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Comparison of CD23 staining patterns in Merkel cell carcinoma and non-cutaneous small cell carcinoma

Background: During our daily practice, we observed that cluster designation 23 (CD23) (clone BU38) labels Merkel cells in normal skin. In this study, we examined the expression of CD23 in Merkel cell carcinoma (MCC) and assessed its usefulness in distinguishing MCC from non-cutaneous small cell carcinoma (SMCC).
Methods: Immunohistochemical staining of CD23 was performed on a total of 33 MCCs, 22 SMCCs and 5 carcinoid tumors.
Results: CD23 reactivity was present in 32 of 33 (97%) MCCs, 18 of 22 (82%) SMCCs and 5 of 5 (100%) carcinoid tumors. In MCC, 19 cases (59%) showed a predominance of perinuclear dot-like staining similar to cytokeratin 20, 3 (9%) showed mostly cytoplasmic staining and 10 (31%) displayed a combination of perinuclear dot-like and cytoplasmic staining. In contrast, all CD23-positive SMCCs and carcinoid tumors showed a diffuse cytoplasmic staining. There was a significant difference in the CD23 staining patterns between MCC and SMCC ($p < 0.0001$).
Conclusion: CD23 is expressed in the majority of MCC, SMCC and carcinoid tumor irrespective of clinical outcome. The distinct punctate CD23 staining for MCC may be helpful in differentiating it from SMCC. To our knowledge, this is the first study to show the expression of CD23 in neuroendocrine tumors.

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Cluster designation 23 (CD23) is the low-affinity receptor for IgE and is well documented as a lymphoid marker.[1,2] Apart from its primary use in diagnosing chronic lymphocytic leukemia/small cell lymphoma,[3] CD23 expression has recently been detected in the glandular epithelium of breast, colon and the eccrine/apocrine secretory coils of sweat glands, as well as in several cutaneous sweat gland tumors.[4,5]

Merkel cell carcinoma (MCC) is a rare primary undifferentiated small cell carcinoma (SMCC) of the skin that arises mostly in sun-exposed skin of elderly individuals.[6] It is an aggressive cutaneous carcinoma with high rates of recurrence and metastasis.[7] In clinical practice, MCC and cutaneous metastatic SMCC of the lung share common histologic features and therefore frequently present a diagnostic challenge. The distinction between MCC and lung SMCC has significant prognostic and therapeutic implications.

Previous studies have shown that cytokeratin 20 (CK20) is positive in the majority of MCC, although lung SMCC may occasionally show CK20 reactivity.[8,9] CK20 has also been shown to be helpful in detecting occult metastases of MCC in sentinel lymph nodes.[10] Thyroid transcription factor-1 (TTF-1) is frequently expressed in lung SMCC[11] but not in

MCC.[12] When used in combination with TTF-1, CK20 is helpful in differentiating MCC from lung SMCC.[13] Recently, some new immunohistochemical markers, such as CD117 and CD56, have been shown to be expressed by neuroendocrine carcinomas, but few of them seem to be specific for MCC.[14–19]

During our study of CD23 expression in cutaneous adnexal tumors, we discovered that CD23 labeled Merkel cells in normal skin. To expand this finding, we investigated the potential utilization of CD23 in diagnosing MCC and perhaps in differentiating MCC from non-cutaneous SMCC. We report herein that CD23 is constantly expressed in MCC (97%) with characteristic punctate staining, a well-recognized reaction pattern for CK20. Although a substantial number of non-cutaneous SMCCs (82%) are also highlighted by CD23, a different staining pattern is observed.

Materials and methods

Case selection

After approval from the University of Michigan Institutional Review Board for human subject research, a search of the laboratory files of the University of Michigan, Pathology Department from January 1996 to May 2006 was performed. We identified 33 cases of MCC in 28 patients of which 25 were primary skin lesions and 8 were lymph node metastases. Among these, we had the corresponding sentinel lymph node specimens available for five skin lesions from five individual patients. As comparison, 22 cases of non-cutaneous SMCC, including 16 pulmonary (10 primary, 6 metastases to skin, liver, adrenal, mediastinum or soft tissue) and 6 extrapulmonary tumors (2 urinary bladder, 1 ethmoid, 1 tonsil, 1 esophagus and 1 vocal cord), were examined. In addition, five cases of carcinoid tumor (four bronchus/lung and one duodenum) were evaluated.

Hematoxylin and eosin sections and all prior immunohistochemical stains (i.e. pancytokeratins, CK20, TTF-1, CD56, chromogranin A or synaptophysin) were reviewed to confirm the original diagnoses.[20,21] All MCCs included in this study were positive for CK20 and/or pancytokeratin and negative for TTF-1.

Immunohistochemical detection of CD23

Immunohistochemical study was performed on formalin-fixed and paraffin-embedded tissue sections on a Ventana Benchmark XT system (Ventana Medical Systems, Tucson, AZ, USA) using the iVIEW diaminobenzidine reaction kit for visualization. All slides were pretreated with protease for 16 min before applying the mouse anti-human monoclonal CD23 antibody (clone BU38; 1 : 10 dilution; The Binding Site, San Diego, CA, USA).

The immunoreactivity of the tumor cells was independently evaluated by two pathologists (L. M. and J. C.) according to the pattern, distribution and intensity of the CD23 staining. Cases were considered positive if more than 1% of tumor cells were immunoreactive. The staining pattern was stratified into three groups: diffuse cytoplasmic labeling, perinuclear dot-like staining or both. The percentage of positive tumor cells was recorded as one of three categories: $> 50\%$, 20–50% and $< 20\%$. The intensity of staining was graded as weak or moderate to strong.

Statistical analysis

Statistical analysis was carried out using SAS 8.2 software (SAS Institute Inc., Cary, NC, USA). The Fisher's exact test was used to assess the difference in CD23 staining patterns between MCC and SMCC. A p value < 0.05 was considered statistically significant.

Results

In normal skin, CD23 expression was detected in the secretory epithelium of sweat glands (data not shown), as well as rare isolated Merkel cells in the basal layer of the epidermis and the outer root sheath of hair follicles (Fig. 1A). In the lung, it was observed in bronchial epithelium and some pneumocytes (data not shown).

We studied CD23 expression in 33 MCCs, 22 non-cutaneous SMCCs and 5 carcinoid tumors that were fully characterized histologically and immunophenotypically. The results are summarized in Table 1.

The MCC patients included in our study were 22 men and 11 women, with an age range of 44–87 years old (mean = 73 years). With the exception of one primary skin tumor, 32 MCCs consistently expressed CD23, with four cases showing weak staining and 28 having moderate-to-strong staining, resulting in an overall sensitivity of 97%. Among cases that had CD23 expression, 19 of 32 (59%) showed a predominance of perinuclear dot-like pattern (Fig. 1C) that closely resembled CK20 reactivity in MCC (Fig. 1B). Ten of 32 (31%) cases displayed a combination of dot-like and diffuse cytoplasmic (some with membrane accentuation) staining, while the remaining three cases (9%) showed a mainly diffuse cytoplasmic/membranous pattern. In 20 of the 32 positive cases (14/24 primary skin tumors and 6/8 nodal metastases), CD23 was detected in greater than 50% of the tumor cells. No differences in staining patterns were observed between 24 primary skin MCCs and 8 lymph node metastases. Similar to primary skin lesions, six of eight nodal metastases showed a characteristic punctate CD23-positivity,

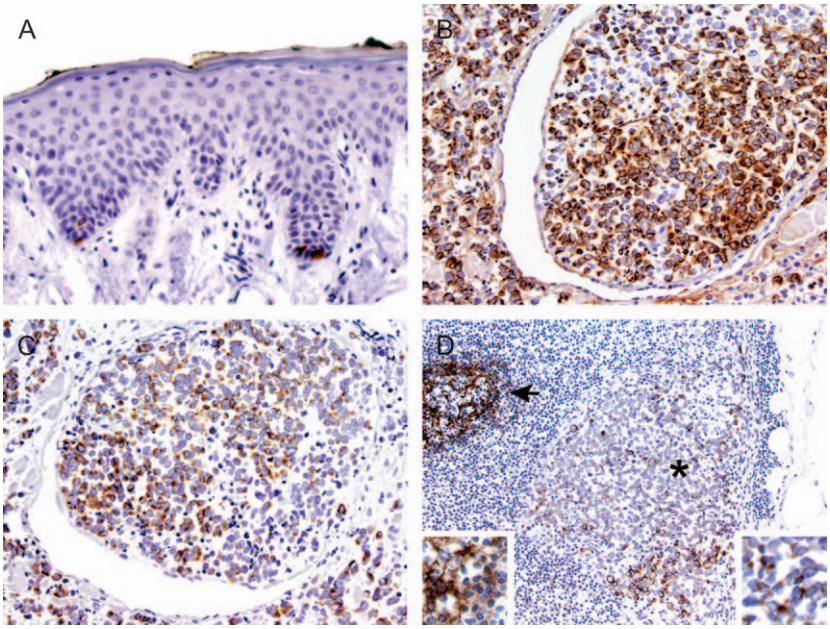

Fig. 1. The expression of cluster designation 23 (CD23) in normal skin and Merkel cell carcinomas (MCCs). A) CD23 labels several small cells with scant cytoplasm exclusively located in the basal layer of skin, features typical of Merkel cells (×400). B) Punctate pattern of cytokeratin 20 staining in a primary MCC (×400). C) Characteristic perinuclear dot-like CD23 staining in a primary MCC (×400). D) Perinuclear dot-like CD23 labeling (indicated by asterisk and inset on lower right corner) in a lymph node metastasis of MCC (×200). Adjacent normal germinal center B cells (indicated by arrow and inset on lower left corner) are also marked by CD23 and can be easily distinguished from the tumor cells.

while two showed a combined cytoplasmic and punctate staining (Fig. 1D). Among 25 cases of primary MCC, 16 had a sentinel lymph node biopsy performed. Twelve of 16 patients had positive sentinel lymph node(s) for metastatic MCC, while four patients showed no evidence of nodal disease. When the primary lesions from these two groups of patients were compared, no appreciable difference in CD23 staining was noted, indicating that CD23 expression does not correlate with MCC progression or metastasis. We also compared the primary skin tumor with its corresponding lymph node metastasis in five different patients and found no difference in the intensity and pattern of CD23 expression. Again, the intensity, distribution and pattern of CD23 expression did not appear to correlate with clinical outcomes in this small cohort of patients.

In the non-cutaneous SMCC group (10 primary lung tumor, 6 metastases of lung tumor and 6 extrapulmonary tumor), there were 15 men and 7 women, with an age range of 42–83 years (mean = 60 years). Typically, the pulmonary and extrapulmonary tumors exhibited identical morphologic features and were composed of sheets of small round/ovoid cells with hyperchromatic nuclei and scant cytoplasm (Fig. 2A). As shown in Table 1, CD23 expression was detected in 18 of 22 (82%) SMCCs (2 weak and 16 moderate to strong) with a sensitivity of 82%. The positive cases included 15 of 16 (94%) lung SMCCs and 3 of 6 (50%) extrapulmonary SMCCs from various sites. The majority (12/18, 67%) of CD23-positive SMCCs had > 50% tumor cells stained. In contrast to MCC, all positive cases displayed a diffuse cytoplasmic/membranous staining (Fig. 2B). Interestingly, the only case of skin metastasis of lung SMCC showed a diffuse cytoplasmic staining rather than a punctate pattern (Fig. 2C). Statistically, the CD23 reaction patterns were significantly different between MCC and non-cutaneous SMCC groups ($p < 0.0001$).

Table 1. The CD23 expression in MCC, non-cutaneous SMCC and carcinoid tumor

	MCC (n = 33)	SMCC (n = 22)	Carcinoid tumor (n = 5)
Staining pattern			
Perinuclear dot	19	0	0
Cytoplasmic	3	18	4
Cytoplasmic and perinuclear dot	10	0	1
Percentage of tumor cells staining			
Negative (< 1%)	1	4	0
< 20%	4	3	0
20–50%	8	3	1
> 50%	20	12	4

CD23, cluster designation 23; MCC, Merkel cell carcinoma; SMCC, small cell carcinoma.

We next studied five carcinoid tumors. CD23 reactivity was detected in all five lesions (two weak and three moderate to strong), with four showing > 50% tumor cells stained (Fig. 2D). All five tumors showed diffuse cytoplasmic staining, although one had very focal punctate labeling. Interestingly, two cases with weak CD23 reactivity were both spindle cell bronchial carcinoid tumors. Our findings indicate that CD23 expression is not restricted to neuroendocrine carcinomas. Instead, it may be a useful marker for neuroendocrine tumors in general.

Discussion

To our knowledge, this was the first study to investigate the CD23 expression in Merkel cells,

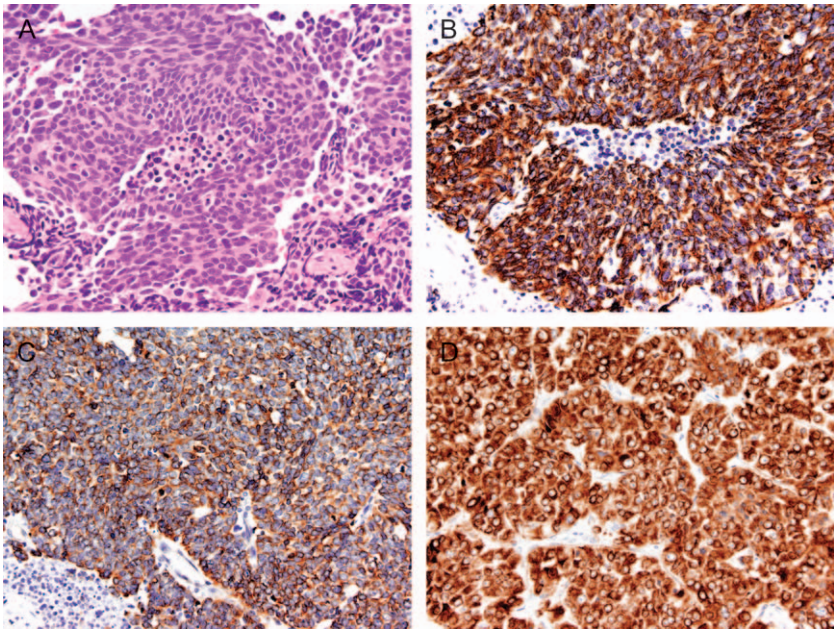

Fig. 2. The expression of cluster designation 23 (CD23) in lung small cell carcinomas (SMCC) and carcinoid tumors. A) Hematoxylin and eosin sections of a lung SMCC showing small neoplastic cells with nuclear molding and frequent karyorrhexis (×400). B) It shows a diffuse cytoplasmic staining with CD23 (×400). C) A case of cutaneous metastatic lung SMCC showing diffuse cytoplasmic CD23 reactivity (×400). D) A lung carcinoid tumor showing strong CD23 staining with a diffuse cytoplasmic pattern (×400).

MCC, non-cutaneous SMCC and carcinoid tumor. We report that the expression of CD23 is not limited to lymphoid cells, but is also detected in Merkel cells in normal skin as well as in some neuroendocrine tumors.

CK20 is a well-accepted marker for primary cutaneous MCC with a reported sensitivity of 95%.[9] However, it is not entirely specific for MCC because it can be identified in roughly 30% of lung SMCCs in less than 25% of tumor cells.[13] Occasional MCCs have been found to be CK20 negative,[9] and in such cases, an expanded battery of neuroendocrine markers, including synaptophysin, chromogranin A, neuron-specific enolase and CD56, may help substantiate the diagnosis.[22] In this study, the detection of strong CD23 expression in MCC and non-cutaneous SMCC is intriguing. Because CD23 displayed a similar sensitivity (97%) as that described for CK20 (95%), it could serve as a useful adjunct marker to CK20 for occasional CK20-negative MCC.[9]

In practice, MCC and non-cutaneous SMCC are diagnosed by their characteristic histologic features and the expression of typical neuroendocrine markers. The expression of CK20 in MCC and TTF-1 in lung SMCC aids in the distinction between the two. In our series, the CD23 expression in MCC is particularly notable for its perinuclear dot-like reactivity, a finding similar to that described for CK20.[23] Although our results indicate that CD23 is not specific for MCC, the distinct punctate CD23 staining for MCC makes it a potential adjunct immunohistochemical marker in distinguishing between MCC and non-cutaneous SMCC.

Recently, Ki-67 was shown to be a prognostic factor in MCC.[24] In this study, we did not find a significant correlation between the intensity and pattern of CD23 reactivity and the metastatic potential of primary MCC. The fact that five nodal metastases of MCC displayed similar CD23 staining to their cognate primary lesions suggests that CD23 expression is not related to disease outcome of MCC. Furthermore, the expression of CD23 in neuroendocrine carcinomas, as well as carcinoid tumors, provides additional support to the notion that CD23 is not related to the pathogenesis or the aggressiveness of these tumors. An additional study with a large number of cases would help substantiate this observation.

Merkel cells are generally considered to be derivatives of either neural crest cells or primitive epithelium, showing both neuroendocrine and epithelial features.[25,26] Similarly, SMCC derived from the amine precursor uptake and decarboxylation cells was also thought to be of neural crest or epithelial origin.[27] The strong expression of CD23 in both MCC and non-cutaneous SMCC suggests a possible related histogenesis of the two.

The mechanism of the CD23 expression in MCC and other neuroendocrine tumors is unclear. CD23 is a type II transmembrane glycoprotein.[1] The unique punctate CD23 staining, a pattern similar to the CK20 and/or pancytokeratin reactivity in MCC, suggests that CD23 may cross-react with keratin/intermediate filaments in the tumor cells. We speculate that the target of the cross-reaction is less likely to be CK20. The reason is that most carcinoid tumors and non-cutaneous SMCCs in our study were strongly reactive for CD23, yet these tumors are generally negative for CK20.[8] Alternatively, CD23 may represent or be associated with an intrinsic molecule expressed in neuroendocrine cells and tumors.

Future studies are necessary to elucidate the mechanism(s) of CD23 expression in neuroendocrine tumors.

Lastly, it is important to be aware that the CD23 expression is not restricted to chronic lymphocytic leukemia/small lymphocytic lymphoma. Interestingly, CD56 and the paired box-5, markers commonly used in evaluating lymphoid malignancies, were also found to be expressed in MCC and non-cutaneous SMCC.[14,16]

In summary, we have shown that CD23 is a very sensitive marker for MCC and other neuroendocrine tumors. CD23 staining patterns were significantly different between MCC and non-cutaneous SMCC. Therefore, it could serve as an ancillary marker in skin tumors suspicious for neuroendocrine origin and may have some utility in differentiating MCC from non-cutaneous SMCC when used in conjunction with CK20 and TTF-1.

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