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Differential Expression of Cancer Testis Antigens on Lentigo Maligna and Lentigo Maligna Melanoma

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Abstract: The cancer/testis antigens (CTA) are a group of antigens expressed on germ cells of healthy testis and malignant tumors. We studied whether CTA are present on lentigo maligna (LM) and LM melanoma (LMM) samples. Immunohistochemical expression of a panel of CTA (MAGE-A1, A2- A3, NY-ESO-1, PRAME, SSX-2, and a MAGE-A antibody reactive with -A1, -A2, -A3, -A4, -A6, -A10, and -A12) was investigated in formalin-fixed paraffin-embedded samples from LMM (n = 20), LM (n = 8), chronically sun-exposed skin (n = 7), and healthy skin (n = 7). In 4 LMM lesions, the MAGE-A marker was positive. Another 3 LMM lesions were positive for MAGE-A1, MAGE-A2, and MAGE-A3. PRAME was positive in 18/20 LMM and 6/8 LM. We did not find expression of MAGE, NY-ESO-1, or SSX-2 in LM, thereby excluding these CTA as diagnostic markers to discern malignant melanocytes in LM from normal melanocytes. LMM did express MAGE, NY-ESO-1, and SSX-2. If a biopsy from a lesion suspect for LM shows positivity for MAGE, NY-ESO-1, and SSX-2, the lesion may actually be LMM. In contrast, PRAME expression was found in LM at low levels and in LMM at much higher levels, and absent in normal melanocytes. PRAME can potentially be used to discern normal melanocytes from malignant melanocytes.

Key Words: cancer/testis antigen, MAGE, lentigo maligna, lentigo maligna melanoma, melanoma in situ, diagnosis

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INTRODUCTION

Cancer/testis antigens (CTA) are a group of antigens expressed by germ cells of healthy testis and by malignant tumors of different histological origin, including cutaneous melanoma. This selective expression pattern qualifies CTA as diagnostic and prognostic tumor markers and makes CTA key candidate targets for immunotherapy.[1] Lentigo maligna (LM) is considered to be a variant of melanoma in situ and a precursor of LM melanoma (LMM).[2] Classically, 4 types of cutaneous melanoma are discriminated, namely superficial spreading melanoma (SMM), nodular melanoma (NM), LMM, and acrolentiginous melanoma.[3] The "divergent pathways" model, postulated in 2003, differentiates melanoma associated with chronic sun damage from melanoma arising in intermittently sun-exposed skin.[4] It has been shown by Stadelmeyer et al[5] that LM more often have BRAFV600K mutations, which are associated to chronic sun damage, than SSM and NM, which are more associated to intermittent sun damage. Based on these facts, LM may warrant a different management approach. Therefore, this study aims to identify phenotypic differences between LM, LMM, and normal melanocytes, that could aid in the diagnosis of LM and LMM.

Currently, the diagnosis of LM is based on the histological presence of melanocytes proliferating along the basal layer of the epidermis. However, a specific marker to discriminate malignant melanocytes from normal melanocytes is lacking.[6]

This study aims to investigate whether CTA are present on LM and LM melanoma (LMM), and to evaluate whether CTA can be used to discern malignant melanocytes in LM from normal melanocytes.

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The authors declare no conflicts of interest.

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MATERIALS AND METHODS

Formalin-fixed paraffin-embedded tissue sections of LMM (n = 20), LM (n = 8), chronically sun-exposed skin (n = 7), and healthy skin (n = 7) were kindly provided by the department of Pathology at the Amsterdam University Medical Centers, VU Medical Center, Amsterdam, the Netherlands. This study was approved by the biobank ethical committee of the VU Medical Center. LM was defined as atypical melanocytes, singly and in nests, usually confined to the basal layer with little pagetoid invasion of the epidermis. LMM was defined as LM with an invasive component composed of spindled melanocytes or epitheloid melanocytes with variable cytological atypia, nuclear pleomorphism, and tumor giant cells, as described by Patterson et al[7] Tissue sections were deparaffinized in xylene and rehydrated by serial passage through graded ethanols. Heat-induced antigen retrieval was performed for 20 minutes at 98°C in TrisEDTA pH9.0 buffer. Antibodies used included anti-MAGE-A mAb 6C1, reactive with MAGE-A1, -A2, -A3, -A4, -A6, -A10, and -A12 proteins, anti-MAGE-A1 mAb MA454, polyclonal anti-MAGE-A2 Ab, polyclonal anti-MAGE-A3 Ab, anti-SSX2

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mAb CL3202 (all obtained from Thermo Fisher Scientific, Waltham, MA), anti-NY-ESO-1 mAb E978; (Santa Cruz Biotechnology, Dallas, TX), and anti-PRAME mAb EPR20330 (Abcam, Cambridge, United Kingdom). Next, tissue sections were incubated with either Poly-AP antimouse or Poly-AP antirabbit (Immunologic) and visualized with Perma Red/AP (Diagnostic Biosystems, Pleasanton, CA). Tissue sections were counterstained with hematoxylin (Sigma Aldrich, St. Louis, MO). Coverslips were mounted using Pertex (VWR International, Radnor, PA). Images were taken with Olympus Cell Sens software (Olympus) (Fig. 1).

RESULTS

MAGE-A showed a nuclear and cytoplasmic staining pattern with no background staining. Four of 20 (20%) LMM stained positive with the anti-MAGE-A antibody. MAGE-A1 was expressed by 50% of tumor cells in 1 LMM sample, whereas in 3 LMM samples MAGE-A1 expression was limited to 5% of tumor cells. Three different LMM lesions expressed MAGE-A1, MAGE-A2, and MAGE-A3 in less than 5% of the tumor tissue. PRAME showed a nuclear and membranous staining pattern and no background staining. PRAME expression was seen in 18 of 20 (90%) LMM. Of these 18 positive samples, 14 showed expression in 90%–100% of the tumor cells, whereas the other 4 showed positive expression in 1%–50% of the tumor cells. We did not find expression of NY-ESO-1 or SSX-2 in LMM. In the LM group, 6 of 8 (75%) LM showed expression of PRAME at low levels. In one of these LM tissues, PRAME was expressed in 20%–30% of the tumor cells, whereas 5 other LM showed PRAME expression in <1% of tumor cells. No expression of MAGE-A, MAGE-A1, -A2, -A3, NY-ESO-1, or SSX-2 was seen in LM. Sun-exposed skin did not show any positive staining for CTAs (MAGE, NY-ESO-1, SSX-2 or PRAME). One of 7 normal skin tissues showed positive expression of PRAME in <1% of melanocytes with a similar staining pattern as in LM and LMM. The results are summarized in Table 1.

DISCUSSION

LM is considered a melanoma in situ and CTA expression can be a feature of malignant tumors. We did not observe any MAGE-A1, -A2, -A3, -A4, -A10, -A12, NY-ESO-1, or SSX-2 expression, but we did observe PRAME expression in LM, albeit at lower levels compared with LMM. This is concurrent with the hypothesis that LM is the precursor of LMM, and perhaps the premalignant stage before LMM.

We demonstrated expression of MAGE-A1, MAGE-A2, and MAGE-A3 antigen on LMM. In our systematic review cutaneous melanoma was described to express MAGE-A1 protein in 7.5%–30% of 659 primary tumors and MAGE-A3 protein was expressed in 15%–37% of 254 primary tumors. In comparison, LMM seems to have a lower expression rate of MAGE-A1 and MAGE-A2 at 1/20 (5%) tumors. In this same review, we found that PRAME expression is reported in 88% of 49 primary cutaneous melanoma and in 95% of 152 metastatic cutaneous melanoma. This review also revealed that primary tumors express CTA at lower rates compared with metastatic tumors.[8] Although the expression of PRAME (90% in LMM) in this study does not differ in comparison with cutaneous melanoma, the pattern of lower MAGE-A expression levels supports the notion that LMM is a distinct entity compared with SSM and NM.

In our samples, we did not find any positive staining of MAGE-A1, -A2, -A3, -A4, -A10, -A12, NY-ESO-1, or SSX2 in LM tissue. Therefore, these specific CTA cannot be used to discern malignant melanocytes from normal melanocytes to confirm the diagnosis of LM. However, it is possible that if a biopsy from a lesion suspect for LM shows positivity for MAGE, NY-ESO-1, or SSX-2, the lesion may actually be LMM. A recent study showed that 9% of biopsy proven LM are reclassified as LMM after surgical excision.[9]

Interestingly enough we found positive PRAME expression in LM at low levels, but not in sun-exposed skin. In a clinical setting, it is difficult to accurately distinguish LM from solar lentigines. Usually a biopsy is taken to confirm or disprove the diagnosis LM. Another uncertain situation is when a sample may not show all the classical characteristics of LM on histopathological examination. PRAME

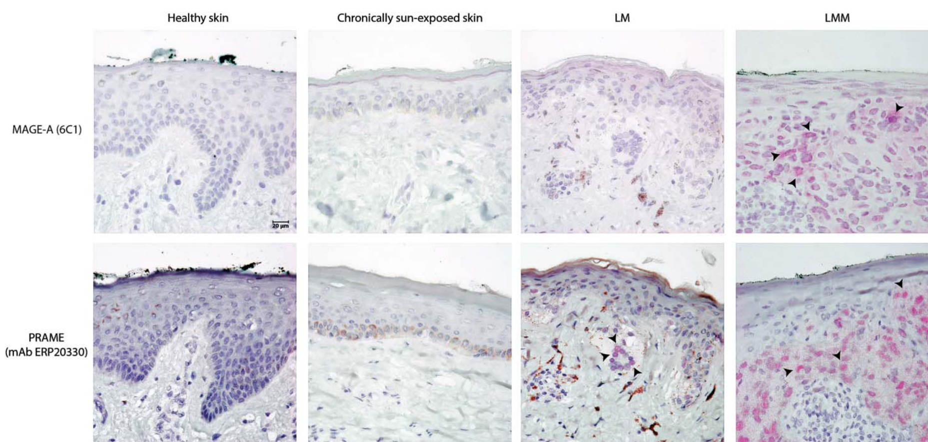

FIGURE 1. MAGE1 and PRAME expression in skin sections. FFPE sections were immunohistochemically stained for MAGE1 (mAb 6C1) and PRAME (mAbERP20330) and visualized using Perma Red/AP chromogen. Images are taken at ×400 magnification.

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TABLE 1. Summary of Expression Patterns of Cancer Testis Antigens in Lentigo Maligna and LM Melanoma

	MAGE-A (mAb 6C1)	MAGE-A1 (MA454)	MAGE-A2 (Polyclonal)	MAGE-A3 (Polyclonal)	NY-ESO-1 (mAb E978)	PRAME (mAb EPR20330)	SSX2 (mAb CL3202)
Staining pattern	Nuclear and cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	—	Nuclear and membranous	—
Healthy skin (N = 7)	Negative	Negative	Negative	Negative	Negative	1/7 samples positive expression in <1% of cells	Negative
Sun-exposed skin (N = 7)	Negative	Negative	Negative	Negative	Negative	Negative	Negative
LM (N = 8)	Negative	Negative	Negative	Negative	Negative	6/8 samples positive expression in <1-30% of cells	Negative
LMM (N = 20)	4/20 samples positive expression in 5%–50% of cells	1/20 samples positive expression in <5% of cells	1/20 samples positive expression in <5% of cells	1/20 samples positive expression in <5% of cells	Negative	18/20 samples positive expression in 50%–100% of cells	Negative

specifically could potentially be used to differentiate normal melanocytes in sun-exposed or chronic sun-damaged skin from malignant melanocytes, indicating LM. Our findings are similar to a recent study by Lezcano et al, in which they found positive PRAME expression in 24 of 27 (88%) LM and 15 of 17 (88%) LMM. They also found rare isolated cases of junctional melanocytes with immunoreactivity for PRAME in benign nonlesional skin.[10]

A single normal skin sample showed positive expression of PRAME. Expression of PRAME has been described in normal skin in an earlier study by Ikeda et al and the aforementioned study by Lezcano et al [10,11] A potential pitfall is that PRAME seems to be less specific for testis and malignancies in comparison to other CTA. If a normal skin sample is false positive, it could lead to the incorrect diagnosis of LM and consequently to overtreatment. Because the staining pattern is the same, it is important to correlate clinical information with the histopathological findings to prevent incorrect diagnosis.

Limitations to this study are a small sample size and the lack of LM samples that consecutively progressed to LMM. We recommend future studies to investigate the prevalence of PRAME in larger cohorts of LM and LMM to analyze whether PRAME can be used as a discerning marker between normal and atypical melanocytes.

In conclusion, we did not find expression of MAGE-A, NY-ESO-1, or SSX-2 in LM, thereby excluding these CTA as diagnostic marker to discern malignant melanocytes in LM from normal melanocytes. LMM does express MAGE, NY-ESO-1, and SSX-2, but at lower levels compared with cutaneous melanoma. If a biopsy from a lesion suspect for LM shows positivity for MAGE, NY-ESO-1, and SSX-2, the lesion may actually be LMM. In contrast, PRAME expression was found in LM at low levels and in LMM at much higher levels. This specific CTA can potentially be used to discern normal melanocytes from malignant melanocytes in LM.

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