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Evaluation of HLA-G immune checkpoint molecule in melanocytic lesions

Avaliação da molécula de checkpoint *imunológico HLA-G em lesões melanocíticas*

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ABSTRACT

The human leukocyte antigen-G (HLA-G) is a non-classical molecule of the major histocompatibility complex. HLA-G has been associated with the process of tumorigenesis and tumor escape. In this study, we aim to evaluate the HLA-G expression in melanocytic lesions and in melanoma for determining when melanocytic lesions start its expression. Twenty-two skin biopsies samples were submitted to immunohistochemistry; HLA-G expression was detected in 63.6% of the samples. This expression in melanocytic cells was significantly higher in melanoma than in benign melanocytic lesions ($p < 0.002$). Our results suggest that HLA-G expression starts late in the process of tumorigenesis.

Key words: melanoma; HLA-G antigens; immunohistochemistry.

RESUMO

*O antígeno leucocitário humano G (HLA-G) é uma molécula não clássica do complexo principal de histocompatibilidade que tem sido associada ao processo de tumorigênese e escape tumoral. Neste estudo, objetivamos avaliar a expressão de HLA-G em lesões melanocíticas e no melanoma para determinar quando as lesões melanocíticas iniciam sua expressão. Vinte e duas amostras de biópsias de pele foram submetidas à imuno-histoquímica; a expressão de HLA-G foi observada em 63,6% das amostras. Essa expressão nas células melanocíticas foi significativamente maior no melanoma do que em lesões melanocíticas benignas (*p *< 0,002). Nossos resultados sugerem que a expressão de HLA-G se inicia tardiamente no processo da tumorigênese.*

Unitermos: *melanoma; antígenos HLA-G; imuno-histoquímica.*

RESUMEN

*El antígeno leucocitario humano G (HLA-G) es una molécula no clásica del complejo principal de histocompatibilidad que ha sido asociada al proceso de tumorigénesis y escape tumoral. En este estudio, nuestro objetivo es evaluar la expresión de HLA-G en lesiones melanocíticas y en el melanoma para determinar cuando las lesiones melanocíticas comienzan su expresión. Veintidós muestras de biopsias de piel se estudiaron mediante inmunohistoquímica; se detectó la expresión de HLA-G en el 63,6% de las muestras. Esa expresión en las células melanocíticas fue significativamente mayor en el melanoma que en lesiones melanocíticas benignas (*p *< 0,002). Nuestros resultados sugieren que la expresión de HLA-G empieza tardíamente en el proceso de tumorigénesis.*

Palabras clave: *melanoma; antígenos HLA-G; inmunohistoquímica.*

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INTRODUCTION

Melanoma is an aggressive tumor, accounting for most of the deaths caused by skin cancer. Although surgical excision is effective for the treatment of primary cutaneous melanoma, metastatic melanoma presents a high mortality rate. Standard chemotherapy has a limited effect in these cases[1].

However, in the last decades there has been a revolution in the treatment of melanoma. Studies on the molecular mechanisms of this disease and cancer immunology have been performed. Currently, two new classes of systemic treatments are available: immunotherapy and targeted therapy[2-4].

Immunotherapeutics target immune molecule checkpoints. However, the response to treatment is not uniform and is directly influenced by the tumor microenvironment. It is fundamental to know the infiltrate of immunocompetent cells in the tumor, as well as the factors and molecules produced by them[5].

Most cancers produce several immunosuppressive factors that allow the immune response to be avoided. Among the mechanisms of immune escape of tumor cells, we can mention the expression of the human leukocyte antigen-G (HLA-G) molecule. HLA-G is distinguished from other HLA class I molecules, mainly because it: has a limited gene polymorphism; undergoes systematic alternative recombination of the primary transcript; presents tissue protein expression restricted to the placenta (invasive cytotrophoblast and amniotic epithelial cells) and certain adult tissues (thymus, pancreas, proximal nail matrix, and cornea) and erythroblasts; presents immune tolerance functions[6].

Notably, the HLA-G molecule expression can be observed ectopically under pathological conditions. HLA-G expression in tumors is capable to protect cancer cells from natural killer (NK) cells and cytotoxic T lymphocytes. Thus, HLA-G expression becomes an important mechanism in which cancer cells are used to escape host immune surveillance. It is now widely accepted that HLA-G is a critical marker of immunotolerance in cancer cell immune evasion and is strongly associated with disease progress and prognosis for cancer patients[7]. HLA-G is considered an immune checkpoint and its heterogeneity in cancers have been related to disease stage and outcomes, metastatic status and response to different therapies[8].

In this context, our aim was to evaluate the HLA-G molecule expression in melanocytic lesions and in melanoma for determining when melanocytic nevus evolution begins its expression.

METHODOLOGY

Samples

This study was approved by the Institutional Ethics Committee for Human Subjects (protocol 419.570). Medical records were reviewed to determine age and gender. These studies were performed at the Preventing Health System, Goiânia, Goiás, Brazil.

The samples were obtained after surgical resection. For this study, 22 melanocytic lesions of different histological subtypes (acral melanoma, extensive superficial melanoma, lentigo maligna melanoma, nodular melanoma, and melanocytic nevus) from 16 patients were evaluated.

Immunohistochemistry

Sections from paraffin-embedded specimens were immunostained with MEM-G/02 monoclonal antibody (1/100) that recognizes the free heavy chain of all HLA-G isoforms (Exbio, Praha, Czech Republic). Invasive cytotrophoblast from the third-trimester human placenta served as a positive HLA-G protein control. The reaction product was visualized using the Ultratech HRP Streptavidin-Biotin Universal Detection System (Immunotech-Coulter, Villepinte, France) according to the manufacturer's recommendations. Ten skin biopsies of healthy women who had undergone breast reduction surgery were used as normal controls.

Evaluation of stained sections

Immunoreactivity was scored by evaluating the percentage of positive cells, using a semi-quantitative scoring method. The scoring system was performed as previously described by Gonçalves *et al*. (2015)[9]. Two independent pathologists (E. L. Z. and B. W. K.) interpreted the HLA-G staining results.

Statistical analysis

The immunostaining scores were compared to the Mann-Whitney *U* test. Significance was defined as a $p < 0.05$ at a confidence interval of 95%. All statistical analyses were performed using the RStudio front-end 0.98.507 software.

RESULTS

The immunohistochemical staining revealed that HLA-G+ cells revealed a cytoplasmic brown-stained pattern. This staining

was also observed in immune-inflammatory cells. From the 22 samples subjected to immunohistochemistry, 14 expressed the HLA-G, whereas no control sample expressed its. The expression in the melanocytic lesions and melanoma is shown in detail in **Table**. HLA-G antigen expression in melanocytic cells was significantly higher in melanoma than in nevi ($p < 0.002$). Female samples presented higher staining intensity than males ($p < 0.001$).

TABLE – Expression of HLA-G in melanocytic and melanoma lesions

Immunohistochemistry	HLA-G positive *n* (%)	HLA-G negative *n* (%)	Total *n* (%)
Patients	11 (68.75)	5 (31.25)	16 (100)
Female	5 (71.43)	2 (28.57)	7 (43.75)
Male	6 (66.67)	3 (33.33)	9 (56.25)
Lesions	14 (63.64)	8 (36.36)	22 (100)
Acral melanoma	1 (100)	0	1 (4.54)
Superficial spreading melanoma	4 (80)	1 (20)	5 (100)
Lentigo maligna melanoma	2 (50)	2 (50)	4 (100)
Nodular melanoma	2 (100)	0	2 (100)
Melanocytic nevus	5 (50)	5 (50)	10 (100)

HLA-G: human leukocyte antigen-G.

DISCUSSION

In this study, lesions characterizing the evolution of melanocytic lesions changes during melanoma development were evaluated. We observed the expression of HLA-G in more differentiated progression rates of melanocytic lesions, which corroborates with previous studies demonstrating the expression of HLA-G in melanoma.

Positivity for HLA-G in benign lesions suggests vigilance over melanocytic nevi. About 30% of melanomas are derived from benign melanocytic lesions. In a retrospective study involving 850 cutaneous patients, Lin *et al*. (2015)[10], demonstrated that 235 patients presented melanoma associated with a melanocytic nevus.

Histological types of superficial extensive melanoma and acral lentiginous melanoma expressed HLA-G, suggesting that HLA-G increasing is associated with malignant transformation in this type of cell, since HLA-G is often expressed in tumor cells, and its expression is associated with unfavorable prognosis in various malignancies[11].

CONCLUSION

Our results suggest that the HLA-G expression begins at late-stage of the tumorigenesis process. HLA-G could allow prediction of malignancy potential and affords new insights on how to improve the targeting of immunotherapies.

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