Human Pathology (2015) **46**, 443–453

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Merkel cell carcinoma: histopathologic and prognostic features according to the immunohistochemical expression of Merkel cell polyomavirus large T antigen correlated with viral load☆,☆☆

Valérie Leroux-Kozal MD[a,*], Nicolas Lévêque PharmD, PhD[b,c], Véronique Brodard PharmD[b], Candice Lesage MD[d], Oriane Dudez Resident[a], Marc Makeieff MD, Phd[e], Lukshe Kanagaratnam MD[f], Marie-Danièle Diebold MD, PhD[a]

[a]*Department of Pathology, Faculty of Medicine, Robert Debré University Hospital, 51092 Reims, France*
[b]*Clinical and Molecular Virology Unit, University Hospital, 51092 Reims, France*
[c]*Faculty of Medicine, EA-4684 Cardiovir SFR-CAP Santé, 51092 Reims, France*
[d]*Department of Dermatology, Robert Debré University Hospital, 51092 Reims, France*
[e]*Department of Otolaryngology–Head and Neck Surgery, Robert Debré University Hospital, 51092 Reims, France*
[f]*Department of Research and Innovation, Robert Debré University Hospital, 51092 Reims, France*

Received 18 September 2014; revised 29 November 2014; accepted 3 December 2014

Keywords:
Merkel cell carcinoma;
Merkel cell polyomavirus;
Immunohistochemistry;
Polymerase chain reaction;
Histopathologic characteristics;
Prognosis

Summary Merkel cell carcinoma (MCC) is a neuroendocrine skin malignancy frequently associated with Merkel cell polyomavirus (MCPyV), which is suspected to be oncogenic. In a series of MCC patients, we compared clinical, histopathologic, and prognostic features according to the expression of viral large T antigen (LTA) correlated with viral load. We evaluated the LTA expression by immunohistochemistry using CM2B4 antibody and quantified viral load by real-time polymerase chain reaction. We analyzed formalin-fixed, paraffin-embedded (FFPE) tissue samples (n = 36) and corresponding fresh-frozen biopsies when available (n = 12), of the primary tumor and/or metastasis from 24 patients. MCPyV was detected in 88% and 58% of MCC patients by real-time polymerase chain reaction and immunohistochemistry, respectively. The relevance of viral load measurements was demonstrated by the strong consistency of viral load level between FFPE and corresponding frozen tissues as well as between primary tumor and metastases. From FFPE samples, 2 MCC subgroups were distinguished based on a viral load threshold defined by the positivity of CM2B4 immunostaining. In the LTA-negative subgroup with no or low viral load (nonsignificant), tumor

☆ Funding/Support: None of the authors of the present manuscript has a commercial or other association that might pose a conflict of interest (eg, pharmaceutical stock ownership and consultancy). This work was supported by a grant for pathology research (Matrice Extracellulaire et Dynamique Cellulaire, UMR CNRS/URCA 7369) from the Medical University and School of Medicine of Reims, France.
☆☆ Competing interests: No authors have direct or indirect commercial financial and personal relationships with other people or organizations that could inappropriately influence this manuscript.
* Corresponding author at: Service d'anatomie et cytologie pathologiques, CHU Reims, Ave du Général Koenig, 51092 Reims, France.
E-mail address: lerouxvaleri@gmail.com (V. Leroux-Kozal).

http://dx.doi.org/10.1016/j.humpath.2014.12.001

cells showed more anisokaryosis (P = .01), and a solar elastosis around the tumor was more frequently observed (P = .03). LTA-positive MCCs with significant viral load had a lower proliferation index (P = .03) and a longer survival of corresponding patients (P = .008). Depending on MCPyV involvement, 2 MCC subgroups can be distinguished on histopathologic criteria, and the CM2B4 antibody is able to differentiate them reliably. Furthermore, the presence of a significant viral load in tumors is predictive of better prognosis.

1. Introduction

Merkel cell carcinoma (MCC) is a rare neuroendocrine skin tumor, which was originally described by Toker in 1972 [1]. Ultrastructural analyses revealed some similarities with the Merkel cell, which was firstly suspected to be the cellular origin of MCC [2]. This hypothesis was highly controversial, and despite much research, the origin of the tumor is still unknown. Skin stem cells appear to be the most probably involved [3]. MCC is an aggressive malignancy that mostly occurs in elderly and immunocompromised individuals as a painless, rapidly growing, and violaceous nodule or papule. However, the clinical presentation can be variable and simulate other skin lesions [4]. On microscopic evaluation, MCC is a "small blue cell neoplasm" composed of monomorphic cells with a scant ill-defined cytoplasm and a round-to-oval nucleus with finely dispersed chromatin. Tumor cells express neuroendocrine immunohistochemical markers and usually show a typical paranuclear dot-like staining with cytokeratin 20 (CK20) [5]. Sun-exposed areas of the skin, especially the head and neck regions, are more frequently affected.

In 2008, Feng et al [6] discovered a novel human polyomavirus called *Merkel cell polyomavirus* (*MCPyV*) in 80% of primary MCCs with monoclonal DNA integration in the host genome. This finding, associated with the detection of high viral genome copy numbers in MCC cells, which express a truncated form of viral large T antigen (LTA), suggested that MCPyV is likely involved in oncogenesis [7,8]. Since then, subsequent studies confirmed the presence of MCPyV in MCCs [9]. The first and most common analytic method used for virus detection was the polymerase chain reaction (PCR), most of the time targeting the LTA coding gene [10-13]. However, MCPyV DNA was also detected in certain nontumor tissue samples from MCC and non-MCC patients. The place of molecular virological analyses in the MCC diagnosis remains to be defined, and determination of a viral load threshold characteristic of the disease could be a key element of the diagnosis. Shortly after the MCPyV discovery, Shuda et al [10] developed a monoclonal antibody (CM2B4) detecting the viral LTA expression by immunohistochemistry (IHC) in formalin-fixed and paraffin-embedded (FFPE) tissues. These 2 MCPyV detection tools have contributed to improve the understanding of the tumor physiopathology. However, correlation between LTA expression and MCPyV DNA load was assessed in a limited number of studies [10,14-17]. Moreover, few studies have investigated clinical and prognostic associations of MCC patients according to the level of viral load [8,14-16], and none has compared histopathologic criteria of tumors related to this parameter.

The present study tested FFPE tissue samples and corresponding fresh-frozen biopsies when available from patients with an MCC primary tumor or MCC metastases or both. The aim of this study was to analyze the correlation between the viral LTA expression assessed by IHC and the MCPyV DNA load determined by quantitative PCR assays. The study also researched potential histopathologic differences as well as clinical and prognostic associations according to MCPyV viral load and LTA expression between MCC tumors and patients, respectively.

2. Materials and methods

2.1. Patients and samples

The study was conducted on patients with MCC included in the pathologic database of the Pathology Department of Reims University Hospital (Reims, France) from 1989 to 2012. All FFPE samples from primary and metastatic MCCs were included, as were fresh-frozen corresponding tumor biopsies when available in the tumor bank of Champagne Ardennes. There were 15 primary and 21 metastatic tumors. Patients with a history of extracutaneous neuroendocrine tumor and cases with unavailable FFPE tissue samples were excluded. Nontumor FFPE tissue samples from MCC patients and FFPE and fresh-frozen tissues from non-MCC patients were used as negative control for quantitative PCR analyses. Clinical data were obtained through consultation of medical records and by sending a clinical data questionnaire to general practitioners and dermatologists. Clinical data included age at diagnosis, sex, site and size of primary MCC, clinical evolution (remission, recurrence, and most recent clinical status of patients), and presence of other skin malignancies (previous and/or concomitant). The stage of the disease at diagnosis was defined according to the seventh edition of American Joint Committee on Cancer (AJCC) staging manual based on primary tumor size (I, $\leq$ 2 cm and II, $>$ 2 cm) and the presence of regional lymph node metastasis (III) or distant metastasis (IV) at diagnosis [18]. The study was in accordance with the Declaration of Helsinki principles.