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CASE REPORT

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Chromosome 6 trisomy as sole anomaly in a primary Merkel cell carcinoma

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Abstract We present a case of Merkel cell carcinoma of the thigh diagnosed by conventional histology, immunohistochemistry, electron microscopy and cytogenetics. A unique chromosome 6 trisomy characterized this primary neoplasm, as confirmed by FISH study. The role of chromosome analysis and interphase cytogenetics is emphasized as an adjunct in the subtyping of tumours and their prognostic evaluation.

Key words Merkel cell carcinoma · Cytogenetics · Chromosomes · FISH

Introduction

Neuroendocrine carcinoma of the skin was first described by Toker in 1972 as a trabecular carcinoma [28]. This tumour, which appears to originate from Merkel cells, is a well-defined aggressive clinico-pathological entity with a 40% of local recurrence rate and frequent regional lymph node or distant metastases. It occurs frequently in elderly subjects and is localized in the head and neck area in 50% of cases, followed by the lower extremities [15, 25]; it can also occur in lymph nodes as a primary site [3]. About 30% of patients die of the disease [1, 7, 16, 25]. Merkel cell tumours have a specific immunohistochemical and ultrastructural profile [6, 16, 24].

Chromosome analysis in solid tumours is developing rapidly, and nonrandom rearrangements appear to be associated with tumour type or biological behaviour so that karyotyping may now be part of the initial diagnosis, at least in some soft tissue tumours [20]. There are few cytogenetic reports on Merkel cell carcinoma (MCC), and most of those available deal with complex rearrangements in lymph node metastases or long-term cell cultures. We report here a chromosomal approach to the diagnosis of MCC, with a trisomy 6 as the sole anomaly in a primary cutaneous MCC.

This analysis could be of help in the diagnostic challenge posed by MCC, contributing to the differential diagnosis of small cell tumours in the adult.

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Case report

Clinical data

An 82-year-old man developed a rapidly growing mass at the root of the right leg in the subcutaneous tissue. The lesion was removed by wide excision. At section, the tumour measured 30×20×15 mm and was firm and white with small haemorrhagic areas adhering to the skin. Three months later, a right inguinal swelling was revealed to be a lymph node metastasis.

Materials and methods

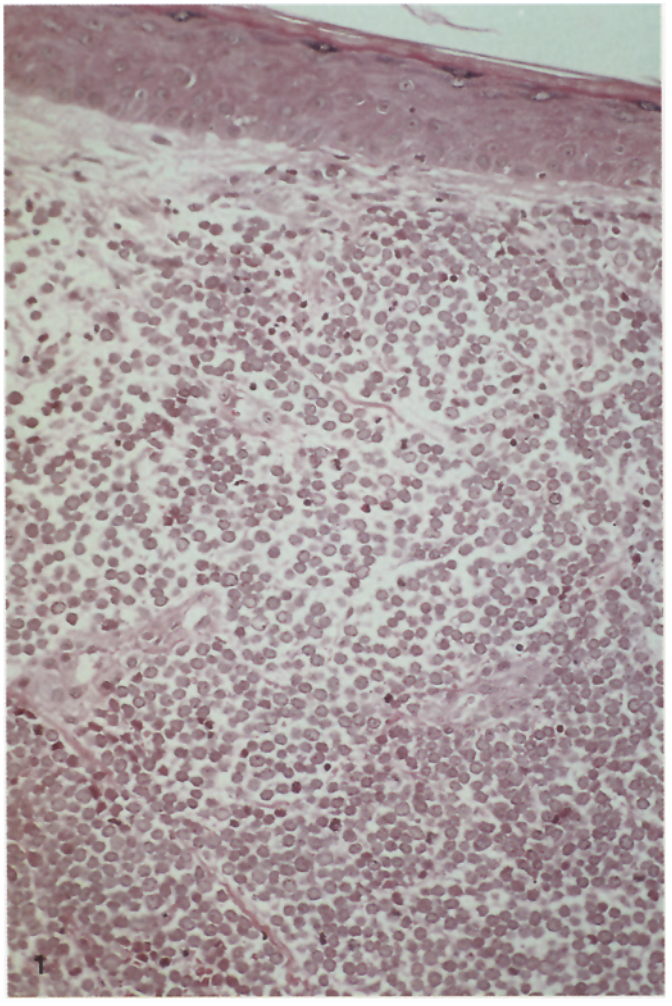
Histology

Tissue was sliced, fixed in 10% buffered formalin and paraffin-embedded. Slides 4 µm thick were stained with haematoxylin and eosin. Immunohistochemical study on fixed material with streptavidin-biotin peroxidase complex was performed for cytokeratins (CAM 5.2, 25 µg/ml, Boehringer Mannheim, Germany); protein S100, 1/50 (Dako, Glostrup, Denmark); neurofilaments, 1/50 (Sigma, St. Louis, Mo.); chromogranin A, 1/80 (Immunotech, Marseille, France); vimentin, 1/20 (Dako); and somatostatin, 1/10 (Novo Nordisk).

For electron microscopy (EM), tissue was fixed in glutaraldehyde 2.5% and washed in 0.1 M cacodylate buffer, postfixed in 1% osmic acid in 1% cacodylate 0.1 M, dehydrated in graded alcohol and embedded in Epon. Ultrathin sections were examined using a transmission electron microscope.

Cytogenetic analysis

A piece of tumour was minced in a collagenase solution; the cell suspension was cultured for 7 days following a standard procedure described elsewhere [12]. In ally 23 metaphase cells stained in G-



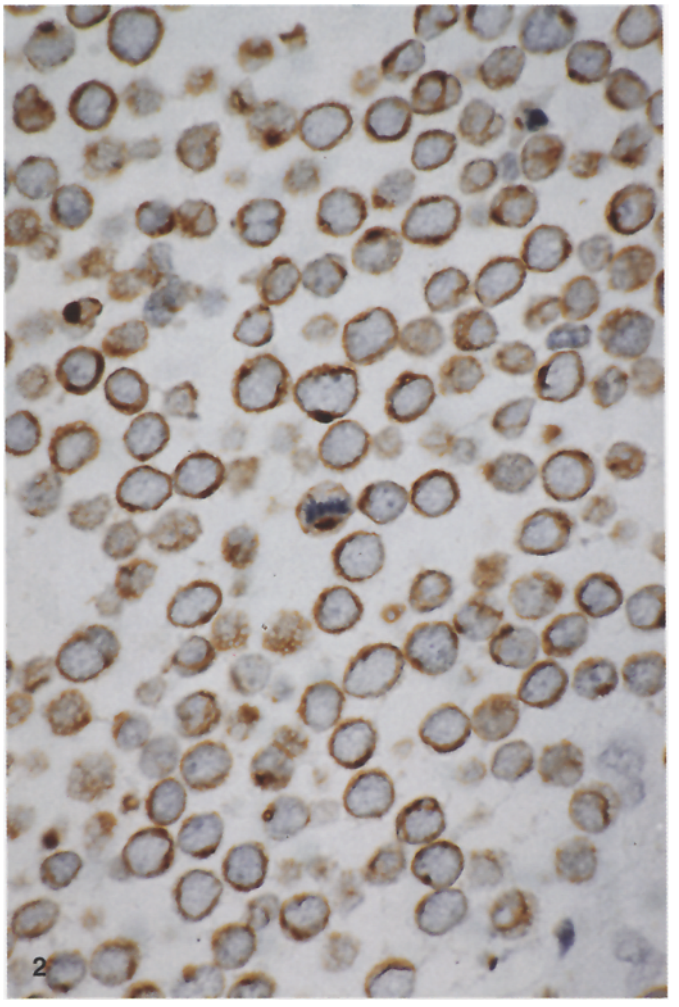


Fig. 1 Cutaneous involvement by a population of uniform noncohesive small cells characterized by a round nucleus with powdery chromatin and fine nucleoli surrounded by a thin ring of cytoplasm

Fig. 2 Cytokeratin (CAM 5.2) positivity with paranuclear dot-like enhancement

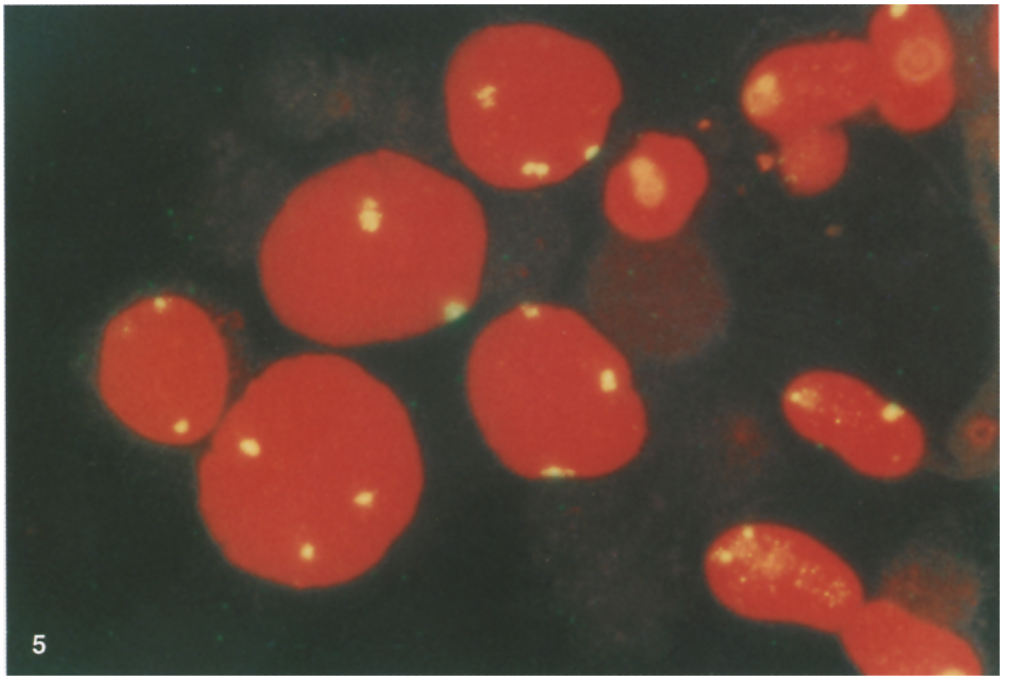


Fig. 5 Smear of tumour cells, showing three nuclear spots using alpha-6 centromeric probe in fluorescent in situ hybridization

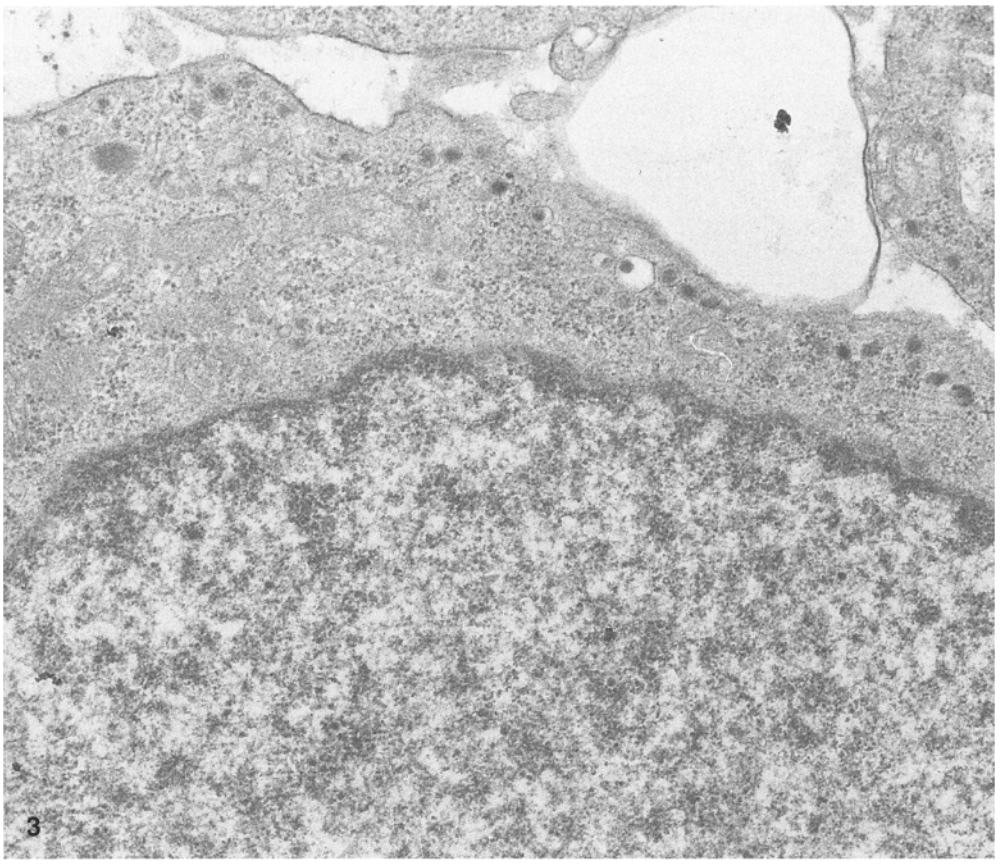


Fig. 3 Electron microscopy reveals dense core granules near the cytoplasmic membrane

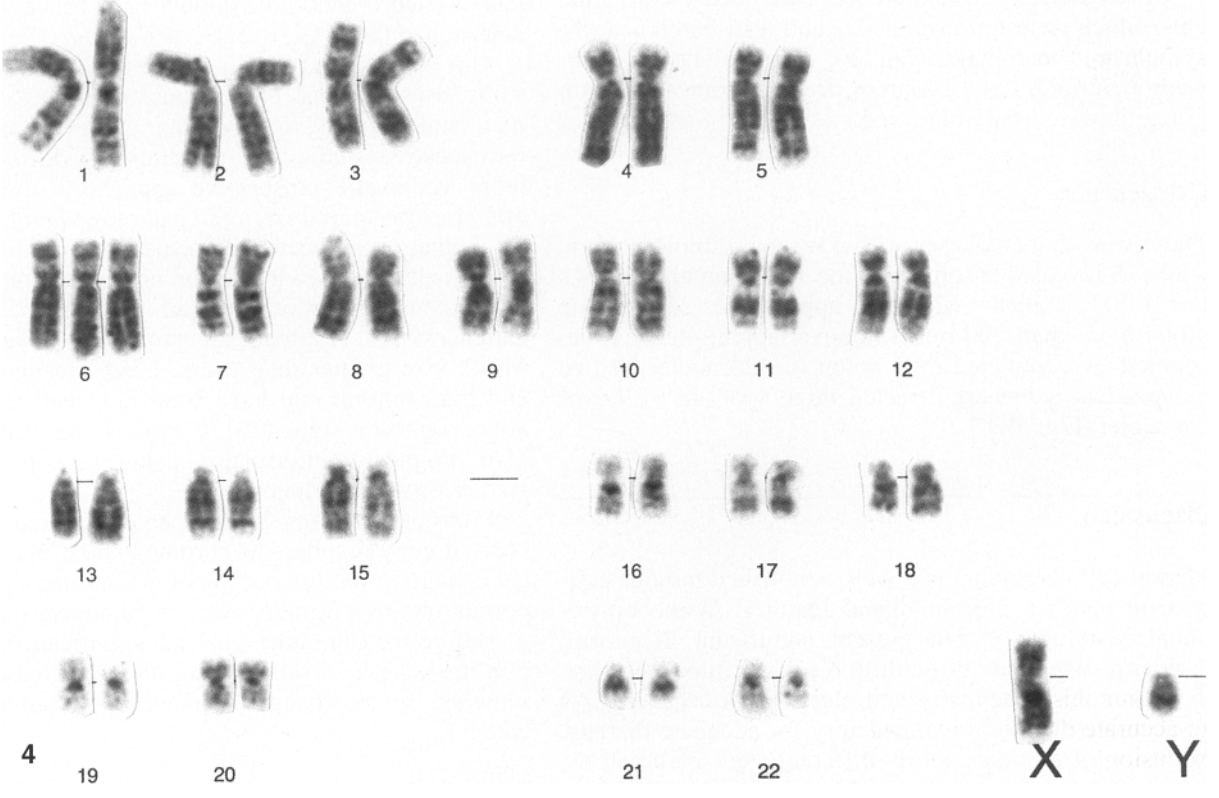


Fig. 4 Full karyotype in G-banding, illustrating trisomy 6

banding were analysed and fully karyotyped according to ISCN guidelines [13].

In situ hybridization

Fluorescence in situ hybridization (FISH) was performed following the procedures of hybridization and detection recommended in the manufacturer's protocol (Oncor, Gaithersburg, Md.); we used a specific alpha-satellite centromeric probe for chromosome 6. Fluorescein-labeled antidigoxigenin was used as detection reagent, and counterstaining was obtained by means of propidium iodide.

The number of bright spots by nucleus was scored by two different observers; a normal blood smear served as control.

Results

Light microscopy

The tumour, which was well delineated, nodular, white, and firm and measured 2 cm in its largest dimension, showed sheets and trabeculae of small undifferentiated cells with round to oval nuclei, inconspicuous nucleoli and characteristic powdery chromatin surrounded by scant cytoplasm on light microscopy (Fig. 1). Mitoses and individual cell necrosis were observed. At the immunohistochemical level, tumour cells revealed positivity for low molecular weight keratins (CAM 5.2) with a paranuclear dot-like pattern (Fig. 2) and diffuse positivity for neuron specific enolase (NSE). All other antibodies revealed negative staining.

Ultrastructurally, the tumour showed dense core granules, which were uniform in size and distributed near the cytoplasmic membrane (Fig. 3). Intermediate filaments were identified. Polyribosomes, free ribosomes and Golgi region were easily observed.

Cytogenetics

There were 5 metaphases with a normal diploid pattern, while 18 revealed trisomy 6 as the sole anomaly (Fig. 4). For FISH analysis with the appropriate centromeric probe more than 200 intact nonoverlapping nuclei were counted. A cumulated total count of 428 nuclei by two independent screeners detected three spots in 51.9% of the nuclei (Fig. 5).

Discussion

Merkel cell carcinoma is a well-established tumour entity with specific morphological features. With conventional histology, it can present significant diagnostic problems, which are difficult to resolve without recourse to immunohistochemistry and electron miscroscopy. As an accurate diagnosis is mandatory for adequate therapy, exclusion of all other poorly differentiated small cell tumours is mandatory; these include lymphoma, metastatic small cell carcinoma, carcinoid tumour, metastatic neuroblastoma, peripheral neuroectodermal tumour and rhabdomyosarcoma.

Chromosome analysis has proved to be useful in discriminating between these tumours, which are often hallmarked by simple but specific karyotypic abnormalities [9, 19, 20]. In MCC multiple chromosome abnormalities have been documented without any consistent aberrations, probably because the majority of the cytogenetic studies deal with metastatic lesions, long-term cultures or xenografted cell lines [5, 11, 27]. These studies have shown the high frequency of aberrations on both arms of chromosome 1 [5, 14, 17, 18, 27] in the same regions as described for other neural crest-derived tumours, such as melanoma [2] and neuroblastoma [4]. It has been suggested that they contribute to the proliferative advantage of the tumour cells [22] rather than reflecting a primary change [22, 27]. The detection by loss of heterozygosity of small deletions on 1p in advanced MCC may be related to a putative supressor gene or to the accumulation of neutral mutations common to a variety of aged tumours [8].

MCC shares electron microscopic and immunohistochemical features with small cell neuroendocrine bronchial carcinoma, as well as either impressive cytogenetic similarities [26] or distinct abnormalities [18]. Others see possible common mechanisms of tumourigenesis with meningioma [23].

The case of Gibas [5] was the first reported with only one anomaly in a primary MCC tumour. In his review of the literature [5], structural aberrations of the short as well as the long arms of chromosome 1 were found in 10 of 14 cases. In 5 of the 14 cases, including 1 primary cutaneous neuro-endocrine tumour [23], trisomy 6 was also determined [10, 11, 21, 27].

Our case is thus the first so far described with a trisomy 6 as the sole early clonal event in MCC. FISH methodology is revolutionizing cytogenetics, allowing retrospective studies on paraffin-embedded tissue and more systematic prospective application to cytological and biopsy material from the pathology laboratory. Once an anomaly is suspected to be specific to and recurrent in a particular disease, it may be confirmed and evaluated as a potential prognostic factor. No histological classification exists to estimate the prognosis of patients with MCC; size greater than 2 cm, head and neck location, and high mitotic rate have been indicated as unfavourable prognostic signs [15]. Chromosome analysis might give prognostic information using retrospective FISH studies on archive material.

From our findings, it might be considered that the increased copy numbers of chromosome 6 may be a critical change in the tumour genotype and may play an important part as a primary event in pathogenesis.

Before we can understand the significance of trisomy 6 in the genesis of this tumour, additional cases with the same karyotypic changes must be sought out and investigated.

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