Increased Risk of Brain Metastases Among Patients with Melanoma and PROM2 Expression in Metastatic Lymph Nodes

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Abstract

Background: Melanoma brain metastases are the main cause of specific death among patients with metastatic melanoma. The biology of melanoma brain metastases remains largely to be deciphered, as there have been only a few genomic studies on brain metastatic samples. In this study, melanoma metastatic lymph nodes were used with the aim to identify biomarkers associated with the occurrence of brain metastases.

Methods: Fifty-one patients with melanoma lymph node metastasis and a median follow-up of 48 months were included in the development cohort. Transcriptomic data were obtained from these metastatic lymph nodes and patients who developed brain metastases and those who did not were compared. Recommendations for tumour marker prognostic studies (REMARK recommendations) were followed.

Results: From transcriptomic data, we identified *PROM2* which was significantly overexpressed in metastatic lymph nodes of patients who developed brain metastases compared to those who did not. Using immunohistochemistry with two different anti-PROM2 antibodies, a PROM2 score was developed for metastatic lymph nodes. Using a cut-off of 5, a PROM2 mean score ≥ 5 was significantly associated with an increased risk of brain metastases and an increased hazard risk of death by 4.

These results were confirmed in an internal validation cohort of 50 additional patients with melanoma lymph node metastases.

Conclusions: In this study, we identified PROM2 expression as a biomarker predictive of the occurrence of distant metastases, particularly brain metastases, among patients with stage III melanoma. Our findings open new perspectives to validate PROM2 as a useful biomarker for clinical trials in the adjuvant setting, and as a potential biotarget for the treatment of metastatic melanoma.

Background

Melanoma is an increasing cause of death by cancer, due to an increased incidence of metastases and particularly brain metastases. Brain metastases occur in the progression of metastatic melanoma in up to 44% of cases (1, 2). In addition, the incidence of brain metastases has increased in the last ten years as a result of better control of localizations outside the central nervous system with immunotherapies (3, 4), and because most anti-cancer drugs fail to cross the blood-brain barrier at relevant pharmacological concentrations (5). Despite evidence of clinical benefit of combined immunotherapies on melanoma brain metastases (6), more than 50% of patients will have brain progression, challenging daily practice in oncology.

The biology of melanoma brain metastases remains largely to be deciphered, mainly due to difficult access to tissue samples. There have been few genomic studies on melanoma brain metastases, and most of them have explored their mutational status, comparing brain metastases with matched primary melanoma or extra-central nervous system metastases (7–9). There are even less transcriptomic data on melanoma brain metastases, characterized by an activation of the *PI3K* pathway (7, 10). In a transcriptomic study on soft tissue, skin or lymph node metastases, *PLEKHA5*, a pleckstrin homology domain-containing protein family A, member 5, was identified as a potential biomarker predictive of the risk of melanoma brain metastases (11).

In our study, we performed transcriptomic analyses on laser-micro-dissected tumour cells from metastatic lymph nodes of patients with metastatic melanoma. Clinical follow-up enabled us to compare molecular results for patients

who developed brain metastases and for those who did not. Our aim was to identify biomarkers associated with the occurrence of brain metastases over a median follow-up of 48 months, and assess their association with survival.

Methods

We have followed reporting recommendations for tumour marker prognostic studies (REMARK recommendations) (12).

Some methods are fully detailed in Supplementary Material and Method file.

Patient data

One hundred and one patients from Saint-Louis Hospital with available tumour samples and follow-up data were included in this study, for the development and the validation cohorts. Informed consent was obtained from each patient. The Clinical-Research-Board-Ethics-Committee approved this study (CPP-Ile-de-France#13218).

All patients were diagnosed between 2009 and 2014, and had a regional macroscopic lymph node metastatic melanoma without distant metastases at the time of inclusion in this study. None of them received systemic adjuvant therapy. Fifty-one patients were included in the development cohort, with samples collected between 2009 and 2013. Fifty additional patients were included in the validation cohort, with samples collected between 2013 and 2014.

On the basis of clinical and imaging data, and a median follow-up of 48 months from the time of the regional lymph node disease, patients with and without distant metastases were separated, including those with brain metastases. As recommended by the National Comprehensive Cancer Network, magnetic resonance imaging (MRI) of the brain was systematically performed at the time of the metastatic disease, and then at least once a year, or in case of neurological symptoms.

Laser-microdissection of tumour cells from metastatic lymph nodes and transcriptomic data processing

For the development cohort, we identified 336 patients with melanoma and regional lymph node metastatic samples collected between 2009 and 2013. Among them, 100 patients had at least one frozen metastatic sample available and 58 patients had a follow-up of at least 36 months from diagnosis of metastatic disease (Supplementary Fig. 1). Cryo-cut sections of each sample were laser-micro-dissected to select tumour cells. Using a PALMMicrobeam/Zeiss-system, a minimum of 1500 tumour cells were laser-micro-dissected for RNA extraction. 51 cases had a mean RNA integrity number of 8.7 (range 7-10) (Supplementary Fig.1).

Transcriptomic analyses were performed using MiltenyiBiotec-Microarray.

RT-qPCR and validation of *PROM2* mRNA expression in metastatic lymph-nodes

For the development cohort, on following sections of the same laser-micro-dissected metastatic lymph nodes, RT-qPCR was performed to validate the transcriptomic results for *PROM2* expression, according to the MIQE guidelines(13).

For the validation cohort, melanoma cancer cells from 50 metastatic lymph nodes were laser-micro-dissected and processed for RT-qPCR.

In situ PROM2 expression in metastatic lymph nodes

Using immunochemistry, PROM2 expression was assessed in the metastatic lymph nodes of the 101 samples of the two cohorts. An indirect immuno-peroxidase method (Discovery/RocheDiagnostics) on 5µm-thick frozen tissue sections was used using anti-PROM2 (ab74997, rabbit polyclonal, Abcam, 1/100) as primary antibody, and anti-rabbit OmniMap detection kit (Roche-Diagnostics). Systematic controls were the absence of a primary antibody and the use of an irrelevant primary antibody of the same isotype. Normal skin and normal liver were used as positive and negative controls respectively. A membranous and cytoplasmic distribution of PROM2 was considered positive. Each sample was given a score by multiplying the stain intensity grade (0 = no staining, 1 = low intensity, 2 = medium intensity, 3 = strong intensity) by the numerical code for the percentage of positive cells (0 = 0%, 1 = under 10%, 2 = 10–50%, 3 = 51–80%, 4 = over 81%).

Statistical analyses

The data were analyzed using R statistical software (version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org).

Transcriptomic data: The SAMR package was used to identify differentially expressed genes in samples with the two-class unpaired method.

We performed the same analyses on transcriptomic data downloaded from public databanks (14),(15),(16),(11).

Factors associated with brain metastases: Patients with and without brain metastases were compared regarding their clinical and biological characteristics. Univariate and multivariate logistic regression were performed with the sample of brain metastatic patients.

Factors associated with mortality: Univariate survival curves were plotted according to the Kaplan-Meier method for brain metastatic status, and “PROM2 IHC score”. Univariate and multivariate Cox proportional hazard regression were performed with the sample of patients deceased.

Results

Patient characteristics in the development cohort

Among the 51 patients selected for this study, after a median follow-up of 48 months from the time of regional lymph node disease, 19 (37%) developed brain metastases while 32 (63%) did not. The characteristics of these 51 patients are summarized in Table 1, comparing patients with brain metastases (Group 3) and patients without (Groups 1 and 2) (Fig.1A).

Each metastatic lymph node was laser-micro-dissected to specifically select a minimum number of 1500 tumour cells, with a mean surface area of 645 000 µm² ± 175 000µm². After RNA extraction, all samples were of good quality, enabling transcriptomic analyses, since the mean RNA integrity number was 8.7 (range 7–10).

The median overall survival calculated from first diagnosis of regional lymph node metastasis was significantly shorter among patients with brain metastases than among patients without brain metastases (39 months *vs*. 76 months, p<0.01) (Fig. 1B). The median survival from the time of brain metastases was 13.3 months (range 2-72 months). Among patients with *BRAF*V600E mutation, the median survival was not significantly different for patients with and without brain metastases (14 *vs*. 17 months).

PROM2 gene expression in metastatic lymph nodes from patients with brain metastases

Transcriptomic analyses were performed on laser-micro-dissected tumour cells obtained from metastatic lymph nodes of the 51 patients. Multivariate analysis was carried out to compare data from patients with brain metastases and those without. Table 2 shows the genes with the highest d-scores and fold-changes, all of them having a *Q*-value <0.001. We decided to focus on the *PROM2* gene, also called prominin-2, with some of the highest d-scores at 4.6 and fold-change of 3.3, and because the PROM2 protein induces membrane protrusions (17) and could thus be implicated in invasive processes.

Using RT-qPCR, we confirmed that the median expression of *PROM2* mRNA was significantly higher in metastatic lymph nodes from patients who subsequently developed brain metastases (ΔCt= 4.9, IQR=6.3) than in those from patients who did not (ΔCt= 2.1, IQR=3.9) (p = 0.005, Fig. 2A).

We then analysed transcriptomic data downloaded from public databanks. In 167 metastatic lymph nodes of the TCGA SKCM cohort, a high *PROM2* expression was found to be associated with poor survival (p=0.007, score logrank test = 7.15) (Supplementary Fig.2A). In the GSE cohorts that were used to construct Lund molecular classification (GSE22155 and GSE65904) (15, 16), a high *PROM2* expression level in 102 metastatic lymph nodes tended to be associated with a poor survival in the first 1000 days of follow-up (score logrank test = 2.99, p=0.08) (Supplementary Fig.2B).

PROM2 protein expression in metastatic lymph nodes is associated with the risk of brain metastases

Using immunohistochemistry, PROM2 expression was assessed on the 51 metastatic lymph nodes. We showed that the PROM2 was only expressed by cancer cells and that the mean “PROM2 IHC score” was significantly higher among patients with brain metastases compared to patients without (8.8 *vs.* 4, p<0.01) (Fig. 2B).

When we chose a cut-off of 5 for the “PROM2 IHC score”, overall survival was significantly longer among patients who had a score <5 than among patients who reached the cut-off of 5 (p < 0.01, Figure 2C).

Overall, PROM2 expression in metastatic lymph nodes from melanomas is associated with the risk of brain metastases and with decreased survival.

PROM2 expression is low in regional lymph nodes without associated distant metastases

We also compared PROM2 protein expression and overall survival across the three groups, and found that the “PROM2 IHC score” differed gradually and significantly across the 3 groups (Fig. 3B). Overall survival was also much longer for Group 1 with only metastatic regional lymph nodes and without distant metastases after a median follow-up of 80 months (Fig. 3C).

Risk factors for brain metastasis were identified first by univariate analysis and then by multivariate analysis. Using multivariate regression, we identified a “PROM2 IHC score” ≥ 5 and the presence of bone metastases as the two variables significantly associated with the risk of brain metastases. For the “PROM2 IHC score”, the odds ratio was particularly high, at 28.2 (p=0.003, Table 3), showing the strongest association with the risk of developing brain metastases.

Among patients with resectable regional lymph node metastases and stage III melanoma, an elevated “PROM2 IHC score” enabled identification of a high-risk group for distant metastases, including brain metastases.

Validation cohort confirms PROM2 as a biomarker for distant metastasis

Between 2013 and 2014, 50 additional patients with stage III melanoma at diagnosis and a frozen biopsy sample from lymph node metastases were included in this validation cohort. After a median follow-up of 48 months from the time of the regional lymph node disease, 19 patients (38%) developed brain metastases while 31 (62%) did not. There was no significant difference between the development and the validation cohorts for clinical characteristics or melanoma features (Supplementary Table 1).

In this validation cohort, when *PROM2* mRNA expression was assessed on melanoma cancer cells laser-micro-dissected from metastatic lymph nodes, it was significantly higher among patients who developed brain metastases (median ΔCt=5.1, IQR=2.3) than among patients who did not (median ΔCt=2, IQR=4.6) ($P < 0.01$). The "PROM2 IHC score" was also significantly higher in case of brain metastases (7.4 vs. 2.1, $P < 0.01$). Using a cut-off of ≥5 for the "PROM2 IHC score" in multivariate regression, the "PROM2 IHC score" was the only factor associated with the risk of brain metastases.

In the two cohorts, in univariate analysis, a high "PROM2 IHC score" ≥5 in metastatic lymph nodes was not associated with the risk of other metastatic sites (lung, liver, bone), except for lung metastases in the validation cohort (Supplementary Table 2).

In both the development and validation cohorts, the presence of brain metastases and a "PROM2 IHC score" of ≥ 5 were the only two factors significantly associated with mortality (Table 4 and Supplementary Figure 3).

Discussion

In this study, among patients with regional lymph node metastases from cutaneous melanoma, we identified PROM2 as a biomarker significantly associated with the risk of distant metastases, particularly brain metastases.

Multivariate analysis showed that the high "PROM2 IHC score" in metastatic lymph nodes and the occurence of bone metastases were the only two factors significantly associated with the risk of brain metastases. In a large observational study of 26,430 cancer patients with brain metastases, bone and lung metastases were more frequently associated with the risk of brain metastases than were liver metastases (1). In the case of lung metastases, cancer cells can enter the pulmonary vein through the lung capillaries, taking cancer cells into the arterial circulation *via* the left ventricle, and thus favoring the development of brain metastases (18). This preferential association of lung or bone metastases with brain metastases is particularly true for melanoma. This could be explained by the short median survival of 4 months in case of liver metastases (1, 19) while the median time lapse to the appearance of brain metastases is 9 months (20). There could also be biological reasons, such as the expression of chemokine receptor in melanoma cells and of its respective ligand in a target site for metastasis (21, 22). It still remains unclear whether the expression of chemokine/chemokine-receptors contributes to a preferential distribution of melanoma metastases in the brain.

In our large series of 101 frozen metastatic lymph nodes, the "PROM2 IHC score" was the strongest marker associated with the development of distant metastases in melanoma patients, particularly brain metastases, and with a shorter survival. In two large series of metastatic lymph nodes, a high *PROM2* expression was found to be associated with poor survival (14–16). In another small series of 14 metastatic lymph nodes, no association was found between PROM2 expression level and the occurrence of brain metastases (11). This association between a high PROM2 expression in metastatic lymph nodes and a pejorative evolution is strength of our study, with potential translational applications among patients with stage III melanoma. Indeed, adjuvant treatment using immunotherapy or targeted anti-BRAF and anti-MEK therapies is recommended for patients with stage III disease. No other marker than regional lymph node involvement is currently included in the therapeutic decision. In our study, we were able to

identify a group of stage III patients without any adjuvant treatment who did not develop distant metastases after a minimum follow-up of 5 years, and these patients had a significantly lower “PROM2 IHC score” than the patients in the other two groups. After validation in a larger cohort, this score could be used to identify high and low-risk patients with stage III melanoma more efficiently, and could thus be included in adjuvant clinical trials.

Despite recent improvements in the management of metastatic melanomas, 20 to 40% of patients remain insensitive to immunotherapy or targeted treatments (23, 24), thus challenging clinical practice. Our study opens new perspectives for the use of PROM2 as a potential therapeutic target for the treatment of metastatic melanoma in resort situations.

PROM2 is a membrane glycoprotein and a second member of the prominin family. PROM2 is a paralogue of PROM1 which co-localizes with PROM1 in epithelial cells. PROM1, also called CD133, is a well-known cancer stem-cell marker (25). Cancer stem-cells have been shown to be linked to drug resistance (26–29) tumour invasion and metastatic processes (30–32). Like PROM1, PROM2 is enriched in plasma membrane protrusions in the adult kidney as well as in various other epithelial tissues, and could thus be implicated in cell adhesion, wound healing, and migration (17). Analyses from online databases show a differential mRNA expression of *PROM2* in various cancer types, and PROM2 mutations have been reported in skin melanoma (33). However, the role of PROM2 in the metastatic process has not been investigated. Further studies are required to elucidate this role and to see whether or not PROM2 provides stemness properties.

In our study, we identified PROM2 as a biomarker predictive of the risk of distant metastases, particularly brain metastases, among patients with stage III melanoma. Our findings open new perspectives for further studies to validate PROM2 as a useful biomarker in adjuvant clinical trials, and as a potential biotarget for the treatment of metastatic melanoma in resort situations.

List Of Abbreviations

Ct: cycle threshold

IQR: interquartile range

TCGA: The Cancer Genome Atlas

IHC: immunohistochemistry

MRI: magnetic resonance imaging

SEM: standard error of the mean

SD: standard deviation

FDR: false discovery rates

GEO: Gene Expression Omnibus

OR: odd ratio

aOR: adjusted OR

CI: confidence interval

HRa: Hazard ratios

aHR: adjusted HRs

Declarations

Ethics approval and consent to participate

All patients had been informed that a part of their remaining samples could be used for research after diagnosis had been established, and none opposed it. Written informed consent was obtained from each patient. The Clinical-Research-Board-Ethics-Committee approved this study (CPP-Ile-de-France#13218).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GB, AJ and MB conceived and designed the study. PT, BB, JD, CL and SM provided patient clinical data. MB provided patient tumour samples. TTN and MEB carried out the laser-microdissections. GG and JPF analysed the transcriptomic data. TTN and ChL performed the immuno-staining. FP did statistical analyses. AJ, GB and MB provided financial support. AJ and GB provided administrative support. TTN, GB and MB drafted the manuscript. All authors read and approved the final version of the manuscript.

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Tables

Table 1
Characteristics of the 51 patients in the development cohort with transcriptomic analyses of metastatic lymph nodes

Variables	**N (%)**	**Brain metastases (Group 3)** **N = 19 (37%)**	**No brain metastasis** **(Group 1 and 2)** **N = 32 (63%)**	***P****
Age (y), mean ± SD	57.8 ± 15.8	55.0 ± 15.6	59.5 ± 15.9	0.3
Gender (women)	28 (55)	11 (58)	17 (53)	0.7
Initial TNM classification[#]:	20 (39)	7 (37)	13 (41)	0.5
IIIB	28 (55)	10 (53)	18 (56)	
IIIC	3 (6)	2 (10)	1 (3)	
IIID				
Primary site of melanoma:	5 (10)	3 (16)	2 (6)	0.6
Head and neck	10 (20)	3 (16)	7 (22)	
Trunk	11 (21)	5 (26)	6 (19)	
Upper limb	23 (45)	8 (42)	15 (47)	
Lower limb	2 (4)	0	2 (6)	
Non-available				
Metastatic site:	24 (47)	12 (63)	12 (37.5)	0.07
Lung	**11 (21)**	**8 (42)**	**3 (9)**	**0.01**
Bone	16 (31)	8 (42)	8 (25)	0.2
Liver				
Breslow index (mm), median (25th -75th)	3.5 (2.0–5.0)	3.25 (2.01-7.0)	3.58 (1.42–4.87)	0.4
Ulceration (yes)	18 (41)	8 (42)	10 (31)	0.7
BRAF status:	26 (51)	9 (47)	17 (53)	0.7
BRAF V600E	25 (49)	10 (53)	15 (47)	
Wild type				

[#] TNM classification according to 2017 AJCC 8th edition

* Chi square test or Fisher's exact test as appropriate for categorical variables and Student's t-test or Wilcoxon's test for quantitative variables as appropriate

Table 2
Genes differentially expressed in metastatic lymph nodes of patients with *versus* without brain metastasis

Gene	Without brain metastasis (mean)	With brain metastasis (mean)	Fold Change	d-score	*P*-value
PROM2	**5,7**	**7,5**	**3,3**	**4.6**	**2.1e-05**
LRRC2	7,4	9,3	3,6	4.2	2.2e-05
CASC15	9,8	11,7	3,6	4.2	2.2e-05
HMBOX1	7,2	9,0	3,5	4.1	2.2e-05
GPR179	5,9	7,7	3,4	4.3	2.2e-05
CD86	9,6	11,4	3,4	4.0	2.3e-05
RPA4	7,1	8,8	3,2	4.3	2.1e-05
GPR182	5,8	7,5	3,1	4.4	2.1e-05
KRT79	5,1	6,7	2,9	3.9	2.3e-05
PPP6R1	8,0	9,4	2,7	3.7	2.5e-05
BICC1	5,1	6,5	2,6	4.1	2.2e-05

Table 3
Univariate and multivariate analyses of factors associated with brain metastasis

Variables	Univariate analysis OR [95%CI]	*P*	Multivariate analysis Adjusted OR [95%CI]	*P*
Metastatic site:	2.86 [0.88–9.25]	0.08	1.41 [0.27–7.27]	0.66
Lung	7.03 [1.57–31.4]	**0.01**	16.2 [1.83–395.9]	**0.02**
Bone				
PROM2 expression, per 1 IQR	1.21 [1.03–1.48]	**0.03**	1.05 [0.91–1.36]	0.62
PROM2 IHC score:	1 (reference)	**0.0002**	1 (reference)	**0.003**
Low (< 5)	16.0 [4.10–83.1]		28.2 [4.33–570.7]	
High (≥ 5)				

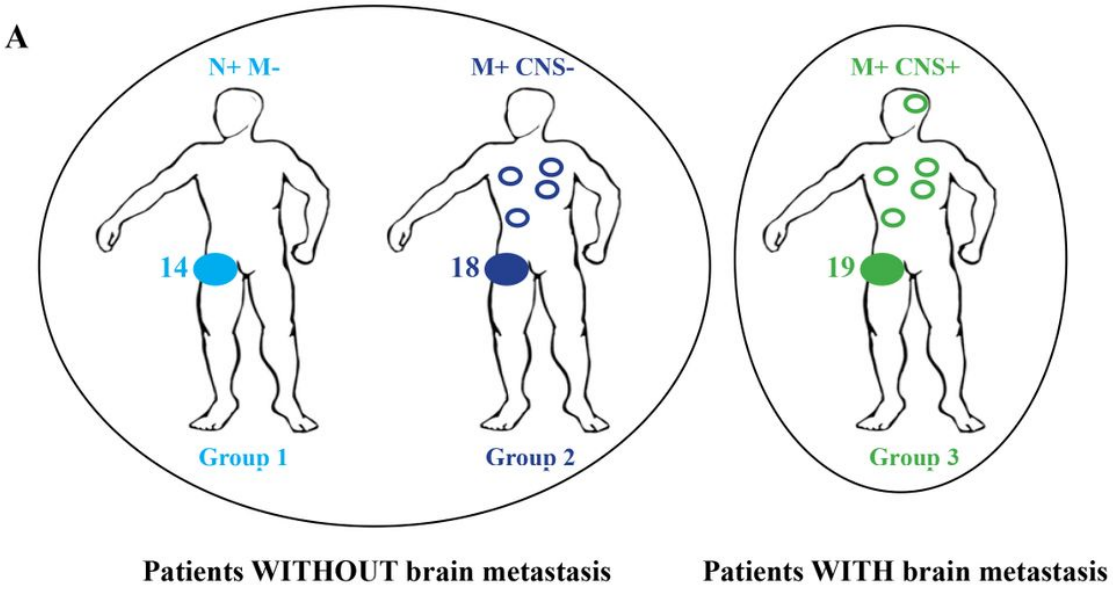
Table 4
Univariate and multivariate analyses of factors associated with mortality in the development and validation cohorts:

Variables	Development cohort (n = 51)				Validation cohort (n = 50)			
	Univariate analysis HR [95%CI]	*P**	Multivariate analysis aHR [95%CI]	*P**	Univariate analysis HR [95%CI]	*P**	Multivariate analysis aHR [95%CI]	*P**
Age (y), mean ± SD	1.01 [0.98–1.03]	0.37			0.98 [0.96–1.01]	0.26		
Gender (women)	0.94 [0.46–1.93]	0.88			0.82 [0.39–1.75]	0.62		
Initial TNM classification	1 (reference)	0.01	-	-	1 (reference)	0.94		
IIIB	1.22 [0.57–2.60]		-		1.12 [0.53–2.34]			
IIIC	7.26 [1.85–28.4]				0.86 [0.11–6.58]			
IIID								
Primary site of melanoma	1 (reference)	0.49			1 (reference)	0.20		
Head and neck	0.91 [0.26–3.15]				0.42 [0.10–1.64]			
Trunk	0.48 [0.13–1.75]				0.80 [0.19–3.26]			
Upper limb	0.46 [0.15–1.44]				1.08 [0.30–3.86]			
Lower limb	0.49 [0.05–4.45]				-			
Unknown								
Metastatic site:	2.61 [1.28–5.34]	0.008	-	-	5.20 [2.39–11.3]	< 0.0001	8.08 [2.49–26.2]	0.0005
Brain	3.82 [1.78–8.18]	0.0005	10.7 [2.86–39.9]	0.0004	2.36 [1.13–4.96]	0.02	-	-
Lung	2.00 [0.91–4.36]	0.08	-	-	2.50 [1.17–5.37]	0.01	-	-
Bone	1.85 [0.90–3.79]	0.09	-	-	2.07 [0.99–4.31]	0.05	-	-
Liver								

Variables	Development cohort (n = 51)				Validation cohort (n = 50)			
Breslow index (mm), per 1 IQR of more	1.19 [1.04–1.36]	0.01	-	-	0.93 [0.82–1.06]	0.28		
Ulceration (yes)	2.75 [1.26-6.00]	0.01	-	-	1.19 [0.53–2.65]	0.67		
BRAF status: BRAF V600E (yes)	1.49 [0.73–3.04]	0.26			1.49 [0.65–3.42]	0.34		
PROM2 mRNA expression, per 1 IQR of more	1.02 [0.99–1.05]	0.11	-	-	1.16 [1.03–1.31]	0.01	-	-
PROM2 IHC score: High (≥ 5)	2.41 [1.16–5.03]	0.01	6.48 [1.65–25.5]	0.007	3.60 [1.69–7.70]	0.0001	3.95 [1.14–13.7]	0.02
Interaction terms: Lung metastases x PROM2 IHC score ≥ 5 Brain metastases x PROM2 IHC score ≥ 5			0.12 [0.02–0.64]	0.01			0.21 [0.04–1.10]	0.06

*Univariate and multivariate Cox proportional hazard regression models were run with the sample of deceased patients. The assumptions of the model were verified. Hazard ratios (HRs) for continuous variables were expressed per 1 SD or 1 interquartile range (IQR) as appropriate. Variables yielding *P* values under 0.2 in the univariate analysis were considered for inclusion in the multivariate analysis. A stepwise selection process of the lowest *P* values was used for the multivariate analysis, also using interaction terms.

Figures



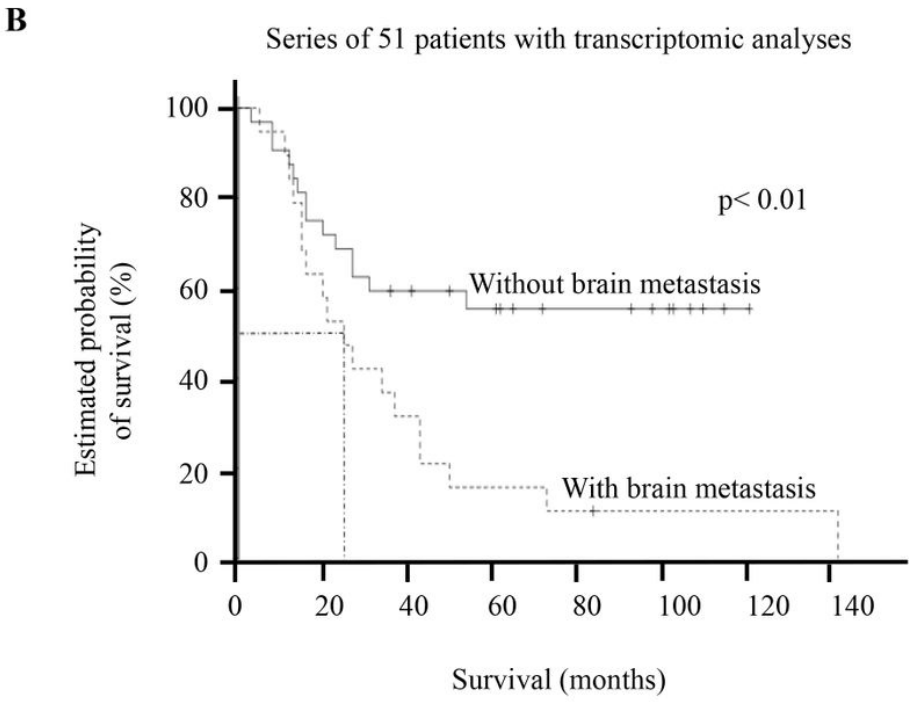


Figure 1

Survival analyses according to presence or absence of brain metastases of the development cohort A. The groups in the development cohort: patients without brain metastases include Group 1 and Group 2. Group 3 includes patients with brain metastases. B. Survival according to brain metastasis status in the series of 51 patients with transcriptomic analyses. The median overall survival is significantly shorter among patients with brain metastases than among those without.

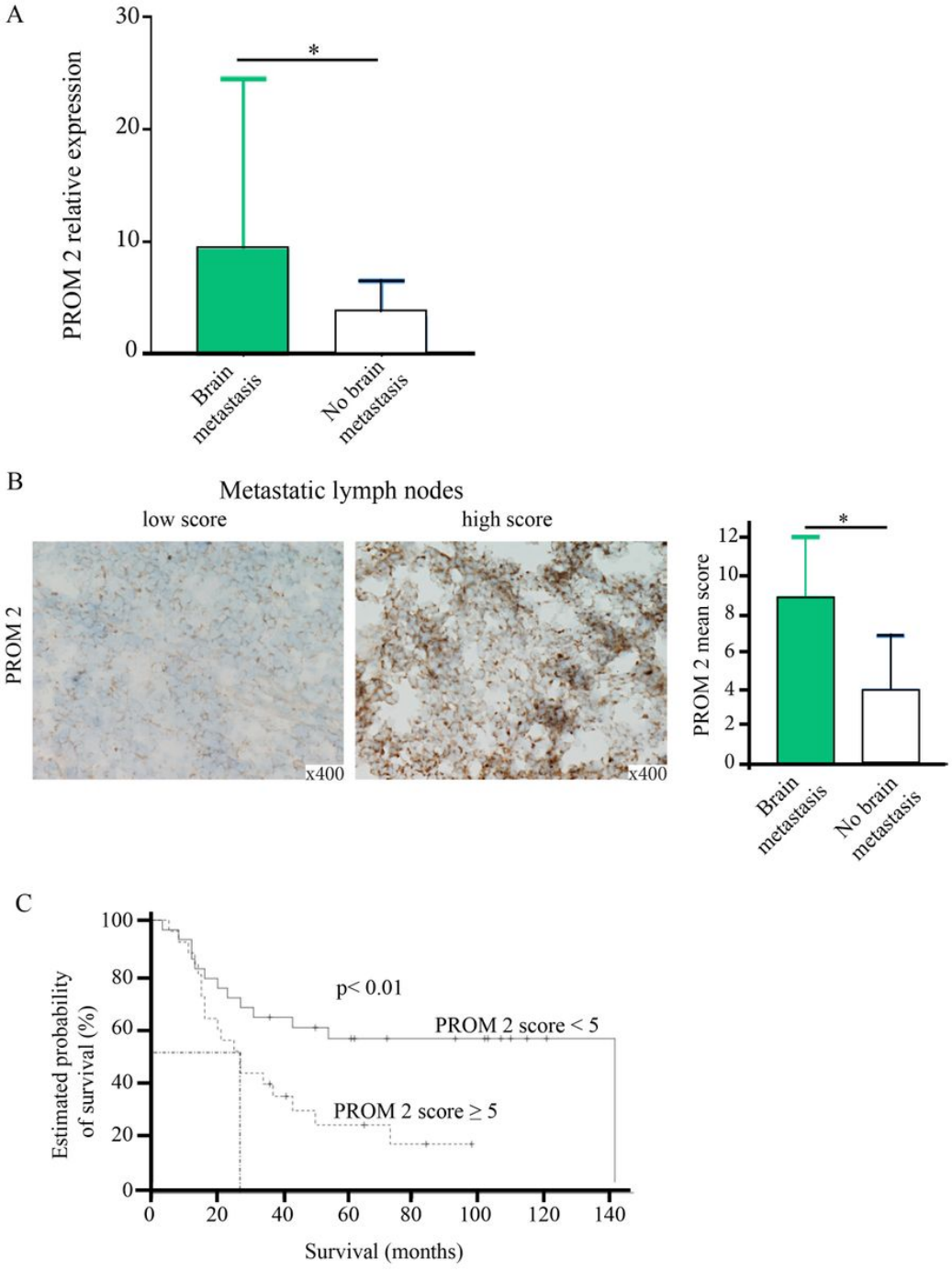


Figure 2

PROM2 mRNA expression, “PROM2 IHC score” in metastatic lymph nodes and survival data in the development cohort A. PROM2 mRNA expression is significantly higher in metastatic lymph nodes from patients with brain metastases than in those from patients without brain metastases ($p < 0.05$). B. Using immuno-staining on metastatic lymph nodes, the mean “PROM2 IHC score” is significantly higher among patients with brain metastases than among those without ($p<0.05$). C. Survival according to the “PROM2 IHC score” level in the development cohort of 51 patients. A “PROM2 IHC score” ≥ 5 is significantly associated with a shorter survival.

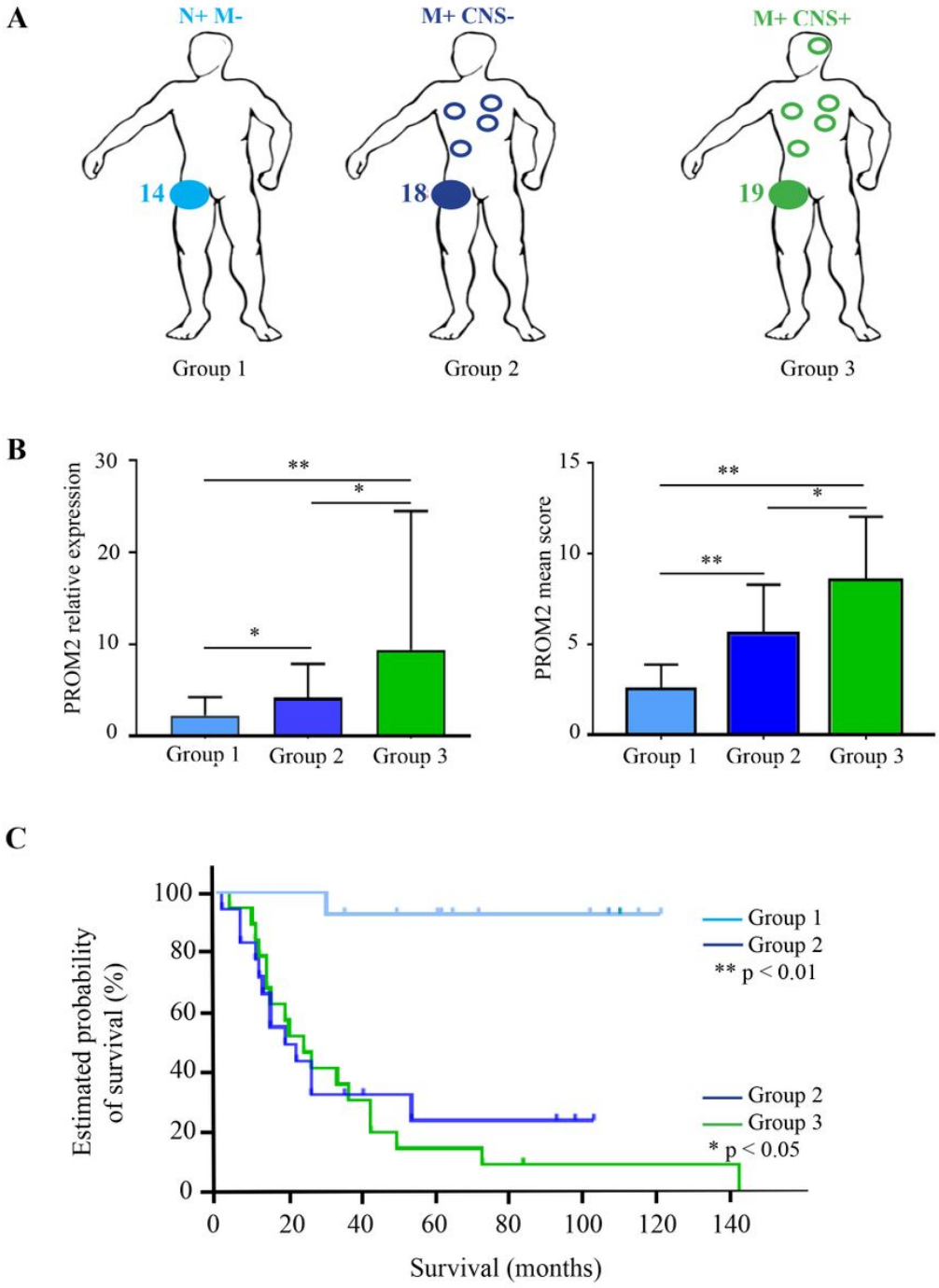


Figure 3

PROM2 expression and survival data according to sub-groups in the development cohort A. Group 1 includes patients with only regional lymph node metastases; Group 2 includes patients without brain metastases but with other distant metastatic localizations; Group 3 includes patients with brain metastases. B. PROM2 mRNA expression and PROM2 mean score in the 3 different groups. PROM2 mRNA expression and PROM2 mean score arere gradually and significantly different in the 3 groups. C. Survival curves according to the 3 different groups. *p<0.05, **p<0.01

Supplementary Files

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