Research Article

Cancer Prevention Research

Genomic Profiling of Human Penile Carcinoma Predicts Worse Prognosis and Survival

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Abstract

The molecular mechanisms underlying penile carcinoma are still poorly understood, and the detection of genetic markers would be of great benefit for these patients. In this study, we assessed the genomic profile aiming at identifying potential prognostic biomarkers in penile carcinoma. Globally, 46 penile carcinoma samples were considered to evaluate DNA copy-number alterations via array comparative genomic hybridization (aCGH) combined with human papillomavirus (HPV) genotyping. Specific genes were investigated by using qPCR, FISH, and RT-qPCR. Genomic alterations mapped at 3p and 8p were related to worse prognostic features, including advanced T and clinical stage, recurrence and death from the disease. Losses of 3p21.1–p14.3 and gains of 3q25.31–q29 were associated with reduced cancer-specific and disease-free survival. Genomic alterations detected for chromosome 3 (*LAMP3*, *PPARG*, *TNFSF10* genes) and 8 (*DLC1*) were evaluated by qPCR. *DLC1* and *PPARG* losses were associated with poor prognosis characteristics. Losses of *DLC1* were an independent risk factor for recurrence on multivariate analysis. The gene-expression analysis showed downexpression of *DLC1* and *PPARG* and overexpression of *LAMP3* and *TNFSF10* genes. Chromosome Y losses and *MYC* gene (8q24) gains were confirmed by FISH. HPV infection was detected in 34.8% of the samples, and 19 differential genomic regions were obtained related to viral status. At first time, we described recurrent copy-number alterations and its potential prognostic value in penile carcinomas. We also showed a specific genomic profile according to HPV infection, supporting the hypothesis that penile tumors present distinct etiologies according to virus status. *Cancer Prev Res; 8(2); 149–56. ©2014 AACR.*

Introduction

Penile carcinoma is an aggressive and mutilating disease, which presents a high incidence in developing countries. In Brazil, 2.9 to 6.8/100,000 inhabitants are affected, representing one of the highest incidences in the world (1). Several risk factors have been associated with this tumor etiology, including phimosis, poor hygiene, and human papillomavirus (HPV) infection (2).

The incidence of HPV in penile carcinoma varies according to histologic subtype, with an overall prevalence of 50% of tumors (3, 4), and the molecular mechanisms behind penile carcinogenesis are still largely unknown. It has been reported amplification and/or high expression of *MYC* levels in HPV-positive cases (5), high levels of ANXA1 protein in high-risk HPV cases (6), strong positive correlation between viral presence and $p16^{INK4A}$ expression, and negative correlation with RB1 protein expression (7), as well as high pEGFR expression in HPV negative samples and HER3 positivity in HPV-positive cases (8).

The diagnosis of penile carcinoma is frequently delayed. Consequently, regional lymph node metastasis and advanced tumors are often observed, resulting in total or partial amputation of the organ with a profound psychologic and social impact on the patient, as well as reduced survival (9). Therefore, the detection of reliable genetic markers would be of great benefit to the patient.

Genetic and epigenetic studies in penile carcinoma are extremely limited. Mutations for *PIK3CA*, *HRAS*, *KRAS*, *TP53*, and *CDKN2A*, as well as methylation of CpG islands in particular genes, including *FHIT*, *RUNX3*, *CDKN2A*, and *THBS-1*, have been reported (10, 11). Even fewer studies have described an association between genetic alterations and clinical findings. Allelic losses on chromosomes 4, 6, 9, 12, and 13 were associated with lower survival and T stage (12).

In this study, DNA copy-number alterations were evaluated by array comparative genomic hybridization (aCGH) combined with HPV infection status, aiming to identify potential molecular markers in penile carcinoma and evaluate the viral role in penile tumors biology.

Materials and Methods

Patients and tissue specimens

Forty-six fresh-frozen penile squamous cell carcinomas (SCC) were collected at A.C. Camargo Cancer Center and Barretos Cancer Hospital (São Paulo, Brazil) between 2000 and 2010. The patients

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

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were advised of the procedures and provided written informed consent. This study was approved by the Human Research Ethics Committees at AC Camargo Cancer Center (CEP 1,230/2009) and Barretos Cancer Hospital (363/2010). The cases were classified histopathologically, according to the recommendations of the World Health Organization (13) and the International Union Against Cancer (14). Clinical and pathologic data are summarized in Supplementary Table S1. The cases were evaluated by an expert pathologist and classified as usual ($N = 41$), papillary ($N = 2$), sarcomatoid ($N = 1$), warty ($N = 1$), and mixed usual/papillary ($N = 1$) SCC. None of the patients have received neoadjuvant radiotherapy or chemotherapy before the surgery. The follow-up time ranged from 0.1 to 67.4 months with a median of 9.7 months (Supplementary Table S2). Ten samples obtained from peripheral lymphocytes from healthy individuals were included as reference for qPCR. Fifteen normal glans obtained from necropsies were used as control samples for transcripts evaluation.

Nucleic acids extraction and HPV genotyping

Genomic DNA was isolated using a standard phenol-chloroform (Invitrogen) extraction and ethanol precipitation procedure. Total RNA was extracted from using TRizol reagent (Life Technologies). cDNA synthesis was performed in final volume of 20 μL containing 1 μg of RNA treated with Dnase I (Life Technologies); 200 U of SuperScript III reverse transcriptase (Life Technologies); 4 μL of SuperScript First-Strand Buffer 5X; 1 μL of dNTP 10 mmol/L each (Life Technologies); 1 μL of Oligo-(dT)18 (500 ng/μL; Life Technologies); 1 μL of random hexamers (100 ng/μL; Life Technologies); 1 μL of DTT 0.1 mol/L (Life Technologies), and 40 U RNAse Out (Life Technologies). Reverse transcription was carried out for 60 minutes at 50°C and subsequently inactivated for 15 minutes at 70°C. The cDNA was stored at −70°C. HPV status was assessed in all penile carcinoma ($N = 46$) by the Linear Array HPV Genotyping Kit (Roche Molecular Diagnostics) according to the manufacturer's recommendations.

Array-based comparative genomic hybridization

High-quality genomic DNA (500 ng) from study specimens ($N = 38$), and a reference sample (male genomic DNA; Promega), were hybridized on Agilent Human 4 × 44 K CGH Microarrays (Agilent Technologies), according to the manufacturer's instructions. Array images were acquired using a DNA microarray scanner with Surescan High-Resolution Technology (Agilent Technologies) and Scan Control (version 8.1, Agilent Technologies) software. The data were analyzed using Nexus Copy Number software (version 6.0, Biodiscovery Inc.). Copy-number alteration was defined as exceeding the significance threshold of 1×10^{-6} and containing at least three consecutive altered probes per segment. Thresholds were defined as the average $\log_2$ CGH fluorescence ratio for copy gains ≥ 0.3, high copy-number gains ≥ 0.6, losses ≤ -0.3, and homozygous losses ≤ -1.0. The Fast Adaptive States Segmentation Technique 2 (FASST2) algorithm and the Significance Testing for Aberrant Copy-number (STAC) statistical method were used to identify nonrandom genomic copy-number alterations (15). Alterations detected in at least 20% of the samples were evaluated in more details. Hierarchical clustering analysis was performed using the Complete Linkage Hierarchical algorithm. Genomic data were deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE50134 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE).

Quantitative real-time PCR

qPCR analysis was used to confirm genomic alterations detected by aCGH for *DLC1* (8p22), *PPARG* (3p25), *LAMP3* (3q26.3–q27), and *TNFSF10* (3q26) genes in 41 penile carcinoma samples (35 aCGH-dependent and six microarray independent samples). The effect of copy-number alterations on gene transcripts was tested in 36 penile carcinoma samples (34 aCGH-dependent and two independent samples) for the four genes by RT-qPCR. Nine primer sets were designed to amplify the altered regions detected by aCGH for each gene. Eight primers flanking microarray altered probes were also constructed intending to determine the extension of the genomic alteration in a certain gene. The primers were designed using Primer-BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/; Supplementary Table S3). *GAPDH* was used as a reference gene for qPCR and *GUSB* and *HMBS* for RT-qPCR amplifications. PCR experiments were performed in an ABI Prism 7500 Sequence Detection System (Applied Biosystems) using SYBR Mix (Applied Biosystems). The reactions were carried out in duplicates in the automated PCR equipment Qiagility (Qiagen). RT-qPCR experiments followed the MIQE guidelines (16). The relative quantification was calculated using the model proposed by Pfaffl (17). The thresholds to classify a fragment as not altered (0.65–1.36), involved in losses (<0.65) and gains (>1.36) were established from the relative copy-number values obtained in control samples (blood samples from healthy individuals). Gains or losses presented in more than 20% of penile carcinoma samples evaluated by qPCR were considered as significant.

Fluorescence *in situ* hybridization

FISH was performed to evaluate *MYC* gene (8q24) gains and chromosome Y losses in seven penile carcinoma samples using even-archived formalin-fixed paraffin-embedded penile carcinoma tumor samples, sectioned to a thickness of 4 μm. The AneuVysion Multicolor DNA Probe (Vysis CEP 18/X/Y; Abbott Molecular) was used to evaluate Y chromosome aneusomy. Gains of the *MYC* gene were assessed using MYC/CEN-8 FISH Probe Mix (Dako). A fluorescence microscope (Olympus BX 61, Olympus Optical), equipped with a CCD camera (Photometrics CH 250) was used to analyze FISH results. At least 50 nonoverlapping tumor cell nuclei were evaluated. Absence of Y chromosome alteration was considered for 1:1:2 (Y:X:18) interphase nuclei labeling. An *MYC*/CEN-8 ratio ≥ 1.5 was considered for gains and ≥ 2.5 was positive for *MYC* amplification.

Statistical analysis

The Fisher exact test ($P \leq 0.05$) was applied to compare genomic alterations with clinicopathologic features. Mann–Whitney ($P \leq 0.05$) was used for transcriptional data analysis. Cancer-specific survival (CSS) and disease-free survival (DFS) were calculated using the Kaplan–Meier method and the log-rank test ($P \leq 0.05$). The follow-up interval was calculated in months from the date of first medical assessment to the date of last follow-up or death by the disease/recurrence. Significant variables in univariate analysis were included in the multivariate analysis model (Cox regression). Statistical analysis was carried out using SPSS version 17.0 (SPSS) and GraphPad Prism 5 (GraphPad Software Inc.) softwares.

Results

Genome-wide profiling

Twenty-eight copy-number alterations were detected in more than 20% of the penile carcinoma cases analyzed. Recurrent copy-number gains were detected on 3q, 5p, 8q, 9p, 20p, and 21p and losses on 3p, 8p, 9p, 21p, and Y (Table 1). The most frequent alterations were found on chromosomes 3, 8, and Y (26% to 66%).

Genomic alterations mapped at 3p and 8p were significantly associated with advanced clinical and T stage, recurrence and death due to disease ($P \leq 0.05$; Table 2). Significantly lower CSS and DFS were observed in cases with losses of 3p21.1–p14.3 ($P = 0.006$ and $P = 0.023$, respectively), and gains of 3q25.31–q29 ($P = 0.017$ and $P = 0.042$, respectively; Table 3, Fig. 1).

Unsupervised hierarchical clustering analysis revealed four distinct groups nearly classified according to chromosome 3 and 8 genomic alterations (Fig. 2A; Supplementary Table S4). However, no significant difference was found between these clusters and the clinicopathologic data.

Copy-number alterations according to HPV status

Sixteen of 46 penile carcinoma (34.8%) were positive for HPV genotyping: HPV16 (12 cases), HPV53 (one case), HPV16/40 (one case), HPV16/62 (one case), and HPV18/40 (one case).

Nineteen genomic regions were more commonly detected in HPV-positive tumors, including losses of 2q33.2–q33.3, 2q35, 2q36.3–q37.1, 2q37.1, 2q37.3, 2q37.3, 2q37.3, 2q37.1, 3p21.1, 4p16.1–p15.2, 4p14–p13, 5q31.1, 17p13.1, 17p12–p11.2, 17q11.2 and gains of 8p12, 9p13.3, 16p13.3, and 19q13.32 (Fig. 2B; Supplementary Table S5). Assuming that high-risk HPV are mostly integrated into the genome, integration sites for HPV16 and HPV18 were investigated as previously described (18). Nine of 19 significant regions corresponded with HPV integration sites in cervical carcinomas or cell lines: 2q33.2–q33.3, 2q35, 2q36.3–q37.1, 3p21.1, 4p16.1–p15.2, 5q31.1, 8p12, 9p13.3, and 19q13.32; (Supplementary Table S5). Furthermore, three corresponded to genomic fragile sites: FRA2I (2q33.2–q33.3), FRA5C (5q31.1), and FRA19A (19q13.32; Supplementary Table S5).

Quantitative analysis of DNA copy number and gene expression

The qPCR analysis was first performed in the same sample set used in microarray ($N = 35$). Regions significantly altered were selected to be investigated in an independent set of samples ($N = 6$). Losses were confirmed for six of eight regions evaluated for *DLC1* and four regions for *PPARG*; gains were confirmed for one region of *TNFSF10* (Supplementary Table S6). Downexpression of *DLC1* and *PPARG* genes ($P \leq 0.001$) and overexpression of *LAMP3* ($P \leq 0.001$) and *TNFSF10* ($P \leq 0.010$) were detected (Supplementary Fig. S1).

Association was found between *DLC1*- and *PPARG*-specific alterations and advanced clinical and T stage and development of lymph node metastasis ($P \leq 0.05$; Table 2). On univariate analysis, *PPARG*-1 ($P \leq 0.001$), *PPARG*-2 ($P = 0.025$), *PPARG*-3 ($P = 0.002$), *DLC1*-4 ($P = 0.048$), and *DLC1*-7 ($P = 0.034$) losses influenced CSS; *DLC1*-3 ($P = 0.045$) and *DLC1*-6 ($P = 0.022$) losses were associated with reduced DFS (Table 3; Supplementary Fig. S2). Multivariate analysis showed *DLC1*-6 as an independent risk factor for recurrence ($P = 0.040$; HR, 5.5; 95% confidence interval, 1.1–27.8; Table 3). No association was observed

Table 1. Frequent genomic alterations (>20% of cases) detected in 38 penile tumors evaluated by aCGH

Genomic region	Start (bp)	End (bp)	Event	Genes	Frequency (%)
3p26.3–p25.1	993,748	14,191,316	Loss	143	26
3p24.3–p22.2	15,550,927	37,778,461	Loss	109	42
3p22.2	38,062,745	38,411,982	Loss	9	26
3p22.2–p21.32	38,941,951	44,629,118	Loss	74	34
3p21.1–p14.3	54,190,324	57,080,457	Loss	15	26
3p14.1	66,457,075	70,311,198	Loss	23	32
3p13–p11.1	72,491,521	90,264,177	Loss	44	34
3q12.3–q13.13	104,132,986	110,515,632	Gain	34	29
3q13.13–q13.31	111,130,268	115,247,042	Gain	46	34
3q13.31–q25.31	116,240,263	157,523,060	Gain	428	42
3q25.31–q29	157,878,836	199,501,827	Gain	382	42
5p13.3	31,077,746	32,825,265	Gain	13	26
5p15.33	0	1,305,409	Gain	33	26
8p23.3–p11.21	0	43,175,310	Loss	444	50
8q11.1–q11.23	47,062,121	55,033,387	Gain	28	34
8q12.1	55,682,239	56,316,934	Gain	2	26
8q12.1–q21.13	56,967,220	82,092,323	Gain	172	34
8q21.2–q24.3	87,183,119	146,274,826	Gain	461	66
8q22.3	101,799,120	103,732,868	Gain	16	61
9p21.3	21,905,405	22,067,827	Loss	6	26
9p12–p11.2	42,014,069	42,702,421	Gain	9	26
9q33.3–q34.11	129,284,384	130,899,653	Gain	79	29
20p12.1	16,381,425	17,709,418	Gain	9	26
21p11.1	10,013,263	10,117,957	Gain	6	26
21p11.1	10,013,263	10,117,957	Loss	6	26
Yp11.2	7,278,858	10,143,912	Loss	19	29
Yq11.21–q11.221	13,924,906	17,801,127	Loss	6	39
Yq11.222–q11.223	19,700,039	23,283,748	Loss	28	39

Abbreviation: bp, base pairs.

Table 2. Genomic alterations associated with clinical and pathologic features in penile carcinomas

Genomic region	Event	P^a				
		Clinical stage III–IV	Recurrence	T stage T3-T4	Lymph node metastasis	Death
aCGH						
3p26.3–p25.1	Loss	0.013	0.033	ns	ns	ns
3p24.3–p22.2	Loss	0.013	0.010	0.001	ns	0.021
3p22.2	Loss	0.013	0.033	0.032	ns	ns
3p22.2–p21.32	Loss	0.030	ns	ns	ns	ns
3p21.1–p14.3	Loss	0.001	0.033	0.032	ns	0.018
3p14.1	Loss	0.022	ns	0.020	ns	0.048
3p13–p11.1	Loss	0.004	ns	0.007	ns	ns
8p23.3–p11.21	Loss	ns	0.041	0.010	ns	ns
qPCR						
DLC1-3	Loss	ns	ns	0.047	0.046	ns
DLC1-7	Loss	ns	ns	ns	0.011	ns
PPARG-1	Loss	0.005	ns	0.019	0.003	ns

Abbreviation: ns, not significant.
[a]Calculated using Fisher exact with $P \leq 0.05$ for significance.

between transcript profile and clinicopathologic features (data not shown).

FISH evaluation of genomic alterations

Recurrent Y chromosome losses and *MYC* gains detected by aCGH were confirmed in all tumors evaluated ($N = 7$). Five cases showed Y chromosome loss, whereas two presented no alterations for both methodologies. Four penile carcinoma showed gains of 8q24 (*MYC* gene), whereas three were unaltered. A summary of aCGH, qPCR, RT-qPCR, and FISH data is presented on Table 4.

Discussion

To the best of our knowledge, this is the first study using a large-scale approach to uncover the genomic alterations with prognostic value in penile carcinomas. Using chromosomal CGH, gains were reported for 8q24, 16p11-12, 20q11-13, 22q, 19q13, and 13q21, and losses for 5p15-22, 4q21-32, and X chromosome in

Table 3. Clinical features and genomic alterations associated with CSS and DFS by univariate and multivariate analysis in penile cancer

Clinical feature/genomic region	Event	Cases	Death (%)	Recurrence (%)	$P_{univariate}$[a]	$P_{multivariate}$[b]/HR (95% CI)
CSS						
Clinical stage	I–II	21	0 (0)	—	0.004	ns
	III–IV	16	6 (38)	—		
Histologic grade	I	8	0 (0)	—	0.085	ns
	II–III	26	6 (23)	—		
3p21.1–p14.3	No alteration	26	3 (11)	—	0.006	ns
	Loss	8	4 (50)	—		
3q25.31–q29	No alteration	19	1 (5)	—	0.017	ns
	Gain	15	6 (40)	—		
DLC1-4	No alteration	24	1 (4)	—	0.048	ns
	Loss	10	3 (30)	—		
DLC1-7	No alteration	19	1 (5)	—	0.034	ns
	Loss	16	4 (25)	—		
PPARG-1	No alteration	26	0 (0)	—	<0.001	ns
	Loss	8	5 (63)	—		
PPARG-2	No alteration	21	2 (10)	—	0.025	ns
	Loss	13	3 (23)	—		
PPARG-3	No alteration	26	1 (4)	—	0.002	ns
	Loss	9	4 (44)	—		
DFS						
Tumor stage	T1-T2	24	—	2 (8)	0.011	ns
	T3-T4	14	—	7 (50)		
3p21.1–p14.3	No alteration	26	—	3 (11)	0.023	ns
	Loss	8	—	4 (50)		
3q25.31–q29	No alteration	19	—	2 (10)	0.042	ns
	Gain	15	—	5 (33)		
DLC1-1	No alteration	8	—	0 (0)	0.083	ns
	Loss	28	—	9 (32)		
DLC1-3	No alteration	15	—	1 (7)	0.045	ns
	Loss	21	—	8 (38)		
DLC1-6	No alteration	23	—	3 (13)	0.022	0.040/5.5 (1.1–27.8)
	Loss	7	—	3 (43)		

Abbreviations: CI, confidence interval; ns, not significant.
[a]Calculated using the log-rank test with $P \leq 0.05$ for significance.
[b]Calculated using Cox regression with $P \leq 0.05$ for significance.

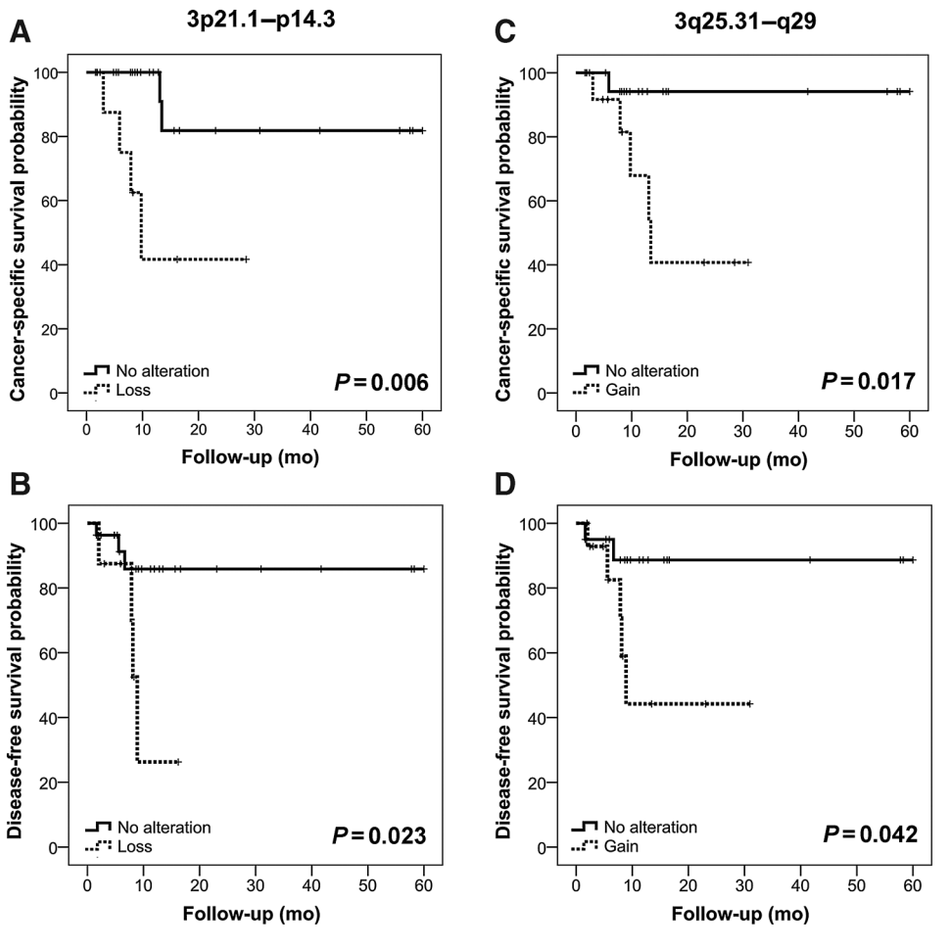

Figure 1.
Recurrent copy-number alterations related to prognosis. Kaplan–Meier curves showing significantly reduced CSS and DFS for patients with penile cancer with losses on 3p21.1–p14.3 (A and B) and gains on 3q25.31–q29 (C and D). *P* values were determined using the log-rank test.

penile carcinoma; however, no association was found between these alterations and clinicopathologic data (19).

The correlation between genomic alterations and clinical data showed interesting and novel results, especially for chromosomes 3 and 8. Copy-number alterations of 3p, 3q, and 8p were related to a worse prognosis, as well as a reduced CSS and DFS. Several studies have reported preliminary evidences that the same regions and genes mapped on chromosomes 3 and 8 may have an important role in the pathogenesis of epithelial tumors, including esophageal, non-small cell lung, head and neck, and bladder carcinomas (20–24). The association between genomic alterations and prognosis may be used to identify patients with penile carcinoma with poor outcome and highlights potential targets for therapy.

Four genes mapped on chromosome 3 and 8 were selected for data confirmation by qPCR due to the frequent association of specific chromosomal regions with prognosis. Using array-dependent and -independent samples sets, *DLC1*, *PPARG*, and *TNFSF10* were confirmed as altered, and *DLC1* and *PPARG* were associated with poor prognosis. Furthermore, copy-number alterations of these genes were translated for altered expression levels by RT-qPCR assays. Although *LAMP3* gains have not been confirmed by qPCR, the transcript evaluation showed gene overexpression, indicating a possible genomic alteration covering a region not evaluated by qPCR or other mechanisms of transcript alteration. In addition to report new putative molecular markers of prognosis in penile carcinoma, our analysis also detected genomic alterations in two relevant therapeutic targets, *PPARG* (25, 26) and *TNFSF10* (27), which may be further studied to design new treatment strategies for penile tumors.

MYC gene gains and Y chromosome losses detected by aCGH were also submitted to validation. The FISH analysis was in agreement with large-scale data and showed the reliability of the gains and losses detected by our microarray approach. The most frequent alteration detected by aCGH was 8q21.2–q24.3 gain (66% of cases), which contains the *MYC* gene (8q24). MYC is a transcription factor responsible for the regulation of approximately 15% of the human gene set, acting in processes such as cell growth and proliferation, cell-cycle progression, transcription, differentiation, apoptosis, and cell motility (28). Previously, *MYC* gains or amplification, as well as increased gene and protein expression related and unrelated to HPV positivity, were described in penile carcinomas (5, 29). It was reported that *MYC* activation was mediated by viral integration, which may be an important event in penile oncogenesis (5). In the present study, *MYC* amplification was detected in both HPV-positive ($N = 7$) and -negative cases ($N = 18$), with no significant correlation between these parameters. The high frequency of this alteration independent of HPV status suggests that *MYC* is a potential therapeutic target in penile carcinoma. Drugs targeting the *MYC* or associated products in related pathways have been extensively evaluated for cancer treatment (30). The FISH genomic data were not submitted

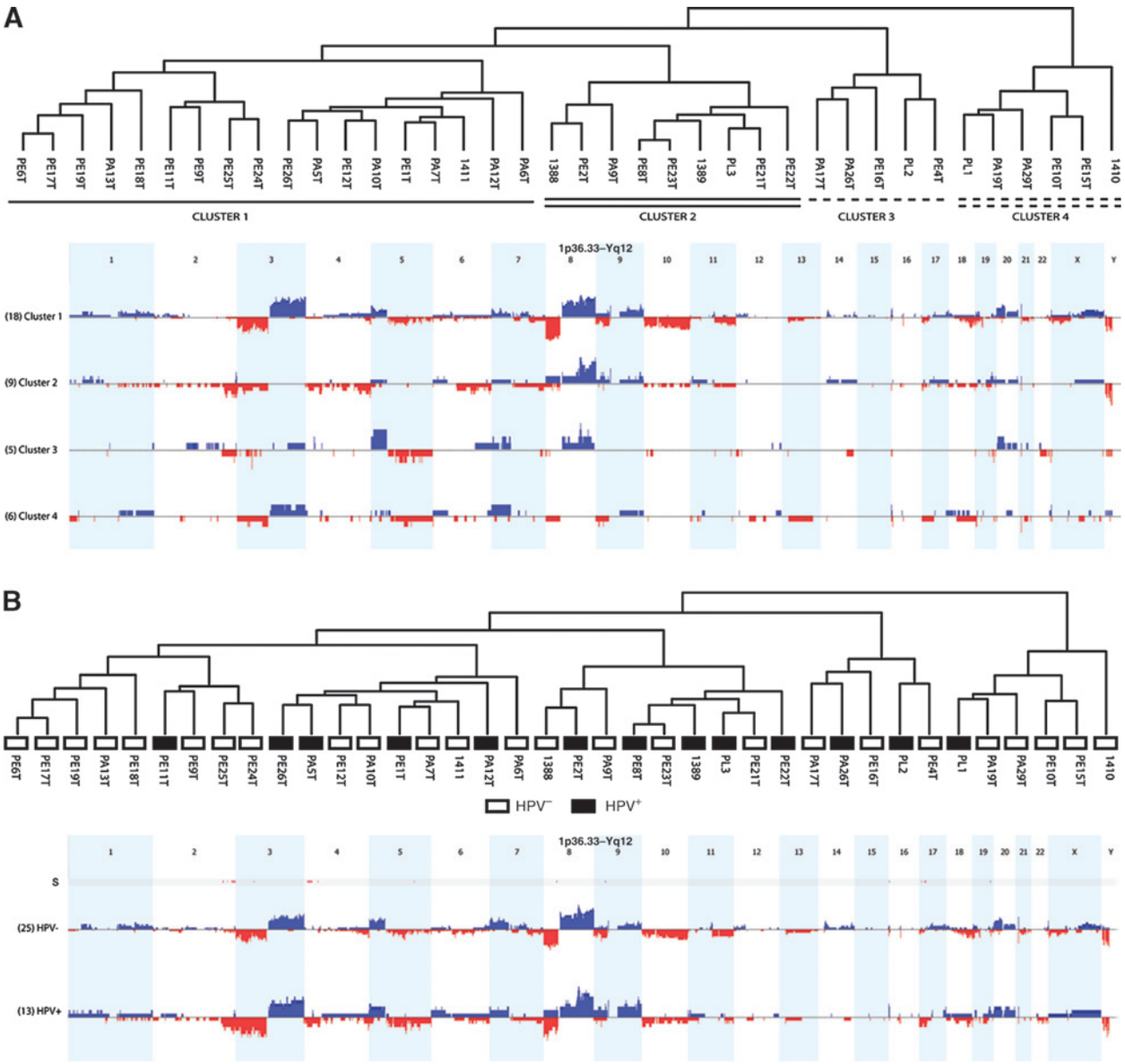

Figure 2.
Genomic profile of 38 penile carcinoma cases evaluated by aCGH (chromosome 1 to Y). Four main clusters were identified on the basis of the genomic profiles for each group (A). Two genomic profiles were generated according to HPV infection (B). Nineteen regions significantly more common in HPV-positive samples are represented in line "S." The top bars (blue) indicate genomic gains, whereas the lower bars (red) refer to chromosomal losses.

to clinical and pathologic features association due to the low sample size ($N = 7$).

Frequent Y chromosome losses detected by aCGH were also confirmed by FISH, in a subset of cases. Despite the significance of this alteration in tumor biology having been controversial, a recent head and neck tumor case–control study revealed that Y chromosome loss is independent of patient age and was significantly associated with cancer cells (31).

HPV is considered an important etiologic factor in penile carcinoma. In this study, 34.8% of penile carcinoma samples were positive for HPV infection; 15 of 16 positive cases showed at least one oncogenic HPV genotype (HPV16 or 18). Three cases presented multiple infections (HPV16/40, HPV16/62, and HPV18/40) and, although its significance remains unknown, the presence of at least one oncogenic HPV genotype in each case supports their relevance in tumor biology.

Comparing tumors according to HPV status, it was revealed that 19 regions were able to differentiate between the groups, nine involving breakpoints previously described as associated with HPV integration sites in cervical carcinomas and cell lineages (18). HPV integration into cellular genome has been associated with the progression of preneoplastic lesions, mainly due to structural changes of the viral genome that allow deregulated expression of the viral oncogenes and confer neoplastic selective

Table 4. Summary of the main alterations detected by aCGH, qPCR, RT-qPCR, and FISH in penile cancer

Chromosome region altered by aCGH (number of genes altered)	Gene (location)	aCGH ($N = 38$)	qPCR ($N = 41$[a])	RT-qPCR ($N = 36$[b])	FISH ($N = 7$[c])
3p26.3–p25.1 (143)	*PPARG* (3p25)	Loss	Loss	Downexpression	—
3q25.31–q29 (382)	*LAMP3* (3q26.3–q27)	Gain	No alteration	Overexpression	—
	TNFSF10 (3q26)	Gain	Gain	Overexpression	—
8p23.3–p11.21 (444)	*DLC1* (8p22)	Loss	Loss	Downexpression	—
8q21.2–q24.3 (461)	*MYC* (8q24)	Gain	—	—	Gain
Chromosome Y (53)	—	Loss	—	—	Loss

[a]35 aCGH-dependent and six array independent samples.
[b]34 aCGH-dependent and two array independent samples.
[c]The samples tested are aCGH dependent.

pressure (18). It is also speculated that critical cellular genes are affected by integration of viral genome (18). The detection of HPV integration sites described herein is in agreement with the importance of these regions in the development and progression of tumors, including penile carcinoma.

The distinct genomic profile between HPV-positive and -negative samples gives additional support for the hypothesis that these tumors have two distinct etiologies: one dependent and the other independent of HPV infection (11, 32, 33). The regions frequently detected as altered in HPV-positive cases must be further explored, aiming to better understand the involvement of HPV in penile carcinoma and its potential application in clinical practice.

There are currently no targets for therapy for clinical use in penile cancer. Using aCGH, we provided insights into novel therapeutic targets for penile carcinoma and identified putative prognostic molecular markers. Distinct genomic profiles were found related to HPV status. In particular, the presence of breakpoints regions previously described in cervical carcinomas, revealed similarities between these two HPV associated diseases. These findings provide novel insights into penile carcinogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S.R. Rogatto
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