Merkel cell carcinoma: The first human cancer shown to be associated with a polyomavirus

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Summary

Merkel cell carcinoma (MCC) is a rare, malignant primary neuroendocrine cancer of the skin, usually affecting elderly, white people in sun-exposed areas. This is a highly aggressive tumor with strong propensity to metastasize. Surgery and radiation therapy remain the mainstay of treatment, with no curative treatment in case of disseminated metastases. Until 2008, MCC was thought to be caused by the malignant transformation of resident Merkel cells, but no investigation of a predominant molecular pathway that could be involved in MCC pathogenesis was successful. A real revolution in MCC understanding and management occurred in 2008, when a new human polyomavirus (MCPyV) was found to be the main etiological agent of this skin cancer. Following the discovery of MCPyV, the association of MCPyV with MCC has been confirmed worldwide, with detection of MCPyV in about 80% of MCCs. At the same time it had been shown that MCPyV infection is almost ubiquitous in healthy subjects, and MCPyV is thought to be persistent resident of the skin microbiome although the route of transmission, the host cell, the viral cycle and/or latency remain unknown. Most studies suggest that there may be two subtypes of MCC: MCPyV-positive (80%) and MCPyV-negative (20%) MCCs, and various studies have reported a better prognosis associated with MCPyV infection. The discovery of MCPyV in MCC patients opens up new therapeutic insights. The necessity and persistence of expression of MCPyV oncoproteins during MCC development make these proteins promising therapeutic targets.
Merkel cell carcinoma (MCC) is a rare skin cancer that has been shown to be caused by a human polyomavirus, i.e. Merkel cell polyomavirus (MCPyV). This discovery in 2008 renewed interest in polyomaviruses, known to be oncogenic in animals but never previously associated with a human cancer. MCPyV infection is ubiquitous, and MCPyV-driven oncogenesis seems to be distinct from other oncogenic viruses and remains widely unknown.

**Merkel cell carcinoma: a rare aggressive skin cancer**

MCC is a malignant primary neuroendocrine cancer of the skin. The denomination “MCC” is related to its presumed origin cell, i.e. Merkel cells, located in the basal part of epidermis. Merkel cells are receptor cells involved in skin mechanoreception, in association with sensory nerve endings in the upper part of dermis. Their cytoplasm contains dense core granules, as do other cells of the dispersed neuroendocrine system. Subsequently, when MCCs were first described in 1972 as “trabecular carcinomas” harboring similar dense coregranules, they were logically speculated to derive from the resident Merkel cells [1]. However, recent studies suggest that MCCs may have another origin, probably an unidentified progenitor cell in the epidermis or in the dermis [2].

Due to its rarity, little information is available on MCC epidemiology. Its incidence in the USA and Europe has been estimated to be 0.24 per 100,000 person-years [3] and 0.3 per 100,000 person-years [4], respectively, and to have increased over the last 20 years. MCC usually affects elderly, white people of both sexes, predominantly in sun-exposed areas. The most frequently affected sites are the head and neck (∼50%), trunk (∼30%), and limbs (∼10%) [3]. MCC can however affect any site of body, including the mucosae. Its clinical appearance is non-specific. The tumor presents as a dome-shaped erythematous or violaceous nodule with a smooth surface, and can be misdiagnosed as an epidermal cyst or a nodular basal cell carcinoma or a spinocellular carcinoma (figure 1). In later stages, the nodule may become ulcerated with satellite in transit metastases. Due to the rapidly growing course, the malignant nature of the nodule is usually obvious and requires the patient to be referred to a dermatologist rapidly for a diagnostic skin biopsy.

Diagnosis of MCC depends on histopathological examination (figure 2). The tumor is round-shaped and located in the dermis, usually without any connection with the epidermis. Tumor cells are hyperbasophilic, monomorphic, with scant cytoplasm and hyperchromatic nuclei. Immunohistochemistry investigation is mandatory for diagnosis, and MCC tumors...
display epithelial and neuroendocrine markers. More than 90% of MCCs express CK20, as well as a range of neuroendocrine markers (chromogranin A, synaptophysin, Neuron Specific Enolase, etc.). Negativity for TTF1 allows differential diagnosis from cutaneous metastases from small cell lung carcinoma.

MCC is a highly aggressive tumor with strong propensity to metastasize (figure 3). At presentation, 66% of patients present local disease, and 27% and 7% present regional and distant metastases, respectively [5]. Interestingly, almost 10% of patients have isolated lymph node MCC metastases with an unknown (or occult) primary [6,7].

Management of MCC patients depends on guidelines developed by American, German and French societies [8–10]. Disease staging at baseline requires clinical examination in order to detect any in transit and distant metastases. This is usually completed with an ultrasonography of the regional lymph nodes and a thoraco-abdominopelvian CT-scan to detect any occult metastases. Additional imaging with 18-FDG positron emission tomography or scintigraphy with somatostatin analogs can be discussed, but are not systematically required. This initial evaluation allows classification according to the AJCC stage that is based on the main prognostic factors identified so far (figure 4). Five-year survival ranges between 50 to 60% [3,5,11]. Five-year relative survival rates for local disease, regional nodal disease and metastatic disease are 64%, 39%, and 18%, respectively [12]. MCC treatment depends

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**Figure 3**
Numerous in transit cutaneous metastases of a MCC of the left thigh in an elderly woman (same patient as in figure 1)
on tumor stage and should be evaluated within multidisciplinary team discussions [8–10]. Surgery remains the mainstay of treatment for both localized and regionally extended disease (stages I, II and III). It requires wide excision of the primary tumor (2–3 cm) and any in transit metastases, completed with lymph node dissection in cases of clinically palpable lymph node metastases. When there are no clinically obvious lymph node metastases, a sentinel lymph node biopsy (SLNB) should be undertaken at the time of excision of the primary tumor. This procedure identifies occult lymph node metastases in about one third of patients, which should lead to a radical lymph node dissection.

Adjuvant radiation therapy (50 Grays) is systematically recommended at the site of the primary tumor. Radiation of the regional lymph nodes is recommended when the SLNB cannot be performed, and also after radical lymph node dissection for microscopic or macroscopic metastases. When the SLNB has been performed and there is no evidence of any metastases, radiation therapy of the lymph nodes can be avoided, although it can be discussed in head and neck locations and in the case of a primary tumor with aggressive features. Adjuvant radiation therapy has shown to be beneficial in terms of recurrence-free survival in several studies [12,13], but its effect on overall survival remains a matter of debate.

In cases of distant metastases (stage IV) that cannot properly be managed by surgery, the prognosis is poor and there is no curative treatment. Due to the rarity of MCC, randomized trials have been difficult to undertake and there is little evidence to determine the optimal strategy. Palliative cytotoxic chemotherapies include anthracyclines (doxorubicin), cyclophosphamide, etoposide, vincristine and platinum derivatives. Chemotherapy regimens, alone or in combination, can lead to tumoral responses in up to 60% of patients, but the duration of the response is limited to a few months and overall survival remains poor [14].

At the time of targeted therapies, many molecular targets and signalization pathways have been investigated in MCCs (C-KIT, PI3 K/AKT/mTOR, regulators of apoptosis, etc.) with no clear evidence of a key pathway. Some case reports have suggested the interest of tyrosine kinase inhibitors, somatostatin analogs, apoptosis analogs, and many early phase studies are currently under investigation in this field (reviewed recently [15]). Similarly, escape from the immune system seems to play a crucial part in MCC progression, and immunotherapies that have hopefully changed the management of metastatic melanoma, such as CTLA-4 or PD1 antagonists, should be soon evaluated in MCC patients [15].

The real revolution in MCC understanding and management occurred in 2008, when Chang and Moore’s team, from the Pittsburgh Cancer Institute, identified the implication of a new human polyomavirus in this skin cancer [16].

**Merkel cell polyomavirus: from discovery to understanding**

Until 2008, MCC was thought to be caused by the malignant transformation of resident Merkel cells, but no investigation of a predominant molecular pathway that could be involved in MCC pathogenesis was successful. Interestingly, Chang and Moore had already discovered that Kaposi sarcoma is closely related to the immune status of the patient is caused by an infectious agent, Human Herpes Virus 8. Knowing that the risk of MCC was increased in cases of immunosuppression (HIV infection, organ transplant recipients), the same team decided to investigate whether an infectious agent could cause this cancer. Using an approach called “digital transcriptome subtraction”, they extracted the transcripts from two MCC specimens and “subtracted” in silico all the known human cDNA sequences to demonstrate two polyomavirus-related sequences. This proved to be the discovery of a new human polyomavirus (the 5th human polyomavirus), which they subsequently called “Merkel cell polyomavirus” (MCPyV) [16]. After completion of the genome sequencing, they evidenced these sequences in 8 out of 10 MCCs. Moreover, they identified that the viral DNA was integrated in a clonal fashion between primary tumors and metastases in the tumor cell genome.

Polyomaviruses (family of Polyomaviridae) are quite close to the papillomaviruses in terms of genome organization, structure, ubiquity as well as oncogenic properties. Polyomaviruses are small (50 nm) non-enveloped viruses, with a circular double stranded 5.4 kb DNA. The genome encodes early non-structural proteins (‘‘T antigens’’, regulating virus replication) and late structural proteins (‘‘VP proteins’’, constituting the viral capsid) (figure 5). Polyomaviruses naturally infect many animals including mammals (particularly rodents and primates) and birds. Some animal polyomaviruses, including the murine polyomavirus (MPyV), the hamster polyomavirus (HαPyV) and the raccoon polyomavirus (RaPyV), have been shown to provoke tumors in their natural hosts. Moreover, the simian polyomavirus SV40 was proven to be oncogenic in rodent models. The oncogenic properties of polyomaviruses are related to the ‘‘T antigens’’ (T standing for ‘‘tumor’’) resulting from alternative splicing of the same transcript from the early region of the viral genome. The oncogenic mechanisms of polyomaviruses are largely unknown. The large T antigen (LTag) of SV40 and MCPyV contains highly preserved domains that bind to the suppressor tumor proteins p53 and pRB. There is here another strong similarity with the oncogenic properties of papillomaviruses, whose E6 and E7 oncoproteins also bind to P53 and Rb, respectively. The role of the small T antigen (sTag) is less understood, but is probably crucial. sTag binds to PP2A, that is involved in regulation of transcription and thus cell proliferation.

The oncogenic properties of polyomaviruses had not been evidenced in humans before 2008. The four polyomaviruses identified in humans were BKPyV (responsible for nephropathy...
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and graft loss in renal organ recipients), JCPyV (responsible for progressive multifocal leuco-encephalopathy in HIV patients), and WUPyV and KIPyV (identified in the respiratory tract with no associated disease). The discovery of MCPyV, the first human polyomavirus found to be associated with a human cancer, led to a huge increase in interest and advances in the field of polyomaviruses. Eight other human polyomaviruses have been identified from 2008, i.e. HPyV6, 7 and 9 (skin of healthy subjects), TSPyV (skin of patients with spinulosis trichodysplasia), MWPyV, SLPyV and HPyV12 (stools of healthy subjects) and NJPyV13 (endothelial cells from an immunosuppressed patient with vasculitis). Following the discovery of MCPyV, the association of MCPyV with MCC has been confirmed worldwide, with detection of MCPyV in about 80% of MCCs [17–19]. At the same time, it had been shown that MCPyV infection is almost ubiquitous in healthy subjects [20,21]. MCPyV DNA is identified in cutaneous swabs from a high proportion of healthy people [21], but MCPyV virions have never been identified in any of the sites investigated. Together with other polyomaviruses and papillomaviruses, MCPyV is currently thought to be a persistent resident of the skin microbiome (so-called, skin “virome”)

although the route of transmission, the host cell, the viral cycle and/or latency remains unknown [22]. Sero-epidemiological studies of MCPyV infection are based on assessment of humoral immunity against the highly immunogenic major capsid protein VP1 (anti-VP1 antibodies) [23]. The first detection of such antibodies, reflecting MCPyV primary infection, occurs before 5 years of age [24,25]. Anti-VP1 antibodies persist throughout life in more than 80% of healthy people [26]. MCPyV infection is thus ubiquitous, the primary infection is asymptomatic, and the virus persists in a latent or at least minimally replicative state throughout life in an unidentified location.

The ubiquity of MCPyV infection has led to controversy regarding its etiological role in MCC pathogenesis. However, its involvement in MCC pathogenesis is supported by several arguments, with the result that MCPyV is classified as a class 2A carcinogen by the IARC [27]. First, MCC patients carry higher MCPyV viral loads than healthy subjects and have higher anti-VP1 antibody titers. More importantly, MCPyV in MCC tumors has unique characteristics, considered as a “molecular signature” specific to MCCs. First, the MCPyV genome is clonally integrated in MCC tumor cells [16]. Genome integration is crucial for other oncogenic viruses (human papillomaviruses, hepatitis B virus) but has not been evidenced in oncogenic polyomaviruses other than MCPyV. MCPyV integration can occur anywhere in the host cell genome, without any integration “hot spots” that could explain activation/inactivation of host gene promoters [28]. MCPyV integration is associated with either mutations or deletions, leading to stop codons that truncate the C-terminal part of the LTAg protein [29]. This double phenomenon (integration, truncation) seems to be necessary for the “harmless resident MCPyV” to become an “oncogenic MCPyV”.

The truncation of the C-terminal part of the LTAg protein leads to loss of replicative properties (abortion of virus cycle and virion production) and overexpression of a truncated LTAg that could promote tumor transformation. Interestingly, LTAg truncation leads to the loss of the P53-binding domain, indicating that the oncogenic mechanism associated with MCPyV is unique and distinct from papillomaviruses and other polyomaviruses. Experimental findings have shown that the expression of the LTAg and STAg in MCPyV-positive MCC cells is necessary for the maintenance of tumor cell lines [30–32]. The Rb-binding domain of the truncated LTAg is also necessary for maintenance of cell lines [33]. Moreover, the expression of STAg is able to initiate transformation of rodent fibroblasts [32]. Further research must be undertaken to understand fully the oncogenic stages of MCPyV in the development of MCC.

Investigation of the Merkel cell polyomavirus in MCC patients

MCPyV markers have been widely investigated in MCC patients since 2008, with conflicting results. First, it is not known whether

MCPyV genome
Circular, double stranded DNA. Early genes encode regulatory, oncogenic proteins (LTAg, STAg). Late genes encode structural proteins (VP1, VP2 and VP3). The NCCR (non-coding control region) regulates viral gene expression and replication.

Source: Reference: Merkel cell polyomavirus isolate MCVw156, complete genome, GenBank: HM355825 1)
all MCC tumors are related to MCPyV infection. MCPyV being the unique cause of MCC was supported by one study reporting nearly 100% MCPyV DNA and LTag expression in the MCC tumors investigated [34]. However, most studies suggest that there may be two subtypes of MCC: MCPyV-positive (80%) and MCPyV-negative (20%) MCCs. MCPyV-negative MCCs are thought to result from alternative oncogenic pathways, mostly involving UV-induced DNA damage and chromosomal aberrations [35]. Another hypothesis is that all MCCs are initiated by MCPyV infection, a small proportion of which subsequently “lose” the MCPyV genome (“hit-and-run phenomenon”) [36]. It seems that MCPyV-positive and MCPyV-negative MCCs display distinct phenotypes and evolution—though the latter issue is a matter of debate [26,37,38]. Various studies have reported a better prognosis associated with MCPyV infection (assessed by higher intratumoral viral loads or LTag expression, and higher plasma anti-VP1 antibody titers). It was also evidenced that the humoral response against TAg (anti-TAg antibodies) was detected in about 40% of MCC patients and not in controls, thus constituting a specific marker of the disease. Interestingly, the anti-TAg antibodies were related to the disease burden. They progressively became undetectable in disease-free patients whereas they were increased in cases of disease progression and recurrence [39]. MCPyV markers may therefore be of prognostic value for this cancer, both at baseline and for disease monitoring. Moreover, the discovery of MCPyV in MCC patients opens up new therapeutic insights. The necessity and persistence of expression of MCPyV oncoproteins during MCC development makes these proteins promising therapeutic targets. 

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