

Merkel cell carcinoma can be distinguished from metastatic small cell carcinoma using antibodies to cytokeratin 20 and thyroid transcription factor 1

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Abstract

Aim—To investigate whether immunohistochemical staining for cytokeratin 20 (CK20) and thyroid transcription factor 1 (TTF-1) is useful in distinguishing Merkel cell carcinomas (MCCs) from metastatic small cell carcinomas (SCCs).

Methods—Eleven cases of MCC and 10 of lung SCC were stained for CK20 and TTF-1.

Results—Ten of 11 MCCs stained with the antibody to CK20. None was positive for TTF-1. No SCC stained with anti-CK20 and all stained strongly with anti-TTF-1.

Conclusions—The use of both anti-CK20 and anti-TTF-1 can reliably distinguish between MCC and metastatic SCC, thus avoiding the need for a detailed clinical investigation of patients with MCC in whom metastatic SCC must be excluded.

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Merkel cell carcinomas (MCCs) of the skin are aggressive malignant skin tumours that are thought to arise from Merkel cells in the skin. They were first described by Toker in 1972.¹ They tend to occur in the elderly in sun exposed sites but can arise anywhere. They recur locally in 26-44% of patients, have regional metastases in up to 75% of patients, and have a five year survival between 30% and 64%.² MCCs often present as solitary purple/red nodules and histologically consist of small round blue cells. The differential diagnosis consists of other tumours with similar morphology from the "small round blue cell" tumour group, in addition to melanoma, but by far the most important histological distinction is from metastatic small cell carcinoma (SCC) of the lung. Once a diagnosis of MCC is made, it is currently recommended that the patient is screened to exclude metastatic SCC. Therefore, a reliable pathological method of distinguishing the two lesions would be useful.

Many immunohistochemical stains have been examined in the search for a suitable marker. Promising results have been reported with markers for cytokeratin 20 (CK20).³ CK20 is a low molecular weight cytokeratin that is only expressed in normal gastrointestinal epithelium, urothelium, and the Merkel cell.⁴ MCC almost always stains with CK20 in contrast to metastatic small cell carcinoma.⁵

Thyroid transcription factor 1 (TTF-1) is a more recently described nuclear transcription factor expressed in epithelial cells of the thyroid and lung. It is expressed in a high proportion of SCCs and is not expressed by MCCs.⁶⁻⁸ Therefore, an ideal panel for the distinction of MCC from SCC might consist of antibodies to CK20 and TTF-1.

The aim of our study was to evaluate a group of patients with MCC or SCC with the use of immunohistochemical markers for CK20 and TTF-1. We also evaluated an antibody against cytokeratins 8/18 (CK8/18), which has also been suggested to be a useful marker.

Methods

Formalin fixed, paraffin wax embedded tissue was taken from 11 MCCs of skin and 10 SCCs of lung. The cases came from the pathology departments of the Royal Victoria Infirmary in Newcastle and Dryburn Hospital in Durham over the past five years.

The histological diagnoses were confirmed before the study. Clinical details on all patients were reviewed. Patients were felt to have definite MCC if they had at least one of the following: a normal chest x ray at diagnosis, regional nodal metastases of MCC, or no evidence of a primary lung lesion within six months of diagnosis.

Paraffin wax embedded sections (5 µm thick) were dewaxed in xylene and rehydrated. Antigen retrieval was performed using pressure cooking with 0.01M sodium citrate (pH 6) for CK20 (1/25 dilution; Novocastra), pressure cooking with 1mM EDTA (pH 8) for TTF-1 (1/100 dilution; Dako, Ely, UK), and pretreatment with trypsin for 10 minutes for CK8/18 (1/20 dilution; Novocastra, Newcastle, UK). Endogenous tissue peroxidase activity was blocked with 3% hydrogen peroxide in methanol. Sections were developed with a streptavidin-biotin-peroxidase detection system and the antibody was detected with freshly prepared diaminobenzidine as the chromagen (brown). Sections were counterstained with haematoxylin.

The stained slides were evaluated by two blinded independent observers with concordant results in all cases.

Results

Figure 1A and B shows staining with anti-CK20 in an SCC of lung and an MCC, respectively. There is no staining for CK20 in the SCC in contrast to the cytoplasmic staining

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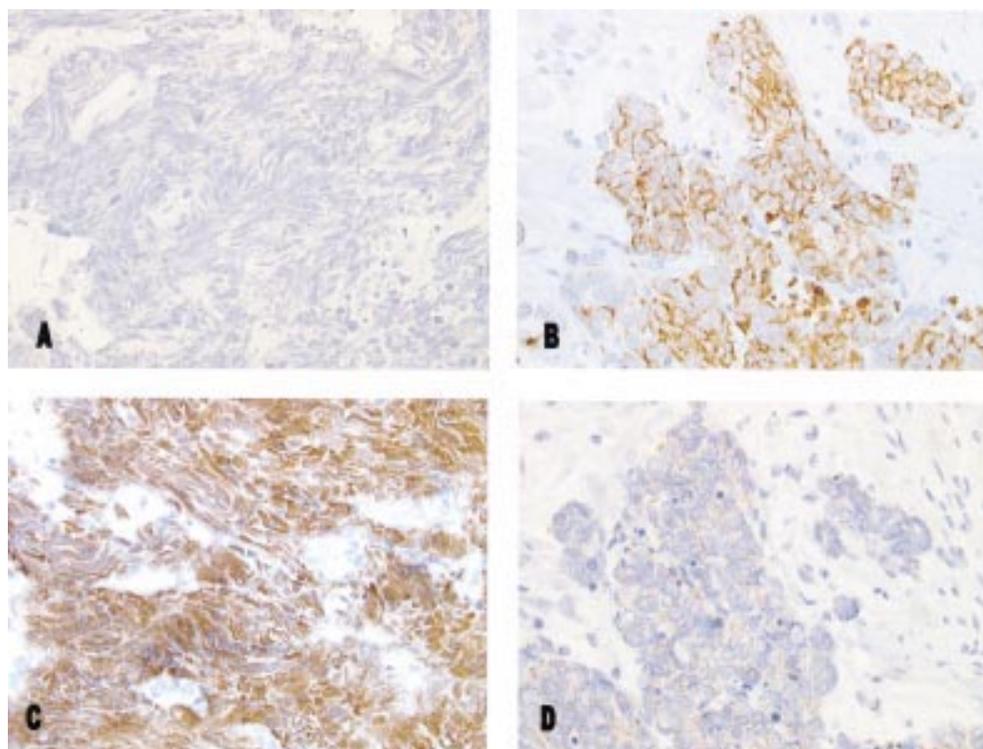


Figure 1 (A) Negative immunostaining with anticytokeratin 20 (anti-CK20) in a metastatic small cell carcinoma (SCC). (B) Positive immunostaining with anti-CK20 in a Merkel cell carcinoma (MCC). Some areas of perinuclear dot-like positivity are seen. (C) Immunostaining with antithyroid transcription factor 1 (anti-TTF-1) in an SCC showing strong nuclear and cytoplasmic staining. (D) Immunostaining with anti-TTF-1 in an MCC showing very weak cytoplasmic staining but no nuclear staining.

Table 1 Immunohistochemical staining of Merkel cell carcinoma (MCC) and metastatic small cell carcinoma (SCC)

Cases	CK20	TTF-1
MCC	10/11	0/11
SCC	0/10	10/10

CK20, cytokeratin 20; TTF-1, thyroid transcription factor 1.

with perinuclear accentuation in the MCC. It should be noted that perinuclear accentuation is seen in a small proportion of cells.

Figure 1C and D shows staining with anti-TTF-1 in an SCC of lung and an MCC, respectively. There is diffuse nuclear and cytoplasmic staining of the SCC in contrast to a faint background blush in the cytoplasm of the MCC.

Anti-CK20 stained 10 of 11 MCCs (table 1). Unusually, one MCC was negative for CK20 but had shown dot-like positivity with a pankeratin marker at initial diagnosis.

Staining for TTF-1 was positive in all of the SCCs and none of the MCCs.

Nine of 11 MCCs stained with anti-CK8/18; however, five of nine SCCs also showed positive staining.

Discussion

MCC is an uncommon skin tumour that behaves aggressively. It can be difficult to differentiate from metastatic SCC. This distinction is important to ensure appropriate management and prognosis.

There are no histological features to distinguish MCC from SCC or other small round

blue cell tumours such as lymphoma, neuroblastoma, extraskeletal Ewing's sarcoma, peripheral neuroectodermal tumour, and rhabdomyosarcoma. All are composed of sheets of small blue cells with vesicular nuclei and inconspicuous nucleoli. Immunohistochemical stains are useful in excluding differentials such as melanoma (S100 positive, keratin negative), lymphoma (leucocyte common antigen (LCA) positive, keratin negative), neuroblastoma (neurone specific enolase (NSE) positive, keratin negative), Ewing's sarcoma (p30/32 positive (CD99)), and rhabdomyosarcoma (desmin positive, muscle specific actin positive, myoglobin positive).⁴ The distinction from SCC is more difficult. This is not unexpected because Merkel cells have immunohistochemical features of both neuroendocrine and epithelial cells. Both tumours are positive for CKs and neurofilaments. Although the perinuclear pattern of CK staining is only seen in MCCs, this pattern is not always present, or is not always clearly identifiable, so that a more reliable method of diagnosis is needed.⁹ Merkel cells contain intermediate filaments in their cytoplasm. Tumour cell lines derived from Merkel cells have been shown to express CKs 8, 18, 19, and 20.¹⁰ This observation has led to the use of antibodies to specific CKs in an attempt to find a discriminatory marker, of which CK20 appears to be the most specific.³⁻⁵ CK8/18 is as sensitive for MCC as CK20, staining a similar proportion of cells, but it is not as specific because it also stained half of the SCCs.

TTF-1 is a newly described nuclear protein that appears to be specific for small cell tumours of lung origin.⁶⁻⁸

Our study has confirmed previous observations regarding the usefulness of CK20 and TTF-1. Anti-CK20 cannot be used alone because one of the MCCs would have been missed. This case was re-examined carefully. Immunohistochemistry with a panel of markers for small round blue cell tumours (as mentioned above) was performed. The tumour was negative for CD99, desmin, smooth muscle actin, NSE, LCA, and myoglobin. It showed faint perinuclear dot positivity for a pankeratin, as it had done at initial diagnosis. Therefore, it was felt to represent a MCC. TTF-1 has 100% specificity for SCC. Thus, positivity for CK20 and negativity for TTF-1 indicates MCC in contrast to negativity for CK20 and positivity for TTF-1, which indicates SCC. The use of two markers, where each one is positive for one of the two tumours, in the differential diagnosis is a more powerful tool than relying on one antibody result alone. Therefore, we recommend the routine use of anti-CK20 and anti-TTF-1 antibodies in all cases of suspected MCC.

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