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Predictive Biomarkers and Personalized Medicine

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Polymorphisms inside MicroRNAs and MicroRNA Target Sites Predict Clinical Outcomes in Prostate Cancer Patients Receiving Androgen-Deprivation Therapy

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Abstract

Purpose: Recent evidence indicates that small noncoding RNA molecules, known as microRNAs (miRNAs), are involved in cancer initiation and progression. We hypothesized that genetic variations in miRNAs and miRNA target sites could be associated with the efficacy of androgen-deprivation therapy (ADT) in men with prostate cancer.

Experimental Design: We systematically evaluated 61 common single nucleotide polymorphisms (SNPs) inside miRNAs and miRNA target sites in a cohort of 601 men with advanced prostate cancer treated with ADT. The prognostic significance of these SNPs on disease progression, prostate cancer-specific mortality (PCSM) and all-cause mortality (ACM) after ADT were assessed by Kaplan–Meier analysis and Cox regression model.

Results: Four, seven, and four SNPs were significantly associated with disease progression, PCSM, and ACM, respectively, after ADT in univariate analysis. *KIF3C* rs6728684, *CDON* rs3737336, and *IFI30* rs1045747 genotypes remained as significant predictors for disease progression; *KIF3C* rs6728684, *PALLD* rs1071738, *GABRA1* rs998754, and *SYT9* rs4351800 remained as significant predictors for PCSM; and *SYT9* rs4351800 remained as a significant predictor for ACM in multivariate models that included clinicopathologic predictors. Moreover, strong combined genotype effects on disease progression and PCSM were also observed. Patients with a greater number of unfavorable genotypes had a shorter time to progression and worse prostate cancer-specific survival during ADT (P for trend < 0.001).

Conclusion: SNPs inside miRNAs and miRNA target sites have a potential value to improve outcome prediction in prostate cancer patients receiving ADT. *Clin Cancer Res; 17(4); 928–36. ©2010 AACR.*

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Introduction

With the advent of prostate-specific antigen (PSA) screening, prostate cancer is being detected and treated earlier. However, approximately 10% to 20% of newly diagnosed prostate cancer patients present advanced disease, and many others will eventually relapse despite local treatments. Androgen deprivation therapy (ADT) is the most commonly used first-line treatment for advanced prostate cancer (1). Despite frequent responses, many patients on ADT progress to castration-resistant disease within 2–3 years (2). Once castration-resistant prostate cancer develops, the life expectancy of the patient is approximately 16–18 months (3). A variety of prediction parameters, such as tumor stage, Gleason score, and PSA kinetics, have been used in clinical practice to define the presentation of prostate cancer and adapt the treatment strategy (4–6). However, their prognostic capabilities are still limited and might be improved by the incorporation of other factors including genetic markers. Germ line genetic

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Translational Relevance

Androgen-deprivation therapy (ADT) is the most common and effective systemic therapy for advanced prostate cancer, but outcome predictors for the efficacy of ADT are still scarce. Recent studies suggest that microRNAs might participate in cancer progression. Therefore, we hypothesized that single-nucleotide polymorphisms (SNPs) in microRNAs and microRNA target sites might have tremendous implications for prognosis after ADT. In the present study, we conducted a genome-wide search for SNPs located in pre-microRNAs and putative microRNA target sites, and investigated their prognostic significance on 3 outcomes of ADT: disease progression, prostate cancer-specific mortality, and all-cause mortality. Multivariate Cox proportional hazards analysis revealed that several SNPs located in microRNA genes and putative microRNA target sites were significantly associated with the 3 outcomes of ADT. Our results suggest that a simple and pretreatment analysis for microRNA SNPs might add significant prognostic value to the currently used indicators for outcome prediction in patients receiving ADT.

variants have been demonstrated to have the potential in identifying predisposition to aggressive prostate cancer and providing insight into biological pathways of initiation and progression of this complex disease (7).

MicroRNAs (miRNAs) are endogenous, small (about 22 nucleotides), nonprotein-coding, single-stranded RNA molecules involved in regulating the expression of other genes. MiRNAs are first transcribed as primary miRNAs (pri-miRNAs) with several hundred nucleotides, processed to the 70- to 100-nucleotides RNA hairpin intermediates, defined as pre-miRNAs, and then exported to cytoplasm and processed to mature miRNAs as part of the RNA-induced silencing complex (8). MiRNAs regulate gene expression by base pairing with sequences within the 3′-untranslated regions of target mRNAs, leading to mRNA cleavage or translation repression (9). Numerous studies have shown that aberrant expression of miRNAs contributes to the etiology of many common human diseases including cancer (10).

Genetic variants within miRNA genes might alter miRNA processing and ultimately change the expression level of the miRNA. Alternatively, genetic variants located in the miRNA binding sites of target mRNAs might disrupt miRNA-target interaction, resulting in the deregulation of target gene expression. In this regard, the most common genetic variation, single nucleotide polymorphisms (SNPs), in miRNA genes and their target sites might be ideal candidate biomarkers for cancer prognosis. To our knowledge, this is the first study conducting a genome-wide search for SNPs located in pre-miRNAs and putative miRNA target sites, and investigating their prognostic significance on disease progression, prostate cancer-specific mortality (PCSM), and all-cause mortality (ACM) in a cohort of prostate cancer patients receiving ADT.

Materials and Methods

Patient recruitment and data collection

The study population was extended from our hospital-based prostate cancer case-control study that has been described previously (11–16). In brief, patients with diagnosed and pathologically confirmed prostate cancer were actively recruited from 3 medical centers in Taiwan: Kaohsiung Medical University Hospital, Kaohsiung Veterans General Hospital, and National Taiwan University Hospital. The prostate cancer patients who had been treated with ADT (orchiectomy or LHRH agonist with or without antiandrogen), including those with disease recurrence after local treatments (radical prostatectomy or radiotherapy), were identified and followed up prospectively to evaluate genetic variants as prognostic predictors of clinical outcomes during ADT. Patients were excluded if the clinicopathologic information or follow-up period were insufficient, leaving 601 patients in this cohort. This study was approved by the Institutional Review Board of the 3 hospitals, and informed consent was obtained from each participant.

Data were collected on patients with disease baseline and clinicopathologic characteristics, as well as 3 treatment outcomes: time to progression, PCSM, and ACM. The PSA nadir was defined as the lowest PSA value achieved during ADT treatment (6, 17). Time to PSA nadir was defined as the duration of time it took for the PSA value to reach nadir after ADT initiation (4). Disease progression was defined as a serial rise in PSA, at least 2 rises in PSA (> 1 week apart), greater than the PSA nadir (18). Initiation of secondary hormone treatment for rising PSA was also considered as a progression event. Time to progression was defined as the duration of time it took to have a progression event once ADT was started. In general, patients are followed every month with PSA tests at 3-monthly intervals. The cause of death was obtained by matching patient personal identification numbers with the official cause of death registry provided by the Department of Health, Executive Yuan, Taiwan. Overall, 145 deaths were identified and 101 of these were from prostate cancer.

SNP selection and genotyping

We identified SNPs within miRNAs by intersection HapMap SNPs CHB (Han Chinese) table with sno/miRNA table (19), and identified SNPs within miRNA target sites by intersection HapMap SNPs CHB table with TS (TargetScan) miRNA sites table (20) from the UCSC table browser (NCBI36/hg18) (21). SNPs with a minor allele frequency less than 5% in HapMap CHB population or inside snoRNAs were excluded. Fourteen SNPs in miRNAs and 59 SNPs in miRNA target sites were initially selected for analysis.

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen) and stored at −80°C until the time of study. Genotyping was

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performed as described previously (14) using Sequenom iPLEX matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry technology at the National Genotyping Center, Academia Sinica, Taiwan. The average genotype call rate for these SNPs was 97.1% and the average concordance rate was 99.8% among 55 blind duplicated quality control samples. Any SNP that did not conform to Hardy–Weinberg equilibrium ($P < 0.001$), below a genotyping call rate of 80%, or with a minor allele frequency less than 3%, was removed ($n = 12$). Thus, a total of 61 SNPs were included for further statistical analyses.

Statistical analysis

Patient clinicopathologic characteristics were summarized as number and percentage of patients or median and interquartile range of values. The continuous factors were dichotomized at the median value within the cohort, with the exception of PSA nadir, which was dichotomized at 0.2 ng/mL because of its correlation with disease progression and PCSM (5–6). The heterozygous and rare homozygous genotypes were collapsed in the analysis if the frequency of the rare homozygote was low (<2%) or if the homozygous and heterozygous genotypes had the same direction of effect. The associations of 61 individual SNPs and clinicopathologic characteristics with time to progression, PCSM, and ACM were assessed using the Kaplan-Meier analysis with log-rank test. Multivariate analyses to determine the interdependency of genotypes and other known prognostic factors, such as age at diagnosis, clinical stage, Gleason score, PSA at ADT initiation, PSA nadir, and time to PSA nadir, were carried out using Cox proportional hazards regression model. As we were testing 61 SNPs, false-discovery rates (q values) were calculated to determine the degree to which the tests for association were prone to false-positives (22). q values were estimated using R q value package (http://genomics.princeton.edu/storeylab/qvalue/) on the observed distribution of P values from the log-rank test for 61 SNPs. Statistical Package for the Social Sciences software version 16.0.1 (SPSS Inc.) was used for other statistical analyses. A 2-sided P value of < 0.05 was considered statistically significant.

Results

The patients' demographic and clinicopathologic characteristics are summarized in Table 1. Four hundred and fifteen (69%) patients had disease progression after ADT initiation, and the median time to progression was 22 months with a mean follow-up of 30.3 months (range, 3–120 months). One hundred and forty-five (24%) patients died, and 101 (17%) died of prostate cancer after a mean follow-up of 39 months (range, 3–125 months). The mean times to PCSM and ACM were 138 and 123 months, respectively. Metastatic stage of the disease, Gleason scores 8–10, higher PSA nadir, and shorter time to PSA nadir were significantly associated ($P \leq 0.006$) with shorter time to progression, PCSM, and ACM. Age at diagnosis was only associated with ACM, and PSA level at ADT initiation was associated with shorter time to PCSM and ACM, but not time to progression.

Of the 61 SNPs evaluated, 4, 7, and 4 polymorphisms showed a statistically significantly correlation with time to progression, PCSM, and ACM respectively, according to the log-rank test (Supplementary Table 1). *KIF3C* rs6728684, *CDON* rs3737336, *ETS1* rs1128334, and *IFI30* rs1045747 were associated with disease progression during ADT (nominal $P \leq 0.031$), and all had a false-discovery rate (q value) less than 0.218 (Table 2). To assess the predictive effects of these SNPs beyond the clinical features to influence disease progression, we performed a multivariate analysis, adjusting for age at diagnosis, clinical stage, Gleason score, PSA level at ADT initiation, PSA nadir, and time to PSA nadir. After adjusting for these predictors, *KIF3C* rs6728684, *CDON* rs3737336, and *IFI30* rs1045747 remained significant ($P \leq 0.035$). A strong gene-dosage effect on disease progression during ADT was observed when these 3 SNPs were analyzed in combination (log-rank $P < 0.001$, Table 2 and Fig. 1A left panel). The time to progression decreased as the number of unfavorable genotypes increased, and the combined genotype remained as a significant predictor after adjusting for clinical factors (P for trend < 0.001, Table 2).

hsa-mir-423 rs6505162, *KIF3C* rs6728684, *PALLD* rs1071738, *ACSL1* rs2292899, *GABRA1* rs998754, *SYT9* rs4351800, and *ZDHHC7* rs3210967 had statistically significant effects on PCSM ($P \leq 0.037$), and all had a q value less than 0.187 (Table 3). Four SNPs, *KIF3C* rs6728684, *PALLD* rs1071738, *GABRA1* rs998754, and *SYT9* rs4351800, and their combined genotype remained as significant predictors for time to PCSM after adjusting for clinical factors ($P \leq 0.039$). A significant combined genotype effect on PCSM was also observed, and the hazard ratios (HRs) for PCSM during ADT increased as the number of unfavorable genotypes increased (P for trend < 0.001, Table 3 and Fig. 1B left panel).

Four SNPs, *ACSL1* rs2292899, *MTRR* rs9332, *GABRA1* rs998754, and *SYT9* rs4351800, were significantly associated with time to ACM in the univariate analysis ($P \leq 0.046$), and all had a q value less than 0.222 (Table 4). However, after adjusting for clinical predictors, only *SYT9* rs4351800 remained a significant predictor for time to ACM in patients receiving ADT (HR 2.55, 95% CI 1.65–3.95, $P < 0.001$). Kaplan–Meier survival curves and log-rank test showed that the *SYT9* rs4351800 CC genotype was significantly associated with poorer overall survival compared with the AA/AC genotypes ($P = 0.001$, Fig. 1C left panel).

To further evaluate the clinical relevance of these miRNA SNPs, a substratification of high-risk patients based on clinical staging is performed. The combined genotypes and *SYT9* rs4351800 still had significant effects on disease progression, PCSM, and ACM in patients with or without distant metastasis, respectively ($P \leq 0.040$, Fig. 1 middle and right panels). This additional information leads to better risk prediction, and also supports that SNPs inside miRNAs and miRNA target sites might be independent

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Table 1. Clinicopathologic characteristics of the study population and analyses of factors that predicted disease progression, PCSM, and ACM during ADT

Variable	No.* (%)	Disease progression			PCSM			ACM		
		No. of events*	Median (months)	$P^{\dagger}$	No. of events*	Mean (months)	$P^{\dagger}$	No. of events*	Mean (months)	$P^{\dagger}$
All patients	601	415	22		101	138		145	123	
Age at diagnosis (years)										
Median (IQR)	73 (67–79)									
≤73	320 (53.2)	228	21	0.219	51	141	0.280	61	**135**	**0.001**
>74	281 (46.8)	186	25		50	127		84	**105**	
Clinical stage at diagnosis										
T1/T2	189 (31.7)	117	**25**	**0.005**	13	**145**	**<0.001**	26	**130**	**<0.001**
T3/T4/N1	184 (30.8)	123	**25**		21	**149**		32	**138**	
M1	224 (37.5)	172	**17**		67	**110**		87	**94**	
Gleason score at diagnosis										
2–6	194 (33.0)	128	**26**	**0.006**	20	**154**	**<0.001**	34	**141**	**<0.001**
7	180 (30.6)	124	**25**		19	**134**		32	**116**	
8–10	214 (36.4)	153	**17**		61	**108**		77	**97**	
PSA at ADT initiation (ng/mL)										
Median (IQR)	35.0 (11.4–129)									
<35	287 (49.6)	184	25	0.083	24	**146**	**<0.001**	44	**132**	**<0.001**
≥35	292 (50.4)	211	19		76	**117**		98	**103**	
PSA nadir (ng/mL)										
Median (IQR)	0.18 (0.01–1.33)									
<0.2	301 (50.8)	186	**31**	**<0.001**	20	**159**	**<0.001**	37	**145**	**<0.001**
≥0.2	292 (49.2)	228	**14**		80	**110**		106	**95**	
Time to PSA nadir (months)										
Median (IQR)	10 (5–18)									
<10	293 (49.4)	220	**10**	**<0.001**	65	**121**	**<0.001**	89	**105**	**<0.001**
≥10	300 (50.6)	194	**33**		35	**150**		54	**136**	

NOTE. $P \leq 0.05$ are in boldface.

Abbreviations: ADT, androgen-deprivation therapy; PCSM, prostate cancer-specific mortality; ACM, all-cause mortality; PSA, prostate-specific antigen; IQR, interquartile range.

*Column subtotals do not sum to 601 for no. of patients, 415 for no. of disease progression, 101 for PCSM, and 145 for ACM due to missing data.

†*P* values were calculated using the log-rank test.

predictors of clinical outcomes following ADT along with current clinicopathologic prognostic markers.

Discussion

In this study, we found that 6 of 61 SNPs inside miRNAs and miRNA target sites were significantly associated with the disease progression, PCSM, or ACM in prostate cancer patients receiving ADT, thus validating our hypothesis. Notably, in multivariate analysis, these SNPs retained their association with the efficacy of ADT while controlling for the known clinicopathologic risk factors (age at diagnosis, clinical stage, Gleason score, PSA level at ADT initiation, PSA nadir, and time to PSA nadir), suggesting that these host genetic factors add information above and beyond currently used predictors. Moreover, strong combined genotype effects on disease progression and PCSM were also observed. To our knowledge, this is the first study to demonstrate a potential value of variants in miRNAs and miRNA target sites as predictors for the outcomes of ADT.

Of the 61 SNPs evaluated, *KIF3C* rs6728684, *CDON* rs3737336, and *IFI30* rs1045747 showed significant associations with disease progression after adjusting for all clinical predictors. KIF3C, kinesin family member 3C, belongs to the family of kinesin motor proteins. Kinesins are microtubule-dependent molecular motors involved in intracellular transport and mitosis (23–24). KIF3C expression is highly enriched in nervous systems. Although the precise functions of KIF3C remain unknown, biochemical studies suggest that KIF3C is an anterograde motor which might be involved in synaptic vesicle trafficking, specialized functions that are associated with the normal

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Table 2. Genotyping frequencies and the association of genotype with disease progression during ADT

Gene SNP	Genotype	No. of patients	No. of events	Median (months)	*P**	*q*	HR (95% CI)	*P*†
KIF3C	TT/TG	577	399	22	0.002	0.056	1.00	
rs6728684	GG	16	12	17			**2.41 (1.31–4.43)**	**0.005**
CDON	TT/TC	543	385	21	0.004	0.056	1.00	
rs3737336	CC	47	24	37			**0.59 (0.38–0.91)**	**0.018**
ETS1	GG/GA	512	343	24	0.011	0.103	1.00	
rs1128334	AA	58	48	16			1.16 (0.84–1.59)	0.368
IFI30	TT/TC	524	361	22	0.031	0.218	1.00	
rs1045747	CC	16	12	14			**1.89 (1.05–3.39)**	**0.035**
No. of unfavorable genotypes present‡								
0		47	24	37	<0.001		1.00	
1		519	367	22			**1.63 (1.06–2.52)**	**0.027**
>1		31	23	14			**3.17 (1.74–5.78)**	**<0.001**
							P-trend	<0.001

NOTE. $P \leq 0.05$ are in boldface.

Abbreviations: ADT, androgen-deprivation therapy; HR, hazard ratio; 95% CI, 95% confidence interval; PSA, prostate-specific antigen.

**P* values were calculated using the log-rank test.

†HRs were adjusted for age, clinical stage, Gleason score, PSA at ADT initiation, PSA nadir, and time to PSA nadir.

‡Unfavorable genotypes refer to GG in *KIF3C* rs6728684, TT/TC in *CDON* rs3737336, and CC in *IFI30* rs1045747.

development of nervous system and the formation of neuroendocrine tumors (25). ADT works through inhibition of androgen receptor in the prostate epithelium, or suppression of the secretion of factors from prostate stromal cells that are critical for the survival of prostate epithelial cells. Because neuroendocrine cells lack androgen receptor and are likely androgen-independent, it is conceivable that ADT will not eliminate these cells. Instead, neuroendocrine cells might be enriched after ADT to stimulate androgen-independent proliferation of prostate cancer, leading to the disease progression. A number of studies have also demonstrated that the presence of neuroendocrine phenotype in tumors is associated with worse prognosis and facilitation of prostate cancer progression during ADT (26). On the other hand, overexpression of KIF3C has been found to mediate docetaxel resistance in breast cancer cells by increasing the pools of free tubulin and promoting the dissociation of tubulin from microtubules to antagonize the effect of docetaxel (27). CDON, cell adhesion molecule-related/downregulated by oncogenes (cdon) homolog, is initially identified as a component of cell surface receptor complex that mediates cell–cell interactions during myogenic differentiation. Recent studies showed that CDON interacts with all Hedgehog (Hh) proteins and positively regulates Hh signaling (28). CDON maps to chromosome 11q23-q24, a region with frequent loss of heterozygosity in lung, breast, and ovarian cancers, suggesting that CDON could play a role in oncogenesis (29–31). Notably, androgen deprivation highly upregulated the expression of Hh ligands and Hh target genes in prostate cancer cells (32). The clinical relevance of this observation is also supported by the increase of Hh ligand production in prostate tumors after neoadjuvant hormone treatment (33). IFI30, interferon gamma-inducible protein 30, encodes a lysosomal thiol reductase that cleaves protein disulfide bonds, and is thought to have an important role in MHC class II-restricted antigen processing in antigen-presenting cells. Establishment of long-term immunity to block tumor recurrence depends on the recruitment and activation of T cells (34). Tumors can constitutively express both MHC class I and II molecules, necessary for tumor antigen presentation to T cells. Yet, malignant cells might evade or avoid T-cell surveillance through modulation of the IFI30 expression to disrupt the pathways for MHC-restricted tumor antigen presentation (35–36). Therefore, it is possible that the effect of these miRNA SNPs on ADT efficacy might be a result of their influence on the gene expressions of KIF3C, CDON, and IFI30, altering tumor neuroendocrine differentiation, Hh signaling, and host immunity.

Four SNPs, *KIF3C* rs6728684, *PALLD* rs1071738, *GABRA1* rs998754, and *SYT9* rs4351800, were significantly associated with PCSM after controlling for known clinical prognostic factors. Of these, *SYT9* rs4351800 also showed significant association with ACM during ADT. PALLD, paladin, encodes a cytoskeletal protein that plays an essential role in the assembly and maintenance of several types of actin-dependent structures to control cell morphology, motility, cell adhesion and cell-extracellular matrix interactions. PALLD knockout mouse displayed defects in actin organization, cell adhesion, and cell motility (37), whereas PALLD is overexpressed in the most invasive population of

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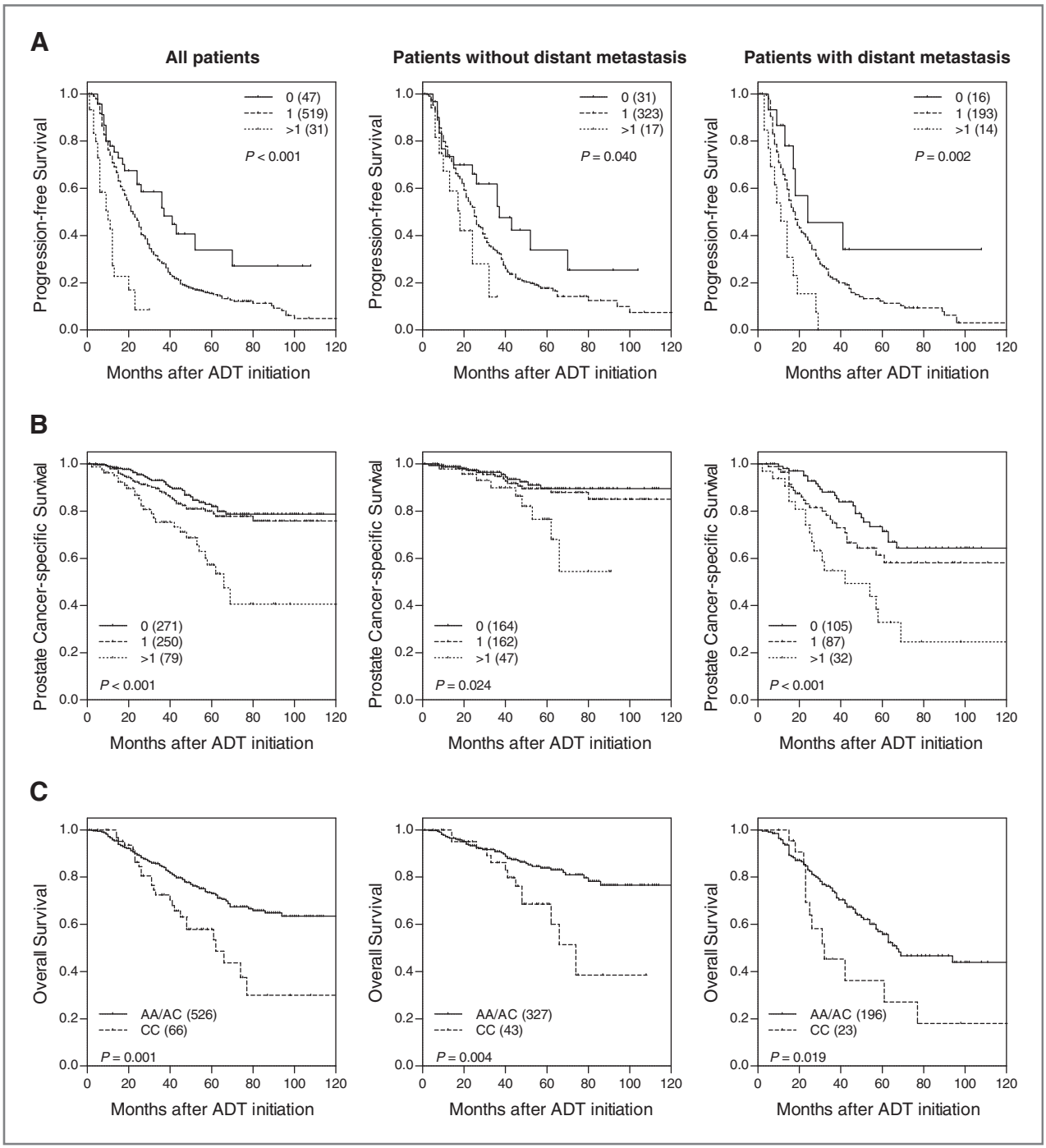

Figure 1. Kaplan–Meier curves of: (A) time to progression during ADT for patients with 0, 1, or >1 unfavorable genotypes at the 3 genetic loci of interest; (B) time to PCSM during ADT for patients with 0, 1, or >1 unfavorable genotypes at the 4 genetic loci of interest; (C) time to ACM during ADT stratified by genotypes at *SYT9* rs4351800; in all patients (left panel), in patients without distant metastasis (middle panel), or in patients with distant metastasis (right panel). Numbers in parentheses indicate the number of patients.

cancer cells (38–40). These correlations suggested that deregulated PALLD might contribute to the aggressive/invasive pathologic cancer cell behavior. GABRA1, gamma-aminobutyric acid (GABA) A receptor alpha 1, is a member of the cys-loop family of ligand-gated ion channels, responsible for mediating the major inhibitory neurotransmitter, GABA, in the brain. GABRA1 maps to chromosome 5q34-q35, the region that has been implicated in the development and progression of bladder cancer (41–42). SYT9 encodes for the vesicular transport

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Table 3. Genotyping frequencies and the association of genotype with PCSM during ADT

Pre-miRNA/Gene SNP	Genotype	No. of patients	No. of events	Mean (months)	*P**	*q*	HR (95% CI)	*P*†
hsa-mir-423	CC	388	74	135	0.037	0.187	1.00	
rs6505162	CA/AA	207	26	141			0.64 (0.40–1.01)	0.054
KIF3C	TT/TG	580	95	139	0.027	0.165	1.00	
rs6728684	GG	16	5	55			**2.65 (1.05–6.70)**	**0.039**
PALLD	GG	464	69	143	0.018	0.165	1.00	
rs1071738	GC/CC	130	31	119			**2.12 (1.36–3.29)**	**0.001**
ACSL1	GG/GA	519	78	141	0.024	0.165	1.00	
rs2292899	AA	73	19	121			1.31 (0.77–2.21)	0.316
GABRA1	TT	172	38	129	0.028	0.165	1.00	
rs998754	TG/GG	400	59	137			**0.59 (0.39–0.90)**	**0.015**
SYT9	AA/AC	526	83	141	0.006	0.165	1.00	
rs4351800	CC	66	18	82			**2.89 (1.70–4.91)**	**<0.001**
ZDHHC7	GG/GA	463	87	134	0.010	0.165	1.00	
rs3210967	AA	133	13	144			0.77 (0.42–1.40)	0.389
No. of unfavorable genotypes present‡								
0		271	35	137	<0.001		1.00	
1		250	39	141			**1.57 (0.97–2.54)**	**0.064**
>1		79	27	85			**4.20 (2.49–7.09)**	**<0.001**
							P-trend	**<0.001**

NOTE. $P \leq 0.05$ are in boldface.

Abbreviations: ADT, androgen-deprivation therapy; HR, hazard ratio; 95% CI, 95% confidence interval; PSA, prostate-specific antigen.

**P* values were calculated using the log-rank test.

†HRs were adjusted for age, clinical stage, Gleason score, PSA at ADT initiation, PSA nadir, and time to PSA nadir.

‡Unfavorable genotypes refer to GG in *KIF3C* rs6728684, GC/CC in *PALLD* rs1071738, TT in *GABRA1* rs998754, and CC in *SYT9* rs4351800.

protein synaptotagmin IX, which regulates exocytosis of synaptic vesicles and appears to serve as a calcium sensor to trigger neurotransmitter release in neuroendocrine cells. A genetic alteration in SYT9, D445N, was found in some colorectal cancers (43). In addition, another closely related synaptotagmin member, SYT7, has also been identified as a prostate cancer-associated gene (44). Interestingly, both GABAergic pathway and synaptotagmins seem to be

Table 4. Genotyping frequencies and the association of genotype with ACM during ADT

Gene SNP	Genotype	No. of patients	No. of events	Mean (months)	*P**	*q*	HR (95% CI)	*P*†
ACSL1	GG/GA	519	116	125	0.046	0.222	1.00	
rs2292899	AA	73	25	109			1.25 (0.80–1.96)	0.326
MTRR	CC/CT	571	132	125	0.033	0.222	1.00	
rs9332	TT	17	7	76			1.27 (0.59–2.75)	0.545
GABRA1	TT	172	53	113	0.020	0.222	1.00	
rs998754	TG/GG	400	86	123			0.70 (0.49–1.00)	0.051
SYT9	AA/AC	526	119	127	0.001	0.032	1.00	
rs4351800	CC	66	26	69			**2.55 (1.65–3.95)**	**<0.001**

NOTE. $P \leq 0.05$ are in boldface.

Abbreviations: ADT, androgen-deprivation therapy; HR, hazard ratio; 95% CI, 95% confidence interval; PSA, prostate-specific antigen.

**P* values were calculated using the log-rank test.

†HRs were adjusted for age, clinical stage, Gleason score, PSA at ADT initiation, PSA nadir, and time to PSA nadir.

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involved in the neuroendocrine differentiation of prostate cancer (45). Taken together, systematically evaluating common variants in miRNAs and miRNA target sites, our research lights up the pathways to influence the survival after ADT, such as KIF3C, GABRA1, and SYT9 in neuroendocrine differentiation, as well as PALLD in cell motility. However, the current findings are hypothesis-generating and further investigation is needed to determine the role of these SNPs/genes during prostate cancer progression.

In summary, we present the first epidemiologic evidence supporting the involvement of genetic variants within miRNAs and miRNA target sites in prostate cancer progression during ADT, and the use of individual as well as combined genotypes of miRNA-related variants to predict clinical outcomes after ADT. The results reported here are limited by analyzing the small number of patients in genetic subset and multiple comparisons. In addition, our homogeneous Chinese Han population might make our findings less generalizable to other ethnic groups. Although this study on miRNA SNPs and efficacy of ADT is at an early stage and the results need replication and laboratory-based functional validation, our findings are nevertheless encouraging in further investigating the genetic candidates implicated by reported SNPs, understanding the pathways of prostate cancer progression during ADT, and ultimately tailoring individual therapeutic interventions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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