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ORIGINAL ARTICLE

HLA-G expression in Merkel cell carcinoma and the correlation with Merkel cell polyomavirus infection

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Abstract

Merkel cell carcinoma (MCC) is a rare aggressive neuroendocrine cutaneous carcinoma with a high mortality rate. The MCC etiology is not fully understood. Merkel cell-associated polyomavirus (MCPyV) was found in MCC patients, indicating a risk factor for the tumor. Caucasian, elderly, and immunocompromised individuals are more likely to develop this tumor. HLA-G consists of a non-classical class I (Ib) HLA molecule with an immunoregulatory function and was associated with tumor escape in different types of tumors, nonetheless, never been studied in MCC. The purpose of this study was to evaluate the HLA-G expression and also to detect the MCPyV in MCC patients and correlate it with the clinical course of the disease. Forty-five MCC patients were included in a retrospective study. Formalin-fixed paraffin-embedded cutaneous skin biopsies were used by immunohistochemistry and RT-PCR to verify the HLA-G expression and MCPyV infection. HLA-G expression was found in 7 (15.6%), while the presence of MCPyV was detected in 28 (62.2%) of the studied patients. No significant association was found between HLA-G expression and MCPyV infection ($p=0.250$). The presence of MCPyV was associated with areas of low sunlight exposure ($p=0.042$) and the HLA-G expression with progression to death ($p=0.038$). HLA-G expression was detected in MCC patients, as well as the MCPyV presence was confirmed. These markers could represent factors with a possible impact on patient survival; however, further studies with a greater number of patients are needed, to better elucidate the possible role in disease progression.

Keywords HLA-G · MCPyV · Merkel cell carcinoma · HLA-G expression

Introduction

Merkel cell carcinoma (MCC) was first described in 1972, as a "trabecular carcinoma" (Toker 1972). It is a rare primary aggressive neuroendocrine cutaneous carcinoma that often spreads to lymph nodes and distal organs (Hanlon 2019). The mortality rate is around 50%, with a worse prognosis than melanoma (Albores-Saavedra et al. 2010; Becker et al. 2017; Mulchan et al. 2019). The MCC incidence has increased in recent decades due to the increase in life expectancy of the population, an increase in sunlight exposure, and immunosenescence (Emge and Cardones 2019). Caucasian immunocompromised individuals are more likely to develop the tumor and the manifestation occurs around the seventh decade of life (Reichgelt and Visser 2011; Uchôa et al. 2017; Neto et al. 2019). MCC often affects head and neck regions (50% of cases), areas with greater sunlight exposure, but it can also affect other parts of the body, such as extremities, trunk, genital, and oral mucosas (Neto et al. 2019). The

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clinical manifestation is described as painless, fast-growing, red-violet, dome-shaped cutaneous nodules (Uchôa et al. 2017; Schadendorf et al. 2017; Neto et al. 2019).

The MCC etiology is not fully understood. Feng et al. (2008) demonstrated the presence of a new type of Merkel cell-associated polyomavirus (MCPyV) in approximately 80% of MCC patients. Although virus DNA and antibody-MCPyV-specific responses are common in the healthy population (60% of the general population), indicating the previous exposition to the virus, patients with MCC have higher levels of antibodies directed against the MCPyV capsid than control subjects (Carter et al. 2009; Pastrana et al. 2009; Schowalter et al. 2010). However, MCC is an uncommon tumor, showing that the presence of the virus is not an exclusive factor for the manifestation of the disease (Robinson et al. 2019). Until now there is not been a consensus about the origin of MCC. Some studies show that the tumor does not originate from mature Merkel cells located at the base of the epidermis, because these cells represent a small portion of the cells, located in the main areas of skin cancer manifestation. Therefore, precursor cells, such as pro-B cells, mesenchymal stem cells, and epidermis progenitor cells, would be responsible to assume the Merkel cell differentiation during the neoplastic process (Harms et al. 2018).

Similar to other malignant tumors, MCC mortality occurs due to the uncontrolled growth of neoplastic cells in normal tissues, which develop specific mechanisms to subvert the host's immune response and escape immune surveillance (Abbas et al. 2018; Silva et al. 2018). Class I MHC (Major Histocompatibility Complex) downregulation is a frequent event for tumor progression. The lack of presentation of the tumor-derived peptide due to the altered expression of class I HLA (human leukocyte antigen) molecules contributes to the LTCD8 + (T Lymphocytes) non-recognition, making tumor clones proliferate and assume control over other cell populations and trigger the expression of immunosuppressive molecules, such as HLA-G (Tripathi and Agrawal 2006).

HLA-G consists of a non-classical class I (Ib) HLA molecule encoded in the MHC region (6p21.3) (Alegre et al. 2014). This molecule shows an immunoregulatory property that was initially recognized in maternal–fetal immune tolerance due to the expression in the trophoblast during pregnancy (Kapasi et al. 2000; Rajagopalan and Long 2012). Seven isoforms are known, four of them membrane-bound (mHLA-G1-G4) and three soluble isoforms (sHLA-G5-G7). Although HLA-G has a similar structure to the classic HLA class I molecules, the main function is to regulate innate and adaptive immune responses (Castelli et al. 2014). The immunoregulatory role occurs through the interaction between the HLA-G molecule and different inhibitory receptors, such as immunoglobulin-like transcript 2 (ILT2), ILT4, and killer immunoglobulin-like receptor 2 (KIR2DL4) present on cell surfaces (Colonna et al. 1998; Rajagopalan and Long 2012). The HLA-G binding with these receptors can trigger different immunological events such as apoptosis and blocking of the cytotoxic activity of LTCD8 + (Kapasi et al. 2000), inhibition of LTCD4 + proliferation (LeMaoult et al. 2004), inhibition of antigen presentation and differentiation of B lymphocytes (LB) (Carosella et al. 2008), modulation of the Natural Killer cells (NK) activity and Dendritic cells (DCs) (Liang et al. 2008), regulation of the T helper 1 (Th1)/Th2 cytokines profile (Liang et al. 2008) and induce of the expansion of regulatory T cells (Treg) (Selmani et al. 2008).

The first HLA-G association with malignant neoplasms was described in Melanoma (Paul et al. 1998). Since then, several studies involving mHLA-G and sHLA-G expression have been performed on different types of cancer such as lymphomas, leukemias, breast, kidney, bladder, ovary, lung, esophagus, and liver cancer (Yan 2011). As a result, HLA-G expression was detected in tumor lesions, and also in some cases, an increase in soluble levels was correlated with aggressiveness and worse tumor prognosis (Yan 2011).

In this way, the purpose of this study was to evaluate the HLA-G expression in the MCC to verify the possible impact of these molecules on the prognosis of the disease, once the immunosuppression is a characteristic present in MCC, and also to detect the MCPyV in these patients to correlate the virus infection with the clinical course of the disease.

Materials and methods

Patients

A retrospective study was carried out with 45 patients with MCC, from January 1997 to December 2018. The patients were diagnosed at Lauro de Souza Lima Institute (ILSL) in Bauru, São Paulo State, and at Amaral Carvalho Hospital (HAC) in Jau, São Paulo State.

The clinical parameters were collected from medical records (age at diagnosis, gender, tumor location, lesion size, staging at diagnosis, extent, angiolymphatic invasion, associated secondary phenotype, surgical margins, number of nodules, evolution time, local recurrence, skin metastases, distal metastases, status disease at the last follow-up, and death). The study included only patients who had paraffin samples from the primary tumor lesion.

The hematoxylin and eosin (HE)-stained slides were located in the respective involved institutions. All of them were reviewed by an experient pathologist, who defined the tumor area. New sections were obtained from formalin-fixed paraffin-embedded biopsies to perform the immunohistochemistry technique and DNA extraction. The Ethics Committees of ILSL (protocol no. 4.243.008) and HAC (protocol no.4.572.219) approved the study.

HLA-G expression

Skin biopsies embedded in paraffin blocks were used for in situ HLA-G detections by immunohistochemistry technique. Tissue Sects. (4 µm) were submitted to successive washes with xylol, ethanol (100%, 70%, and 50%), and water (MiliQ). Hydrogen peroxide (H_2O_2) 3% and methanol were used to block endogenous peroxidase, and the antigen retrieval was performed with citric acid (0.01 M pH 6.0) in flowing steam for 30 min. The samples were incubated for 30 min (37 ºC) and 24 h (4 ºC) with the primary anti-HLA-G monoclonal antibody (MEM-G/2 clone, Exbio, Prague Czech Republic) in a 1:100 dilution. This antibody is capable of recognizing the free heavy chain of all HLA-G isoforms. After this, a secondary biotinylated antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was added and then the reaction was amplified with the CSA kit (Dako, Carpinteria, CA) in 3 steps: (i) incubation with streptavidin and biotin–peroxidase, (ii) incubation with biotinylated tyramine, and (iii) streptavidin-peroxidase, interspersed with 3 washes in PBS. The revelation was done using H_2O_2 and 3,3-diaminobenzidine (DAB), counterstaining with Harris Hematoxylin. To validate the technique, positive controls were used from tissues that constitutively express HLA-G (placenta) and the negative controls were obtained by omitting the primary antibody. The images were captured using the Axiocam 234 HRc camera (Carl Zeiss, DE) attached to the Axiophot 2 photomicroscope (Carl Zeiss, Germany).

Polyomavirus associated with Merkel cell (MCPyV) detection

DNA extraction was performed from paraffin-embedded histological sections, with the previously delimited tumor tissue, carefully removed with a needle (40 × 12 mm). The GenElute FFPE DNA Purification Kit (Sigma-Aldrich) was used according to the manufacturer's instructions. The concentration and the purity of the nucleic acids were obtained in a NanoDrop TM 2000 spectrophotometer (Thermo Fisher Scientific).

MCPyV detection was performed by real-time polymerase chain reaction (RT-PCR) with the GreenMaster lowROX qPCR kit (Cellco Biotec). The sequence of primers used for amplification was forward 5′-CCCTTTGGA GCAAAT TCCA–3′ and the reverse 5′–CTGACCTCATCAAACATA GAGAA–3′ (Invitrogen). These primers amplify the t-small gene of the MCPyV (Arvia et al. 2017). The reactions were tested in duplicate with Viia7 Real-time equipment (Applied Biosystems). The cycling conditions were 10 min at 95 ºC, followed by 45 cycles of 30 s at 90 ℃, 30 s at 60 ℃, and 60 s at 72 ℃. Fluorescence was detected after every 72 ℃ extension. The melting curve (Tm) was determined at 76.8 ℃. The primer sensitivity was defined by the detection curve in quadruplicate (negative control and serial dilutions of the positive control at concentrations of 100 ng/µL, 10 ng/µL, 1 ng/µL, 0.1 ng/µL, 0.01 ng/µL, and 0.001 ng/µL). The amplifications were detected in the four samples with the highest concentration (Cycle Threshold (CT) values: 29.5, 32.4, 35.55, and 38.67); therefore, samples with DNA concentrations below 0.01 ng/µL were considered negative. The DNA suppression in the reaction was the negative control, while the positive control, a sample of MCC previously diagnosed and confirmed by sequencing was used. The control of DNA extraction was made by amplification of endogenous control with the Beta-Actin gene.

Statistical analysis

The HLA-G expression and the MCPyV presence were performed by chi-square test. The comparison between these markers (HLA-G and MCPyV) with the clinical parameters was submitted to nonparametric Kruskal–Wallis analysis, with Dunn's post-test using the jamovi software (https://www.jamovi.org). The Kaplan–Meier test was used to compare the survival curve between the different studied groups with GraphPad Prism 7.04 software (GraphPad Software, Inc., CA, USA). *P* value <0.05 was considered significant.

Results

Considering the 45 patients, the age at diagnosis ranged from 51 to 95 years old (median = 77), 24 (53.3%) individuals were female, with the ranged age from 61 to 95 years old (median = 80), while 21 (46.7%) were males with ranged age from 51 to 90 years old (median = 70), the evolution time of tumor ranged from 1 to 161 months since the diagnosis (median = 24 months).

The lesions were grouped according to sunlight exposure areas: 23 (51.2%) patients had a high sunlight exposure area and 22 (48.8%) had a low sunlight exposure area. Fourteen (34.1%) patients had a local recurrence, 13 (32.5%) had skin metastases, and 11 (27.5%) had distal metastases, 30 (66.7%) had active metastatic disease in the last follow-up, and 29 (65.9%) had MCC as a registered cause of death. HLA-G expression was found in 7 (15.6%) of the studied patients (Figs. 1 and 2), while the MCPyV was detected in 28 (62.2%) patients (Table 1). This association was not significant ($p = 0.250$) (Table 2).

The clinical parameters analysis showed a significant association between low sunlight exposure areas and the MCPyV presence ($p = 0.042$). The MCPyV was found in 17 (77.27%) of the total studied samples. HLA-G expression was correlated with death progression ($p = 0.038$) (Table 3).

Survival analysis showed that HLA-G and MCPyV negative patients had a survival median of 57 months

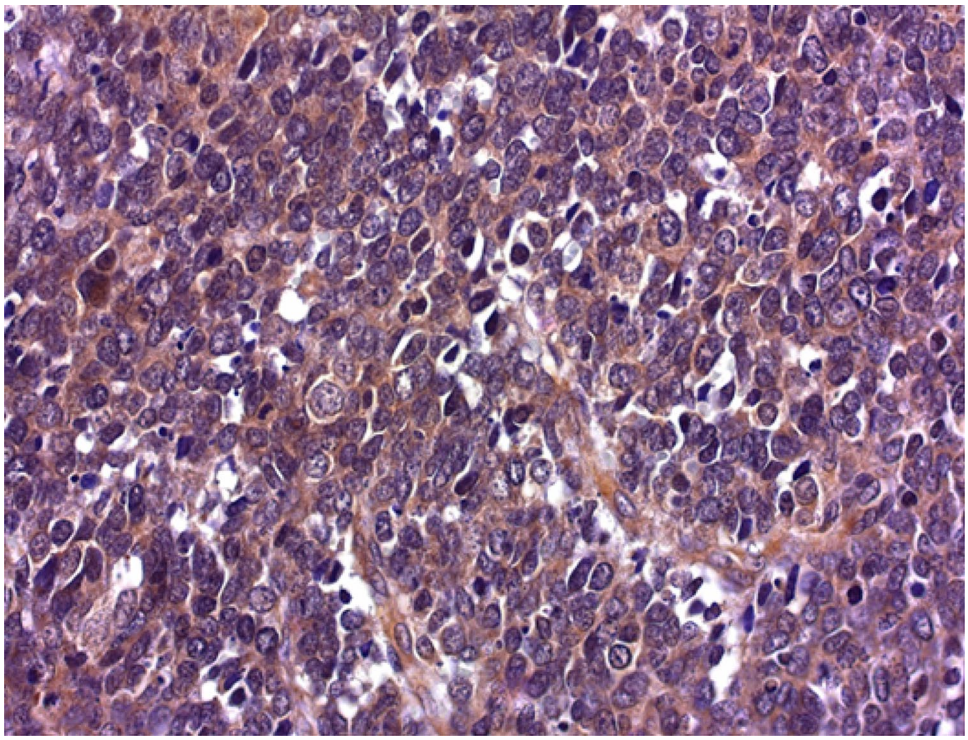


Fig. 1 HLA-G molecule expression in tumoral tissue from patients with MCC. Skin biopsies were analyzed by immunohistochemistry using the anti-HLA-G monoclonal antibody (MEM-G/2 clone, Exbio, Prague Czech Republic) in a 1:100 dilution

(median = 59), while the HLA-G and/or MCPyV-positive group had a survival median of 29.18 months (median = 22.5). However, when these results were analyzed (HLA-G and MCPyV), no significant differences were found ($p = 0.174$; HR = 1.64 and $p = 0.09$; HR 1.99) (Fig. 3a, b). HLA-G expression and tumor staging also did not reveal any significant association ($p = 0.436$), as well as the presence of MCPyV ($p = 0.756$).

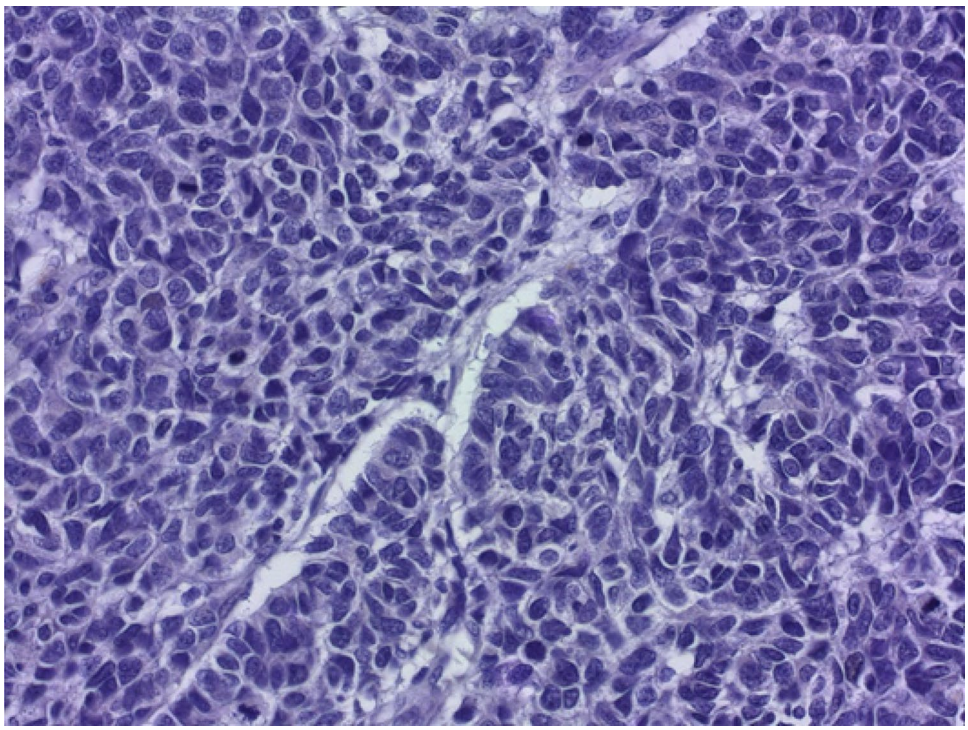


Fig. 2 Absence of HLA-G molecule expression in tumoral tissue from patients with MCC. Skin biopsies were analyzed by immunohistochemistry using the anti-HLA-G monoclonal antibody (MEM-G/2 clone, Exbio, Prague Czech Republic) in a 1:100 dilution

Table 1 Clinical and demographic characteristics of the 45 MCC patients

Clinical and demographic characteristics					
Age (51 a 95 years old)	***N*=45**		**Cancer staging**	***N*=36**	*%*
Mean	76.3		CS-I	11	30.6
Median ± SD	77±10.40		CS-II	7	19.4
Male median (51–90)	70		CS-IIA	3	8.3
Female median (61–95)	80				
Gender	***N*=45**	*%*	CS-III	5	13.9
Female	24	53.3	CS-IIIA	2	5.6
Male	21	46.7	CS-IIIB	1	2.8
Evolution time (1–161 months)			CS-IV	5	13.9
Mean	37.2		CS-X	2	5.6
Median	24±34.90		**Compromised surgical margins**	16/45	39
Last segment	***N*=45**	*%*	**Local recurrence**	14/45	34.1
Metastatic disease presence	30	66.7	**Metastases**	***N*=45**	*%*
No evidence of disease	13	28.9	Cutaneous	13	32.5
Not specified	2	4.4	Distal	11	27.5
			No metastasis	21	40
Primary lesion location area	***N*=45**	*%*	**Secondary neoplasms**	15/45	11.11
High sun exposure	23	51.2			
Low sun exposure	22	48.8	**Death**	29/45	65.9
HLA-G	***N*=45**	*%*	**MCPyV**	***N*=45**	*%*
HLA-G-negative	38	84.4	MCPyV-positive	28	62.2
HLA-G-positive	7	15.6	MCPyV-negative	17	37.8

SD standard deviation, *N* number of patients, *%* frequency, *CS* cancer staging, *MCPyV* Merkel cell-associated polyomavirus

Discussion

The present study evaluated the HLA-G expression in MCC patients to correlate the expression of this molecule with the clinical evolution of the neoplasm. Studies of HLA-G expression are already found with other types of tumors and in MCC have never been studied (Paul et al. 1998; Dellambra et al. 2021). The MCPyV infection, a virus strongly associated with the oncogenic process of the MCC, is considered a predisposing factor for the development of the disease and was also analyzed, to verify the possible correlation between these two variables (Robinson et al. 2019).

Table 2 Comparison between the HLA-G expression and the MCPyV presence in the 45 patients with MCC

HLA-G	**MCPyV**		*p*
	N (%)	*N* (%)	
	Negative	**Positive**	
Negative	13 (34.2)	25 (65.8)	
Positive	04 (57.1)	03 (42.9)	
Total	17(37.8)	28 (62.2)	0.250

N number of patients, *%* frequency, *p* chi-square ($p \leq 0.05$); *MCPyV* Merkel cell-associated polyomavirus

Although studies indicate that the frequency of MCC is higher in males, in our study, MCC was more frequent in women, of more advanced age. This characteristic has also been evidenced in other studies conducted in Brazil and can be justified by the fact that aging is a predisposing factor to the disease, the older, the greater the chance of disease manifestation, and women tend to live longer than men (Álvarez-Argüelles et al. 2017; Uchôa et al. 2017; Neto et al. 2019; Dellambra et al. 2021).

HLA-G expression was observed in only 15.6% of MCC patients, a similar study evaluated the presence of this same marker in melanoma and showed that 30% of patients expressed HLA-G. In the same way, 41% of gastrointestinal tumor patients were positive for this marker (Rebmann et al. 2007). Farjadian et al. (2018) evaluated the HLA-G expression in colorectal tumors and found 9% of positivity in the analyzed samples. This variation suggests that in addition to HLA-G expression, other escape mechanisms from neoplastic cells may be involved in tumor progression. The HLA-G expression is, therefore, variable among the different tumors already studied (Yan 2011). In melanoma, the maintenance or loss of expression of this molecule seems to be directly involved with the conditions of the tumor environment such as hypoxia, inflammation, stress, and hormones, since serial samples of metastases originating from positive tumors

Table 3 HLA-G expression and MCPyV presence according to the parameters: sunlight exposure, lesion extension, secondary phenotype, gender, age, angio-lymphocytic invasion, margin involvement, skin metastases, distal metastases, secondary neoplasms, deaths, local recurrence, and evolution time

MCPyV						**HLA-G**				
	Positive		**Negative**			**Positive**		**Negative**		
	N	***%***	***N***	***%***	***p***	***N***	***%***	***N***	***%***	***p***
Sunlight exposure					****0.044***					0.731
Yes	11	24.40	12	26.70		04	8.90	19	42.20	
No*	***17***	***37.80***	***05***	***11.10***		03	6.70	19	42.20	
Lesion extension	08	17.77	04	8.88	0.225	02	4.44	11	24.44	0.743
Secondary phenotype	01	2.22	00	-	0.427	00	-	00	-	0.664
Gender	*F* = 16	35.55	*F* = 6	13.33	0.516	*F* = 4	8.88	*F* = 20	44.44	0.828
	M = 12	26.66	*M* = 7	15.55		*M* = 3	6.66	*M* = 18	40.00	
Age	75.5	-	77	-	0.400	80	-	75,5	-	0.229
Angio lymphocytic invasion	07	15.55	03	6.66	0.681	01	2.22	09	20.00	0.530
Margin involvement	10	22.22	06	13.33	0.684	03	6.66	13	28.88	0.556
Skin metastases	07	15.55	06	13.33	0.749	04	8.88	09	20.00	0.130
Distal metastases	08	17.77	03	6.66	0.236	01	2.22	10	22.22	0.395
Secondary neoplasms	10	22.22	05	11.11	0.677	02	4.44	13	28.88	0.596
Deaths					0.895					****0.040***
Yes*	18	67.00	11	64.70		***07***	***15.55***	**22**	**59.50**	
No	09	33.00	06	35.30		00	-	15	40.50	
Local recurrence	08	17.77	06	13.33	0.897	02	4.44	12	26.60	0.736
Evolution time	22.50	-	59	-	0.069	25	-	23.50	-	0.938

N number of patients, % frequency, *p* Kruskal–Wallis (p value ≤ 0.05), *MCPyV* Merkel cell-associated polyomavirus

*significant result

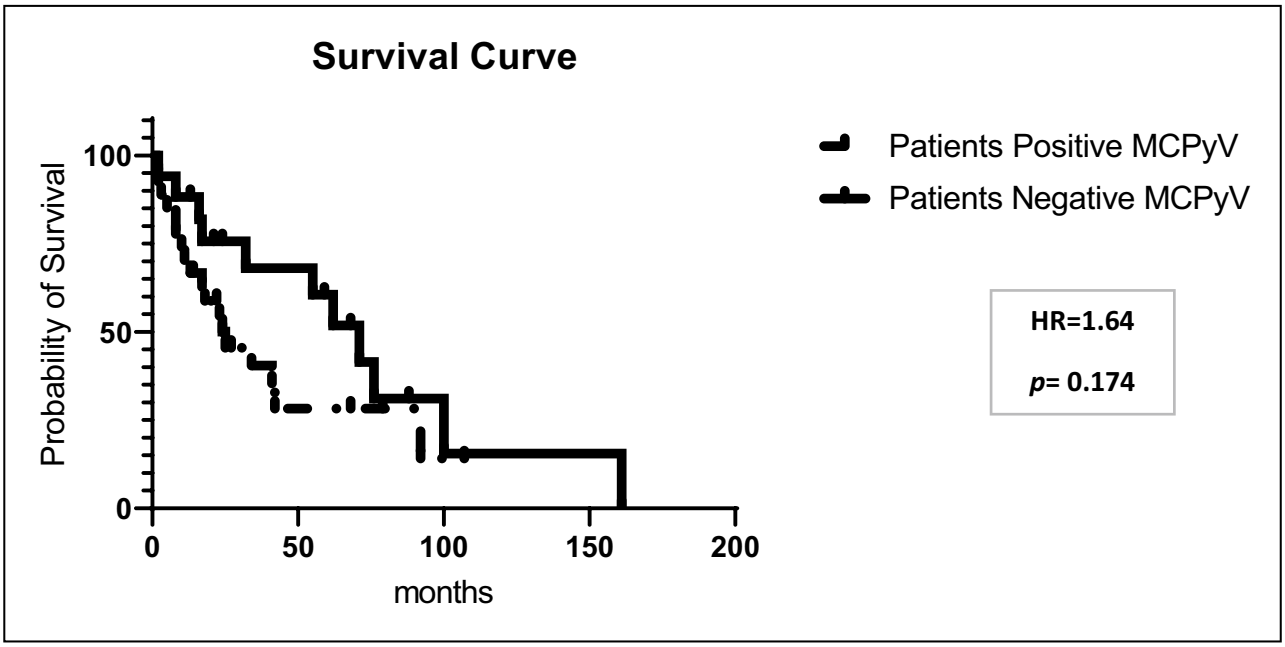
maintain the expression of the HLA-G (Paul et al. 1999; Rebmann et al. 2007; Farjadian et al. 2018; Liu et al. 2020). The immune response inhibition occurs in multiple ways, mainly in the elimination phase, when the immune system attacks the tumor. The HLA-G expression can inhibit the LT, LB proliferation, NK cells, and phagocytic activity of neutrophils. Pro-inflammatory cytokines such as IFN-gamma can increase the HLA-G expression, as well as the immunosuppressive IL-10 cytokine (Rouas-Freiss et al. 2014). These different pathways can induce tumor tolerance, favor tumor enlargement, and disseminate to healthy tissues, even in immunocompetent individuals (Loumagne et al. 2014).

A significant association between the MCPyV virus presence and HLA-G expression in MCC patients was not demonstrated in this study. The correlation of HLA-G expression and the positivity of *Helicobacter pylori* bacteria was also reported by Farjadian et al. in gastrointestinal tumors, which did not find a significant association (Farjadian et al. 2018).

HLA-G positivity was correlated with the patient's death ($p = 0.04$). Seven patients were HLA-G-positive, of these, five patients had metastatic disease (MD) status at the last date of follow-up. The HLA-G expression in tumor tissue and the absence of this expression in adjacent healthy tissues is indicative of the harmful effect of HLA-G on tumor escape, mainly due to the ability to inhibit the immune response through immunosuppressive mechanisms, with a worse prognosis of the disease (Paul et al. 1999; Yan et al. 2011; Farjadian et al. 2018; Liu et al. 2020). In colorectal tumors, the presence of soluble HLA-G was detected and was correlated with a worse prognosis and more advanced staging of the disease. This fact makes it possible to compare malignant and normal transformations, suggesting the participation of this molecule as a laboratory marker (Dhouioui et al. 2022). The host's immune activity is decisive for the progression of the disease. Some cases of MCC regression tumor have been described in the literature; however, the mechanisms have not been elucidated yet, and so far, tumor regression is associated with immune stimulation, which can be triggered by the inflammatory process that occurs after the excision of the lesion (Dellambra et al. 2021).

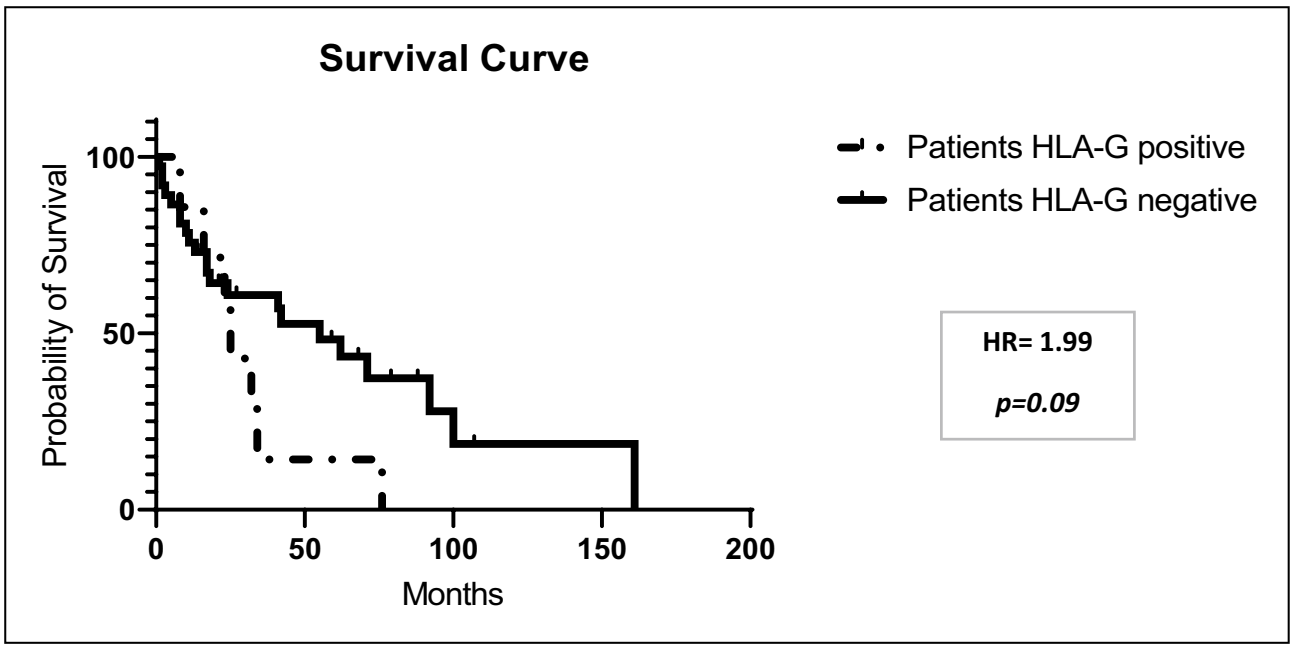
MCPyV infection was detected in 62.2% of the MCC samples. Costa et al. (2021), in a study also conducted in Brazil, found a similar value (65%), reinforcing the prevalence of the virus in the manifestation of this neoplasm in the Brazilian population. When the location of lesions and frequency of exposure was taken into account, MCPyV was found in 77.27% of low sunlight exposure patients. This information corroborates with previous studies that

(a) Survival analysis between 45 MCPyV positives (28) and negative (17) MCC patients.



MCPyV: Merkel cell-associated polyomavirus; HR: Hazard Ratio and p-value: Kaplan-Meier test.

(b) Survival analysis between 45 HLA-G positives (7) and negative (38) MCC patients.



HLA-G: Histocompatibility Leukocyte Antigen-G; HR: Hazard Ratio and p-value: Kaplan-Meier test.

Fig. 3 **a** Survival analysis [LGA1] between 45 MCPyV positives(28) and negative (17) MCC patients. MCPyV Merkel cell-associated polyomavirus, HR hazard ratio and *p* value: Kaplan-Meier test. **b** Survival analysis between 45 HLA-G positives (7) and negative (38) MCCpatients. HLA-Ghistocompatibility leukocyte antigen-G, HR hazard ratio and *p* value: Kaplan-Meier test

demonstrate viral DNA integration as a risk factor for the oncogenic process of MCC (Feng et al. 2008; Costa et al. 2021). Hesbacher et al. (2016) demonstrated that the MCPyV when integrated with the host DNA induces the expression of the oncogenes. Viral oncogenesis, mediated by MCPyV large and small antigens, can inhibit the retinoblastoma suppressor protein (RB1), triggering cell proliferation in the MCC (Hesbacher et al. 2016; Emge and Cardones 2019; Neto et al. 2019). This mechanism is specific but not exclusive to the development of the disease (Hesbacher et al. 2016). In MCPyV-negative patients, chronic exposure to UV rays is believed to be responsible to cause cumulative DNA damage, resulting in several genetic changes in the skin cells throughout life and consequently mutations that can trigger the oncogenic process (Silva et al. 2018). Therefore, the location of the lesions seems to confirm two distinct oncogenic processes, one arising from the viral DNA incorporation into the human genome, activating oncogenic pathways, and the other from exposure to solar radiation (Hesbacher et al. 2016).

Patient survival after diagnosis ranged from 1 to 161 months (median = 24 months). Oncological staging, in our study, did not influence the progression of the disease and HLA-G expression. This fact can be verified through the survival curves of positive and negative patients for both factors (MCPyV and HLA-G) (data not shown). The patient's immunological competence is considered a decisive factor for metastatic dissemination and low survival after diagnosis, due to immune surveillance mechanisms (Paul et al. 1998). Although our results were not statistically significant, we observed that patients who were negative for both variables (MCPyV and HLA-G) had a median survival of 57 months, while patients who were positive for MCPyV and/or HLA-G had shorter survival (median = 29 months), suggesting possible participation in the manifestation of MCC. Maybe if the number of patients was higher, we could find a significant result.

In summary, although the HLA-G expression was detected in a small number of MCC patients, these finds could suggest the participation of this molecule as an inducer of more aggressive solid tumors and in metastatic dissemination. The MCPyV presence in low sunlight exposure areas patients suggested MCPyV infection as a risk factor for MCC. Both these markers (HLA-G and MCPyV) represent factors with a possible impact on patient survival; however, further studies with a greater number of patients are needed, as well as analyzes regarding the maintenance of HLA-G expression in metastases, to better elucidate the role of the molecule in disease progression.

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Declarations

Conflict of interest The authors declare no competing interests.

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