

Epigenetic reprogramming and aberrant expression of PRAME are associated with increased metastatic risk in Class 1 and Class 2 uveal melanomas

Supplementary Materials

Supplementary Table S1: Association of clinical features with PRAME status in 123 uveal melanoma cases from our practice

Variable	All Classes (n = 123)	Association with PRAME+ (P-value)	Class 1 (n = 63)	Association with PRAME+ (P-value)	Class 2 (n = 60)	Association with PRAME+ (P-value)
Age at diagnosis, y						
Mean	62.4	0.3	58.0	0.1	67.1	0.7
Median (Q1–Q3)	64.6 (52.2–74.0)		61.0 (48.2–69.2)		68.7 (61.1–76.0)	
Sex, No.(%)						
Female	67.0 (54.5)	0.6	39.0 (61.9)	0.8	28.0 (46.7)	0.8
Male	56.0 (45.5)		24.0 (38.1)		32.0 (53.3)	
Ciliary body involvement, No. (%)						
Yes	58.0 (47.2)	0.2	22.0 (35.5)	0.8	36.0 (60.0)	0.06
No	63.0 (51.2)		40.0 (64.5)		23.0 (38.3)	
Not Available	2.0 (1.6)		0.0 (0.0)		1.0 (1.7)	
Extra-ocular extension, No. (%)						
Yes	17.0 (13.8)	> 0.9	4.0 (6.4)	0.6	13.0 (21.7)	> 0.9
No	92.0 (74.8)		54.0 (85.7)		38.0 (63.3)	
Not Available	14.0 (11.4)		5.0 (7.9)		9.0 (15.0)	
LBD, mm						
Mean	15.5	0.01	14.7	0.03	16.3	0.1
Median (Q1–Q3)	16.0 (12.0–19.1)		14.0 (11.0–18.0)		17.0 (14.0–19.8)	
Tumor thickness, mm						
Mean	9.5	0.02	8.8	0.7	10.1	0.002
Median (Q1–Q3)	10.0 (6.4–12.0)		10.0 (6.9–12.0)		11.0 (8.1–12.7)	
Metastasis, No. (%)						
Yes	28.0 (22.8)	NA	8.0 (12.7)	NA	20.0 (33.3)	NA
No	95.0 (77.2)		55.0 (87.3)		40.0 (66.7)	
Not Available	0.0 (0.0)		0.0 (0.0)		0.0 (0.0)	
Melanoma-specific mortality, No. (%)						
Yes	21.0 (17.1)	NA	4.0 (6.3)	NA	17.0 (28.3)	NA
No	102.0 (82.9)		59.0 (93.7)		43.0 (71.7)	
Not Available	0.0 (0.0)		0.0 (0.0)		0.0 (0.0)	
All-cause mortality, No (%)						
Yes	23.0 (18.7)	NA	5.0 (7.9)	NA	18.0 (30.0)	NA
No	100.0 (81.3)		58.0 (92.1)		42.0 (70.0)	
Not Available	0.0 (0.0)		0.0 (0.0)		0.0 (0.0)	
Time to last follow-up or death, mo						
Mean	29.6	NA	37.3	NA	9.2	NA
Median (Q1–Q3)	21.0 (13.0–33.5)		22.0 (13.5–54.0)		20.0 (10.8–26.1)	

NA, not applicable (Kaplan-Meier survival analysis shown in article).

Supplementary Table S2: Association of clinical features with *PRAME* status in 80 TCGA uveal melanoma cases

Variable	All Classes (<i>n</i> = 80)	Association with PRAME+ (<i>P</i> -value)	Class 1 (<i>n</i> = 39)	Association with PRAME+ (<i>P</i> -value)	Class 2 (<i>n</i> = 41)	Association with PRAME+ (<i>P</i> -value)
Age at diagnosis, y Mean Median (Q1–Q3)	61.7 61.5 (51.0–74.3)	0.2	58.5 60.0 (50.5–71.0)	0.04	64.7 64.0 (55.0–76.0)	> 0.9
Sex, No.(%) Female Male	35.0 (43.8) 45.0 (56.2)	> 0.9	17.0 (43.6) 22.0 (56.4)	> 0.8	18.0 (43.9) 23.0 (56.1)	> 0.8
Ciliary body involvement, No. (%) Yes No Not Available	24.0 (30.0) 56.0 (70.0) 0.0 (0.0)	> 0.9	8.0 (13.1) 31.0 (50.8) 22.0 (36.1)	> 0.7	16.0 (39.0) 25.0 (61.0) 0.0 (0.0)	> 0.5
Extra-ocular extension, No. (%) Yes No Not Available	7.0 (8.8) 68.0 (85.0) 5.0 (6.2)	0.4	1.0 (2.6) 36.0 (92.3) 2.0 (5.1)	> 0.9	6.0 (14.6) 32.0 (78.1) 3.0 (7.3)	> 0.2
LBD, mm Mean Median (Q1–Q3)	16.9 17.0 (14.8–19.4)	0.02	16.3 16.5 (14.0–18.5)	0.4	17.6 17.5 (15.5–20.0)	> 0.03
Tumor thickness, mm Mean Median (Q1–Q3)	10.4 10.5 (8.5–12.0)	0.2	10.0 10.0 (6.9–12.0)	> 0.9	10.8 11.0 (8.1–12.7)	> 0.1
Metastasis, No. (%) Yes No Not Available	12.0 (15.0) 65.0 (81.2) 3.0 (3.8)	NA	0.0 (0.0) 38.0 (97.4) 1.0 (2.6)	NA	12.0 (29.3) 27.0 (65.8) 2.0 (4.9)	NA
Melanoma-specific mortality, No. (%) Yes No Not Available	11.0 (13.8) 68.0 (85.0) 1.0 (1.2)	NA	0.0 (0.0) 39.0 (62.9) 23.0 (37.1)	NA	11.0 (26.8) 29.0 (70.7) 1.0 (2.5)	NA
All-cause mortality, No (%) Yes No Not Available	13.0 (16.2) 67.0 (83.8) 0.0 (0.0)	NA	1.0 (2.6) 38.0 (97.4) 0.0 (0.0)	NA	12.0 (29.3) 29.0 (70.7) 0.0 (0.0)	NA
Time to last follow- up or death, mo Mean Median (Q1–Q3)	15.0 12.9 (2.4–23.4)	NA	17.4 13.3 (2.4–25.3)	NA	7.8 12.8 (2.7–19.7)	NA

NA, not applicable (Kaplan-Meier survival analysis shown in article).

Supplementary Table S3: Associations between *PRAME* status and copy number gains and losses in 106 Class 1 and Class 2 uveal melanomas

Variable	All Classes (<i>n</i> = 106)	Association with <i>PRAME</i> + (<i>P</i> -value)	Class 1 (<i>n</i> = 51)	Association with <i>PRAME</i> + (<i>P</i> -value)	Class 2 (<i>n</i> = 55)	Association with <i>PRAME</i> + (<i>P</i> -value)
1p loss, No. (%)						
Yes	33 (31.1)	0.7	11 (21.6)	> 0.9	22 (40.0)	> 0.9
No	73 (68.9)		40 (78.4)		33 (60.0)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	
1q gain, No. (%)						
Yes	18 (17.0)	0.07	11 (21.6)	0.04	7 (12.7)	> 0.9
No	88 (83.0)		40 (78.4)		48 (87.3)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	
Chr3 loss, No. (%)						
Yes	53 (50.0)	0.3	0 (0.0)	0.5	53 (96.4)	0.5
No	53 (50.0)		51 (100.0)		2 (3.6)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	
6p gain, No. (%)						
Yes	55 (51.9)	0.1	44 (86.3)	0.02	11 (20.0)	0.05
No	51 (48.1)		7 (13.7)		44 (80.0)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	
6q loss, No. (%)						
Yes	32 (30.2)	< 0.0001	15 (29.4)	< 0.0001	17 (30.9)	< 0.0001
No	74 (69.8)		33 (70.6)		38 (69.1)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	
8p loss, No. (%)						
Yes	25 (23.6)	0.02	3 (5.9)	> 0.9	22 (40.0)	0.03
No	81 (76.4)		48 (94.1)		33 (60.0)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	
8q gain, No. (%)						
Yes	80 (75.5)	< 0.0001	29 (56.9)	< 0.0001	51 (92.7)	0.07
No	26 (24.5)		22 (43.1)		4 (7.3)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	
16q loss, No. (%)						
Yes	24 (22.6)	< 0.0001	6 (11.8)	0.004	18 (32.7)	0.01
No	82 (77.4)		45 (88.2)		37 (67.3)	
Not Available	0 (0.0)		0 (0.0)		0 (0.0)	

Supplementary Table S4: Associations between *PRAME* status and common driver mutations in 139 Class 1 and Class 2 uveal melanomas

Variable	All Classes (<i>n</i> = 139)	Association with <i>PRAME</i> + (<i>P</i> -value)	Class 1 (<i>n</i> = 63)	Association with <i>PRAME</i> + (<i>P</i> -value)	Class 2 (<i>n</i> = 60)	Association with <i>PRAME</i> + (<i>P</i> -value)
BAP1, No. (%)						
Yes	60 (43.2)	0.02	1 (1.6)	0.5	59 (77.6)	0.2
No	64 (46.0)		54 (85.7)		10 (13.2)	
Not Available	15 (10.8)		8 (12.7)		7 (9.2)	
SF3B1, No. (%)						
Yes	28 (20.1)	0.05	21 (33.3)	< 0.0001	7 (9.2)	0.4
No	88 (63.3)		35 (55.6)		53 (69.7)	
Not Available	23 (16.6)		7 (11.1)		16 (21.1)	
EIF1AX, No. (%)						
Yes	16 (11.5)	0.02	14 (22.2)	0.004	2 (2.6)	0.5
No	90 (64.8)		38 (60.3)		52 (68.4)	
Not Available	33 (23.7)		11 (17.5)		22 (29.0)	
GNAQ, No. (%)						
Yes	67 (48.2)	0.5	38 (60.3)	0.6	29 (28.7)	0.3
No	64 (46.0)		24 (38.1)		40 (52.6)	
Not Available	8 (5.8)		1 (1.6)		7 (9.2)	
GNA11, No. (%)						
Yes	55 (39.6)	> 0.9	20 (31.7)	> 0.9	35 (46.1)	> 0.9
No	74 (53.2)		41 (65.1)		33 (43.4)	
Not Available	10 (7.2)		2 (3.2)		8 (10.5)	
CYSLTR2, No. (%)						
Yes	3 (2.2)	NA	1 (1.6)	NA	2 (2.6)	NA
No	77 (55.4)		38 (60.3)		39 (51.3)	
Not Available	59 (42.4)		24 (38.1)		35 (46.1)	
PLCB4, No. (%)						
Yes	2 (1.4)	NA	0 (0.0)	NA	2 (3.3)	NA
No	78 (56.1)		39 (61.9)		23 (38.3)	
Not Available	59 (42.4)		24 (38.1)		35 (58.4)	

NA, not applicable (insufficient number of cases for statistical analysis).

Supplementary Table S5: Validation of differential methylation at 5 CpG sites within the *PRAME* promoter region in *PRAME*⁺ versus *PRAME*⁻ tumors

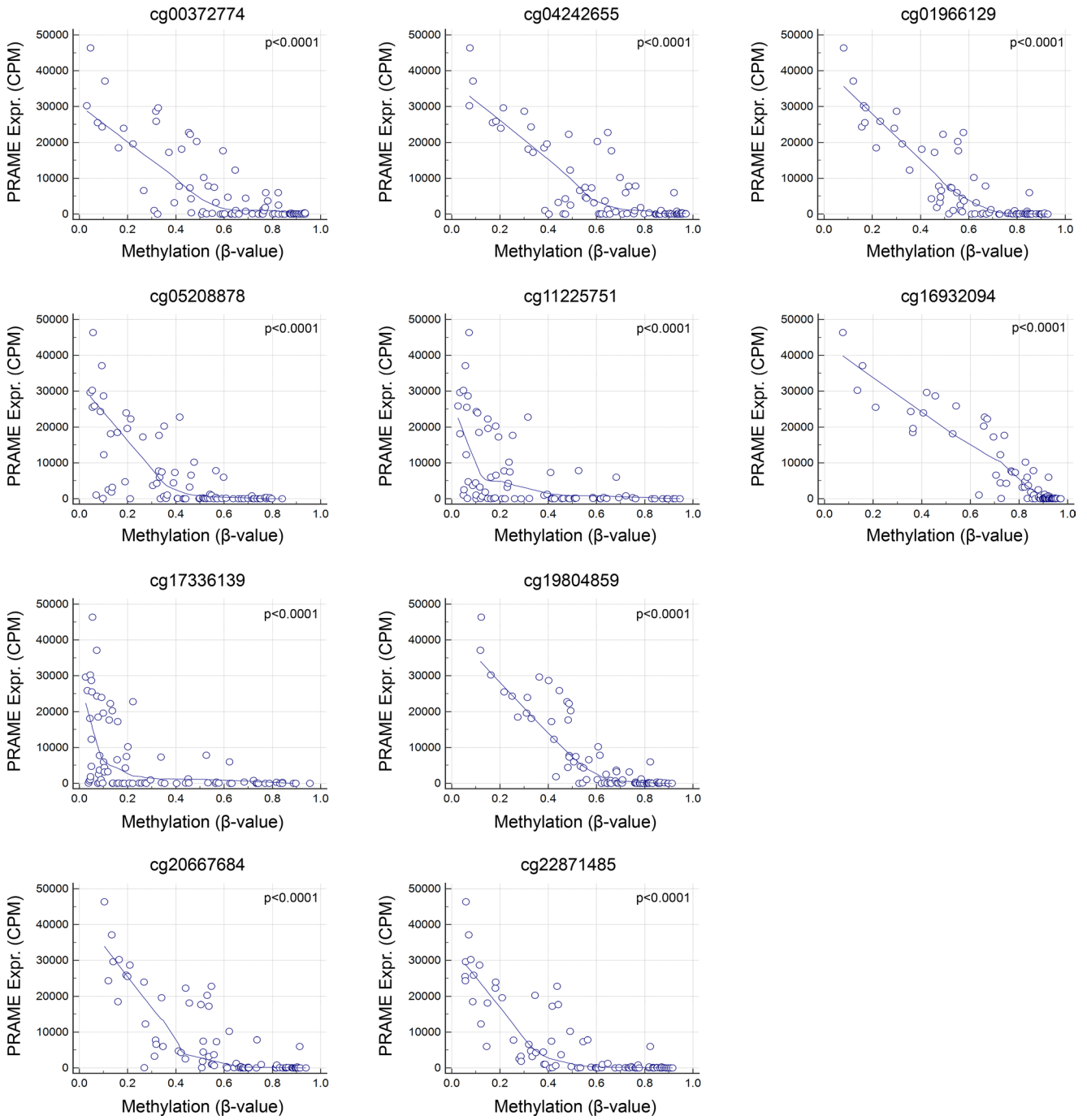
Genomic location on chromosome 22

		22901898	22901907	22902166	22902216	22902255
PRAME ⁺ tumors	MM10	X	X		X	X
	MM92			X	X	
	MM89					X
	MM16					
PRAME ⁻ tumors	MM28					
	MM49					
	MM50					

X, indicates hypomethylation of indicated nucleotide.

Supplementary Table S6: Sources and numbers of uveal melanoma samples for the various data analyses

Variable	Our Practice	Castle	TCGA
Number of samples	123	555	80
Class 1/Class 2 data	Yes	Yes	Estimated
Class 1A/1B data	Yes	Yes	No
Clinical annotations	Yes	No	Yes
Whole-exome data	24	No	80
Mutation data	59	No	80
Chromosomal copy number data	26	No	80



Supplementary Figure S1: Association between PRAME mRNA expression and promoter hypomethylation. Scatter plots showing the relationship between PRAME mRNA expression levels (obtained from the TCGA RNA-Seq data) and PRAME promoter methylation (obtained from the TCGA Infinium Human Methylation 450 Bead Chip Array data) at ten additional methylation sites represented by the indicated probes. Spearman's rank correlation coefficient was used to determine P -values.