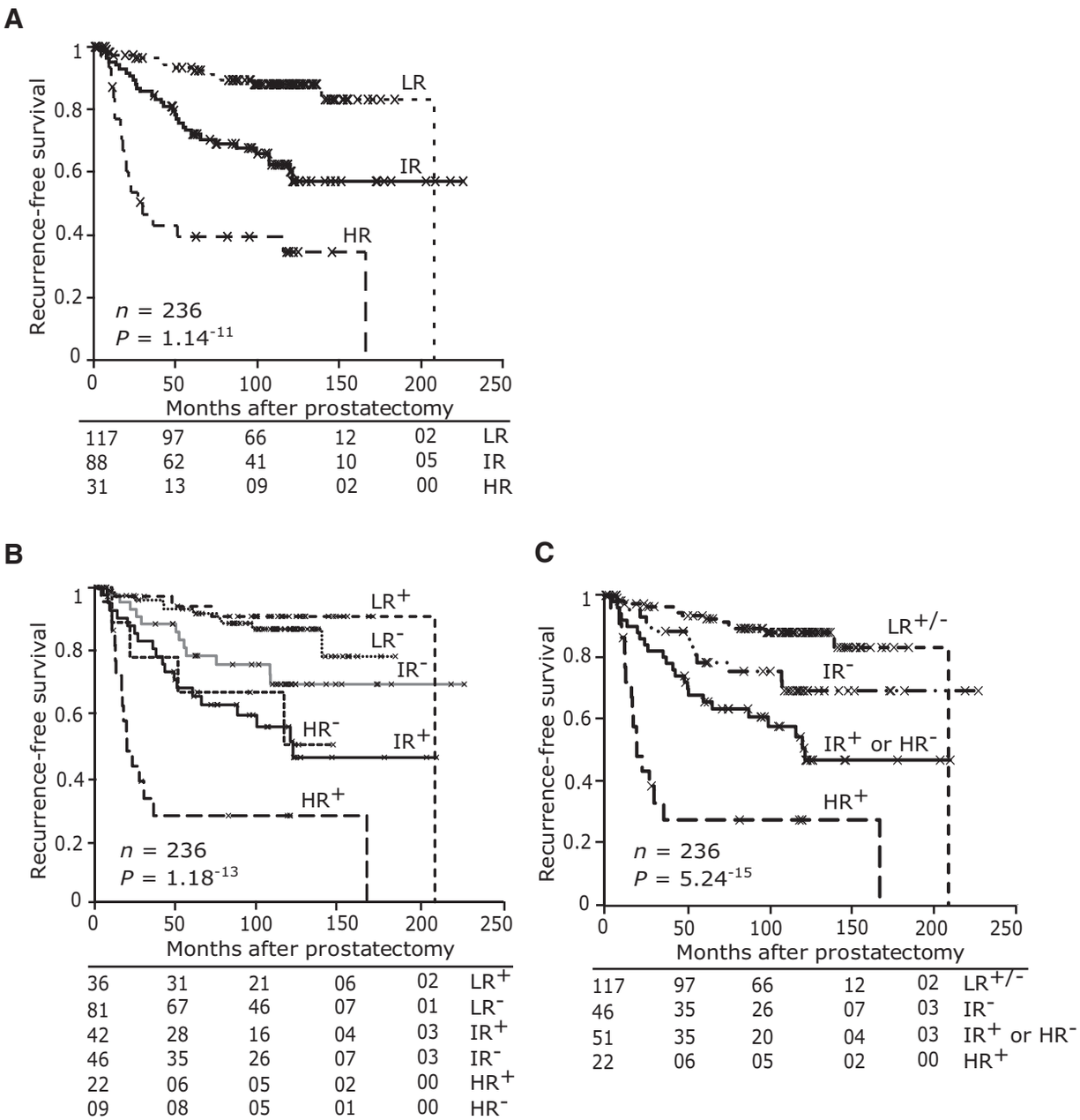


Figure 4.

16p13.3 gain status affords additional prognostic information to the preestablished CAPRA-S score risk groups. **A,** Kaplan–Meier curves validating the prognostic significance of CAPRA-S risk groups when stratified as low risk (LR, CAPRA-S score 0–2), intermediate risk (IR, CAPRA-S score 3–5), and high risk (HR, CAPRA-S score $\geq$ 6). **B,** Each CAPRA-S risk group was further subdivided on the basis of the presence (+) or absence (−) of the 16p13.3 gain. **C,** Four risk groups were derived by merging the groups with the overlapping risk of BCR from **B** as follows: $LR^{+/-}$; IR^{-}; IR^{+} or HR^{-}; and HR^{+}; respectively. Number of patients at risk at respective time points and *P* value (log-rank test) are indicated.

which reached its maximum for patients with four adverse factors, including the 16p13.3 gain. This improved stratification was observed in patients with intermediate and high risk of disease progression based on their CAPRA-S score. These results are in line with previous reports showing that genomic markers can further stratify subsets of patients classified by the CAPRA-S score (31, 32). The fact that the CAPRA-S score is already a very strong multiparameter predictor of BCR and that the low-risk group was not further stratified by the 16p13.3 gain may explain the limited increase of the C-index observed by the addition of this single variable to the model. Our model analysis was exploratory in nature, and further validation

Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

studies are warranted on larger cohorts to fully evaluate the added prognostic value of the 16p13.3 gain to the clinicopathologic predictors. Nevertheless, the addition of the 16p13.3 gain status to CAPRA-S identified a subset of patients at very high risk of recurrence, who may benefit from adjuvant treatments after RP.

Although very few metastatic events were observed in the McGill cohort (~5%), the 16p13.3 gain was also predictive of the increased risk of developing distant metastases following RP. Recently, using the whole-genome sequencing approach, Beltran and colleagues detected the 16p13.3 gain in 52% of the metastatic CRPC tumor samples (13). These results are in line with our previous studies detecting gain in about 50% prostate cancer lymph node metastases, an overrepresentation as compared with unpaired primary tumors (11, 20). These observations further support a role of 16p13.3 gain in cancer progression and warrant future studies in the context advanced diseases and response to therapies including androgen ablation.

The association of 16p13.3 gain with features of aggressive tumors was in line with studies in the breast (33), lung (34), and colon cancer (35), wherein the 16p13 gain was linked to poor prognosis. Our previous study defined the minimal region of 16p13.3 gain spanning 19 genes. *PDPK1*, encoding PDK1, was shown to be a likely driver of the gain but other genes shown to be involved in other types of cancer reside at this locus as well (20). Of these, *RAB26* is a Ras oncogene family member found to be upregulated in non–small cell lung carcinoma (36) and uveal melanoma (37). Similarly, *CCNF* (G2-mitosis–specific cyclin-F) was reported to be overexpressed in breast and esophageal cancer, respectively (38, 39). *ABCA3*, a known drug efflux pump belonging to the p-gp family, is overexpressed in acute myeloid leukemia (AML) and different cancer cell lines. Notably, ABCA3 overexpression conferred drug resistance in these AML cases (40–42). The relevance of these genes in prostate cancer remains to be investigated through functional studies.

A potential application for the 16p13.3 gain would be a FISH biomarker to identify patients requiring adjuvant or neoadjuvant therapies and to improve pretreatment prognostication given that accurate GS can be challenging to obtain on biopsies. Studies on needle biopsy cohort would be required for its implementation in the presurgical setting. The spatial resolution afforded by a histology-based assay such as FISH would facilitate sensitive assessment of individual cancer foci (43, 44) in a context of tumor heterogeneity and bypass the need for nucleic acid extraction from bulk tumor tissue required by several recently developed commercial assays based on gene expression signatures, such as Prolaris and Decipher (45–48). Of course, head to head comparison with these emerging assays would have to be done to select the best test for a given clinical context. Importantly, further studies are needed to demonstrate that genomic biomarker results can predict response to specific therapies and thus orient clinical decisions.

Taken together, our results support a role for 16p13.3 gain in prostate cancer progression and as a relevant prognostic biomarker. Incorporating 16p13.3 gain status with routinely used clinicopathologic variables could potentially be a step toward improving our ability to stratify patients into different prognostic groups.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: Y.M. Bramhecha, J. Lapointe
Development of methodology: Y.M. Bramhecha, K.P. Guérard, J. Lapointe
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y.M. Bramhecha, E. Scarlata, S. Chevalier, L. Hamel, A.G. Aprikian, J. Lapointe
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y.M. Bramhecha, K.P. Guérard, S. Rouzbeh, F. Brimo, S. Chevalier, A. Dragomir, A.G. Aprikian, J. Lapointe
Writing, review, and/or revision of the manuscript: Y.M. Bramhecha, K.P. Guérard, S. Rouzbeh, F. Brimo, S. Chevalier, A. Dragomir, A.G. Aprikian, J. Lapointe
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E. Scarlata, L. Hamel, A.G. Aprikian, J. Lapointe
Study supervision: J. Lapointe

Acknowledgments

This work was supported by the U.S. Department of Defense (DoD, W81XWH-11-1-0638) and the Fonds de Recherche du Québec - Santé (FRQS, Chercheur-boursier; to J. Lapointe) and by the Canadian Institutes of Health Research (CIHR) System Biology and McGill Division of Urology studentships to (Y.M. Bramhecha).

The authors would like to thank Walead Ebrahimizadeh, a member of J. Lapointe's laboratory, for his constructive feedback and discussion during the execution of this project. Special thanks to our colleague, Shraddha Solanki, for patiently proofreading every single draft of this manuscript.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 17, 2017; revised August 27, 2017; accepted October 4, 2017; published OnlineFirst October 9, 2017.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7–30.
2. Klotz L, Vesprini D, Sethukavalan P, Jethava V, Zhang L, Jain S, et al. Long-term follow-up of a large active surveillance cohort of patients with prostate cancer. J Clin Oncol 2015;33:272–7.
3. Hutchinson L. Closing the controversies gap in prostate cancer? Nat Rev Clin Oncol 2014;11:299.
4. 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;61:1019–24.
5. Wong AT, Schwartz D, Osborn V, Safdieh J, Weiner J, Schreiber D. Adjuvant radiation with hormonal therapy is associated with improved survival in men with pathologically involved lymph nodes after radical surgery for prostate cancer. Urol Oncol 2016;34:529.
6. DeMarzo AM, Nelson WG, Isaacs WB, Epstein JI. Pathological and molecular aspects of prostate cancer. Lancet 2003;361:955–64.
7. Holcomb IN, Young JM, Coleman IM, Salari K, Grove DI, Hsu L, et al. Comparative analyses of chromosome alterations in soft-tissue metastases within and across patients with castration-resistant prostate cancer. Cancer Res 2009;69:7793–802.

8. Kim JH, Dhanasekaran SM, Mehra R, Tomlins SA, Gu W, Yu J, et al. Integrative analysis of genomic aberrations associated with prostate cancer progression. Cancer Res 2007;67:8229–39.
9. Robbins CM, Tembe WA, Baker A, Sinari S, Moses TY, Beckstrom-Sternberg S, et al. Copy number and targeted mutational analysis reveals novel somatic events in metastatic prostate tumors. Genome Res 2011;21:47–55.
10. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22.
11. Lapointe J, Li C, Giacomini CP, Salari K, Huang S, Wang P, et al. Genomic profiling reveals alternative genetic pathways of prostate tumorigenesis. Cancer Res 2007;67:8504–10.
12. Lapointe J, Li C, Higgins JP, van de Rijn M, Bair E, Montgomery K, et al. Gene expression profiling identifies clinically relevant subtypes of prostate cancer. Proc Natl Acad Sci U S A 2004;101:811–6.
13. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016;22:298–305.
14. Choucair K, Ejdelman J, Brimo F, Aprikian A, Chevalier S, Lapointe J. PTEN genomic deletion predicts prostate cancer recurrence and is associated with low AR expression and transcriptional activity. BMC Cancer 2012;12:543.
15. Yoshimoto M, Cunha IW, Coudry RA, Fonseca FP, Torres CH, Soares FA, et al. FISH analysis of 107 prostate cancers shows that PTEN genomic deletion is associated with poor clinical outcome. Br J Cancer 2007;97: 678–85.
16. Krohn A, Diedler T, Burkhardt L, Mayer PS, De Silva C, Meyer-Kornblum M, 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;181:401–12.
17. Das K, Lau W, Sivaswaren C, Ph T, Fook-Chong S, Sl T, et al. Chromosomal changes in prostate cancer: a fluorescence in situ hybridization study. Clin Genet 2005;68:40–7.
18. Reid AH, Attard G, Ambroisine L, Fisher G, Kovacs G, Brewer D, et al. Molecular characterisation of ERG, ETV1 and PTEN gene loci identifies patients at low and high risk of death from prostate cancer. Br J Cancer 2010;102:678–84.
19. Sato H, Minei S, Hachiya T, Yoshida T, Takimoto Y. Fluorescence in situ hybridization analysis of c-myc amplification in stage TNM prostate cancer in Japanese patients. Int J Urol 2006;13:761–6.
20. Choucair KA, Guerard KP, Ejdelman J, Chevalier S, Yoshimoto M, Scarlata E, et al. The 16p13.3 (PDPK1) genomic gain in prostate cancer: a potential role in disease progression. Transl Oncol 2012;5:453–60.
21. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM, et al. REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 2005;93:387–91.
22. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA, et al. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol 2016;40:244–52.
23. Cooperberg MR, Hilton JF, Carroll PR. The CAPRA-S score: a straightforward tool for improved prediction of outcomes after radical prostatectomy. Cancer 2011;117:5039–46.
24. Punnen S, Freedland SJ, Presti JC, Aronson WJ, Terris MK, Kane CJ, et al. Multi-institutional validation of the CAPRA-S score to predict disease recurrence and mortality after radical prostatectomy. Eur Urol 2014;65: 1171–7.
25. Moyzis RK, Albright KL, Bartholdi MF, Cram LS, Deaven LL, Hildebrand CE, et al. Human chromosome-specific repetitive DNA sequences: novel markers for genetic analysis. Chromosoma 1987;95:375–86.
26. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996;15:361–87.
27. Abel F, Ejeskar K, Kogner P, Martinsson T. Gain of chromosome arm 17q is associated with unfavourable prognosis in neuroblastoma, but does not involve mutations in the somatostatin receptor 2(SSTR2) gene at 17q24. Br J Cancer 1999;81:1402–9.
28. An G, Xu Y, Shi L, Shizhen Z, Deng S, Xie Z, et al. Chromosome 1q21 gains confer inferior outcomes in multiple myeloma treated with bortezomib but copy number variation and percentage of plasma cells involved have no additional prognostic value. Haematologica 2014;99:353–9.
29. Bown N, Cotterill S, Lastowska M, O'Neill S, Pearson AD, Plantaz D, et al. Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma. N Engl J Med 1999;340:1954–61.
30. Towle R, Tsui IF, Zhu Y, MacLellan S, Poh CF, Garnis C. Recurring DNA copy number gain at chromosome 9p13 plays a role in the activation of multiple candidate oncogenes in progressing oral premalignant lesions. Cancer Med 2014;3:1170–84.
31. Cooperberg MR, Davicioni E, Crisan A, Jenkins RB, Ghadessi M, Karnes RJ. Combined value of validated clinical and genomic risk stratification tools for predicting prostate cancer mortality in a high-risk prostatectomy cohort. Eur Urol 2015;67:326–33.
32. Lennartz M, Minner S, Brasch S, Wittmann H, Paterna L, Angermeier K, et al. The combination of DNA ploidy status and PTEN/6q15 deletions provides strong and independent prognostic information in prostate cancer. Clin Cancer Res 2016;22:2802–11.
33. Maurer M, Su T, Saal LH, Koujak S, Hopkins BD, Barkley CR, et al. 3-Phosphoinositide-dependent kinase 1 potentiates upstream lesions on the phosphatidylinositol 3-kinase pathway in breast carcinoma. Cancer Res 2009;69:6299–306.
34. Shen H, Zhu Y, Wu YJ, Qiu HR, Shu YQ. Genomic alterations in lung adenocarcinomas detected by multicolor fluorescence in situ hybridization and comparative genomic hybridization. Cancer Genet Cytogenet 2008;181:100–7.
35. Mampaey E, Fieuw A, Van Laethem T, Ferdinande L, Claes K, Ceelen W, et al. Focus on 16p13.3 locus in colon cancer. PLoS One 2015;10:e0131421.
36. Valk K, Vooder T, Kolde R, Reintam MA, Petzold C, Vilo J, et al. Gene expression profiles of non-small cell lung cancer: survival prediction and new biomarkers. Oncology 2010;79:283–92.
37. Marshall JC, Nantel A, Blanco P, Ash J, Cruess SR, Burnier MN. Transcriptional profiling of human uveal melanoma from cell lines to intraocular tumors to metastasis. Clin Exp Metastasis 2007;24:353–62.
38. Tamoto E, Tada M, Murakawa K, Takada M, Shindo G, Teramoto K, et al. Gene-expression profile changes correlated with tumor progression and lymph node metastasis in esophageal cancer. Clin Cancer Res 2004;10: 3629–38.
39. Williams C, Edvardsson K, Lewandowski SA, Strom A, Gustafsson JA. A genome-wide study of the repressive effects of estrogen receptor beta on estrogen receptor alpha signaling in breast cancer cells. Oncogene 2008;27:1019–32.
40. Chapuy B, Koch R, Radunski U, Corsham S, Cheong N, Inagaki N, et al. Intracellular ABC transporter A3 confers multidrug resistance in leukemia cells by lysosomal drug sequestration. Leukemia 2008;22:1576–86.
41. Steinbach D, Gillet JP, Sauerbrey A, Gruhn B, Dawczynski K, Bertholet V, et al. ABCA3 as a possible cause of drug resistance in childhood acute myeloid leukemia. Clin Cancer Res 2006;12:4357–63.
42. Yasui K, Mihara S, Zhao C, Okamoto H, Saito-Ohara F, Tomida A, et al. Alteration in copy numbers of genes as a mechanism for acquired drug resistance. Cancer Res 2004;64:1403–10.
43. Bishop R. Applications of fluorescence in situ hybridization (FISH) in detecting genetic aberrations of medical significance. Biosci Horiz 2010;3: 85–95.
44. Gozzetti A, Le Beau MM. Fluorescence in situ hybridization: uses and limitations. Semin Hematol 2000;37:320–33.
45. Cuzick J, Berney DM, Fisher G, Mesher D, Moller H, Reid JE, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. Br J Cancer 2012;106:1095–9.
46. Irshad S, Bansal M, Castillo-Martin M, Zheng T, Aytes A, Wenske S, et al. A molecular signature predictive of indolent prostate cancer. Sci Transl Med 2013;5:202ra122.
47. Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. Eur Urol 2014;66:550–60.
48. Klein EA, Haddad Z, Yousefi K, Lam LL, Wang Q, Choeurng V, et al. Decipher genomic classifier measured on prostate biopsy predicts metastasis risk. Urology 2016;90:148–52.

Published OnlineFirst October 9, 2017; DOI: 10.1158/1541-7786.MCR-17-0270

Molecular Cancer Research

Genomic Gain of 16p13.3 in Prostate Cancer Predicts Poor Clinical Outcome after Surgical Intervention

Yogesh M. Bramhecha, Karl-Philippe Guérard, Shaghayegh Rouzbeh, et al.

Mol Cancer Res 2018;16:115-123. Published OnlineFirst October 9, 2017.