MERKEL CELL CARCINOMA: ADVANCES IN PATHOGENESIS, DIAGNOSIS AND PROGNOSTIC FACTORS

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Rezumat

Carcinomul cu celule Merkel (CCM) este o tumoră neuroendocrină a pielii cu grad ridicat de agresivitate, caracterizându-se prin o probabilitate mare de recurență și metastazare. Cu toate că aceasta este considerată o formă rară de cancer, recent s-a observat o creștere rapidă a incidenței. De aceea lumea de specialitate depune eforturi sporite în definirea ei. Articolul de față tratează atât aspectele cunoscute ale acestei tumori, cât și aspecte controversate legate de patogenia, diagnosticul și factorii de prognostic ai CCM.

Cuvinte cheie: *Carcinom cu celule Merkel, Merkel poliomavirus celule, căi oncogenice, imunohisto-chimie, prognostic.*

Summary

Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine skin tumor, characterized by high incidence of recurrences and metastases. It is a relatively unusual form of cancer however, its incidence is rapidly increasing. Therefore, the interest in defining this disease has escalated rapidly. This article addresses what is known and what is still controversial about the pathogenesis, diagnosis and prognostic factors in Merkel cell carcinoma.

Keywords: *Merkel cell carcinoma, Merkel cell polyomavirus, oncogenic pathways, immunohisto-chemistry, prognosis.*

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Introduction

Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine skin cancer, characterized by high incidence of local recurrences, regional nodal and distant metastases. Mortality rates are estimated to 33% within 5 years, the highest of any dermatological cancer [1]. It is a relatively uncommon form of cancer however its incidence had increased in the last years which had triggered a higher interest in better defining the pathogenesis of this disease and searching for new therapies. MCC has been first described in 1972 by Toker and named trabecular carcinoma [2]. Later studies found neurosecretory granules in the cytoplasm of tumor cells, similar to those seen in non-neoplastic Merkel cells (MC) which resulted in renaming the tumor as MCC.

Although it is believed that MCC originates from MC, there is some controversy regarding the "cell-of-origin". The traditional view is sustained by a similar (but not identical) immunophenotype. It is also generally accepted that MCs are of neuroendocrine origin, being the only cutaneous cells that form electron-dense neurosecretory granules, a histologic feature also

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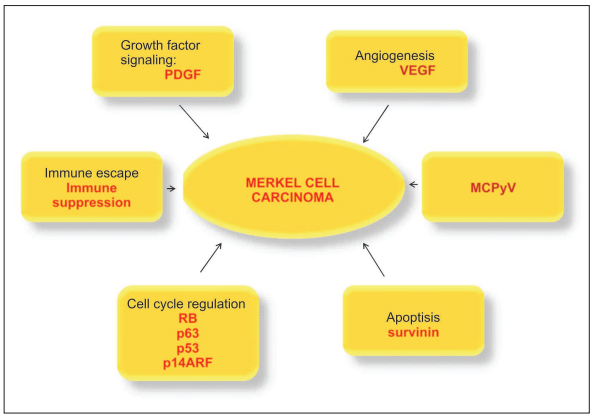

Fig. 1. Main factors implicated in the pathogenesis of Merkel cell carcinoma

discovered in MCC. Some authors advance the hypothesis that MCC originates in the pluripotent epidermal stem cells. This idea is supported by the squamous differentiation observed in certain cases of MCC and its metastases and by the frequent association of MCC with squamous cell carcinoma [6]. Recently, both ideas have been linked by the proof that mammalian MCs develop from epidermal stem cells, rather than from neural crest progenitors [3-5].

The pathogenesis of MCC is not completely elucidated. Frequent associations with exposure to ultraviolet (UV), ionizing, and infrared radiation have been reported [6-8]. Many studies in the literature have described the association between MCC and iatrogenic immunosuppresion, the tumor being noted in almost every autoimmune disease, with an increasing incidence with the use of tumor necrosis factor (TNF) inhibitors [9]. MCC has also been reported after organ transplantation, especially in renal-transplant recipients. These observations support the idea that chronic immunosuppression increases the risk of MCC [10-11]. One of the most important step in understanding the etiology of the disease was the discovery of a novel polyomavirus in MCC tumor specimens [12]. Several oncogenic pathways have also been studied [13-16]

The clinical presentation of this tumor is nonspecific. It is usually described as a painless indurated pink to reddish-blue or brown intradermal nodule with a rapid growth, on the sun-exposed areas of elderly. The unspecific manifestations, mimicking many benign and malignant skin tumors make MCC difficult to diagnose clinically before biopsy. The most common presumptive diagnosis at early stages is cyst. Thus, the diagnosis is made by histopathologic examination and immunohistochemistry [3,17].

Various factors have been described as indicators of a poor prognosis. In the current paper, we review the advances in pathogenesis diagnosis, and potential prognostic factors. (Fig. 1)

Merkel cell polyomavirus and its role in pathogenesis

As mentioned before, MCC was clearly associated with UV exposure and immunosuppression but little was known about the pathological mechanisms involved in tumorigenesis. However, Feng et al [12] revolutionized our understanding of this tumor by discovering a novel viral agent that integrates in the genome of MCC tumor cells: Merkel cell polyoma virus (MCPyV). Further studies demonstrated that viral integration preced clonal expansion, MCPyV being found at distinct sites in different MCCs [18].

MCPyV is a member of Polyomaviridae family, now constituted as a single genus (Polyomavirus). It is a 40 nm non-enveloped double stranded DNA viruse, whose genome consists of approximately 4700-5400 base pairs and codes for 3 structural proteins and few early and late proteins. Early proteins, particularly small and large T antigens, promote genome replication and tumorigenesis, while the late region encodes viral capsid proteins [19]. Large T antigen has the ability to bind DNA polymerase, primase, topoisomerase I and tumor suppressor proteins, such as retinoblastoma protein (pRb) and p53. This antigen seems to possess features commonly seen in oncogenic polyomaviruses, such as the LxCxE motif which can directly bind pRb. Large T antigen also possesses a helicase motif required for virus replication. MCPyV small T cell antigen has the property to inactivate protein phosphatase 2A. Upon integration into the host genome, MCPyV DNA generally harbors tumor-specific mutations, including deletion or truncation at carboxyl-terminal half of the large T antigen... Taken together, these findings lend support to the hypothesis that MCPyV viral proteins are involved in oncogenesis [20-22].

However, approximately 20% of MCCs are negative for this virus, so it is not "necessary" for tumorigenesis. From another point of view, only a small proportion of people infected with MCPyV develop the disease [23]. It was suggested that MCPyV-negative MCCs have fewer genomic deletions than MCPyV-positive MCCs [24]. Added to this, it was highlighted that expression of p53 and KIT was accompanied by the absence or low copy numbers of MCPyV DNA [25].

Seropositivity rates for antibodies against viral capsid proteins 1 (VP1) range from 40 to 88% [26-29]. Despite this relatively high prevalence, the antibody titers are higher in patients with MCC than in general population and seem to correlate with the presence of MCPyV in the skin [30-31]. In contrast, there was no correlation between tumor characteristics and viral load or antibody titer. However, higher antibody titers seem to improve the prognosis, suggesting a strong immune response [32]. A number of studies have shown that MCPyV-positive cases tend to have a better clinical outcome than the negative ones [33,34], while other studies did not find any differences [35]. Cases with MCPyV-positive tumors and an aggressive course have also been described [36].

Oncogenic pathways

The most important processes involved in carcinogenesis are the activation of growth-stimulating oncogenes and inactivation of tumor suppressor genes. However, many studies revealed little involvement of several known oncogenic pathways such as: antiapoptotic Bcl-2 family, Wnt pathway, MAP kinase signaling pathway [37]. PTEN does not seemingly have an important role in MCC oncogenesis, although loss of heterozygosity is a frequent event [38]. Mutations in other tumor suppressor genes such as p73 and CDKN2A were rarely observed in MCC [39,40]. Wnt pathway has also been evaluated, but mutations in β-catenin, APC, AXIN1 or AXIN2 were not identified [41].

Although several studies reported that p53 is not a key protein in the pathogenesis of MCC, Lassacher et al [14] suggested the involvement of p14ARF/mdm2/p53 pathway in a subset of MCCs. The authors demonstrated hypermethylation in the promoter region of p14ARF in 42% of cases, resulting in an altered expression pattern of the gene. The classical mitogen-activated protein (MAP) kinase signaling pathway, one of the most studied pathways in oncogenesis, has a central role in proliferation, cell-cycle arrest, suppresion of apoptosis, migration and teminal differentiation. It is activated in several cancers through mutations involving of tyrosine kinase receptors (EGFR, KIT, HER2), RAS family (H-RAS, K-RAS, N-RAS), and B-RAF [42]. However, several studies showed negativity for H-Ras, K-Ras, N-Ras [13]. B-RAF, [43], EGFR, and Her2 expression [44].

In contrast, KIT protein, a receptor belonging to the PDGFR family, is overexpressed in 59-95% of MCCs. KIT protooncogene, located on chromosome 4p, encodes 976 amino-acid protein (CD117 or KIT) containing an extracellular, transmembrane, juxtamembrane and tyrosine kinase domain. KIT activation is followed by processes like receptor dimerization and internalization, substrate phosphorylation and autophosphorylation, activation of protein kinases and phospholipases and transcription of different proto-oncogenes. Mutations in KIT gene have been identified in several tumors such as gastrointestinal stromal tumors (GISTs), mast cell neoplasms or melanoma and are believed to play a central pathogenetic role [45-48]. Activating mutations of KIT gene in GISTs were identified in exon 11 encoding the juxtamembrane domain, exons 13 and 17 that encode the tyrosine kinase domain, and less frequently in exon 9 enconding the extracellular domain [49,50]. Investigations of the same exons have failed to find any mutations in MCCs [48,51]. More recently, activating mutations of the platelet-derived growth factor receptor alpha have been identified in 33% of MCC cases [52].Activation of the PI3K/AKT signaling pathway represents one of the most frequent events in human cancer. Oncogenic activity is sustained by the identification of somatic mutations in the PIK3CA gene in a wide range of tumors [53-54]. Oncogenic mutations have also been noted in certain domains of AKT1[55]. Hafner et al [56] included MCC in the list of cancers harboring PIK3CA mutations. The authors demonstrated that AKT phosphorylation and

PI3K/AKT pathway activation was not correlated with the presence of MCPyV and oncogenic PIK3CA mutations in MCC. The low frequency of PIK3CA mutations indicates that additional mechanisms contribute to the activation of this pathway in MCC. Additionally, mutations in ATOH1, a tumour suppressor gene encoding a transcription factor involved in Merkel cell differentiation, have been reported [57].

Histologic parameters and prognosis

The typical histological finding is a dermal tumor with extensions to underlying subcutaneous tissue. The epidermis, papillary dermis and adnexal structures are usually spared. Apoptotic cells and mitotic figures are frequently seen. The small blue cells with sparse cytoplasm are characteristic. The nuclei are hyperchromatic without prominent nucleoli and there is a very high nuclear cytoplasmic ratio. The chromatin is displayed in typical salt and pepper pattern. Histologic variants include a large cell type (with a trabecular pattern), an intermediate-cell type, and a small-cell type with resemblance to skin metastases of small-cell lung carcinoma (SCLC). If the architecture is considered, MCC are classified into a solid or organoid variant, a diffuse variant, and a trabecular variant (the least common pattern) [58].

Regarding tumor staging, low stage predicts a relatively good prognosis , but it is not an ideal indicator as a significant proportion of patients with stage I still die within 5 years from diagnosis [59]. Furthermore, five inconsistent staging systems have been proposed during the past 20 years [60]. Thus, alternative independent pathologic markers of prognosis are helpful, especially for low stage tumors,. Tumor size has been found to correlate with survival in clinical stage I, but it loses significance in higher stages [61,62]. Sandel et al [63] found no correlation between tumor size and prognosis.

Histologic features associated with a poor prognosis include a mitotic rate of more than 10 per high power field, small cell size, and a diffuse growth pattern. Other studies failed to find any association between prognosis and tumor pattern or mitotic rate. Regarding the depth of invasion, not all studies agree. Average tumor thickness associated with the risk of distant metastases has been reported in MCC to be close to 10 mm, althowgh other studies have found no correlation between tumor thickness and overall survival . The extension of the tumor into the underlying subcutaneous tissue was correlated with a shortened survival, but another study failed to confirm this finding. It was also suggested that mast cell infiltrates have a negative prognostic value in MCC. Lymphovascular invasion was found to affect the prognosis in one study, but in other studies it was not significantly associated with prognosis. The relation between the associated inflammatory infiltrate and prognosis is contradictory reported. Other histological markers such as ulceration, tumor necrosis, or apoptosis did not reveal any correlation with prognosis [62-67].

Immunohistochemistry: diagnostic and prognostic tool

The diagnosis of MCC is challenging, often being confused with benign conditions such as cyst, lipoma, dermatofibroma or vascular lesions [68]. Therefore, the immunohistochemistry is necessary for diagnosis. The tumour expresses both neuroendocrine and epithelial markers. Neuroendocrine differentiation is sustained by the positivity for neuronspecific enolase (NSE), chromogranin A (CrA) and synaptophysin. These markers show a diffuse cytoplasmic pattern. The tumor can also express other neural markers such as neurofilaments, microtubule-associated protein (MAP) 2, CD56, and CD57. S-100 is negative in most cases [69]. CK20 is the most important epithelial marker, the paranuclear dot-like staining being characteristic. MCC is also frequently positive for other epithelial markers, including AE1/AE3, CAM5.2, and Ber-EP4 [70]. CK7 is rarely positive, and a few CK7+/CK20- cases have been reported [71]. (Table 1)

MCCs share many histological features with small cell carcinomas of other organs, including

Table 1. Positive IHC markers in MCC

Neuroendicrine markers	Epithelial markers
NSE	CK20
CrA	AE1/AE3
Synaptphysin	Ber-EP4
MAP2	CK7 (rarely)
CD56	
CD57	

SCLC [72]. For this reason, IHC plays an essential role for differentiation between these entities. CK7/CK20 staining patterns are useful in some cases. A significant proportion of MCC show a CK7(-)CK20(+) pattern, whereas some of the small cell carcinomas of the lung are CK7(+)CK20(-). There are lots of cases of both entities that express neither CK7 nor CK20. Even CK7(+)CK20(-) or CK7(+)CK20(+) MCC cases have been reported [72,73]. Therefore, thyroid transcription factor-1 (TTF-1), a tissue specific transcription factor expressed in epithelial cells of the thyroid, lung and brain, is a very useful marker for differentiation. Combining TTF-1 with CK-20 offers a strong basis for diagnosis. MCC is CK-20 positive and TTF-1 negative, while SCLC is TTF-1 positive [74-79]. Ralston *et al* [80] proposed MASH1 (achaete-scute complex-like 1, ASCL1) for discriminating between the two conditions. According to their study, 83% of small cell carcinomas of the lung expressed MASH1, and only 73% expressed TTF-1. MASH1 was negative in all MCC, while TTF-1 was expressed in 3%of MCC. MASH1 can be used together with TTF-1, CK7 and CK20 in a panel of immunostains, to facilitate the diagnosis [81]. A monoclonal antibody (CM2B4) can identify MCPyV-positive MCCs with diffuse nuclear staining in immunohistochemistry, with approximately 80% sensitivity compared with polymerase chain reaction [82].

Immunohistochemistry can also be to investigate the prognosis in MCC. To date, various prognostic markers have been reported. However, the lack of standardized methods makes the results considerably variable, only a few markers sustaining their prognostic value [70].Ki-67, the most useful marker of cell proliferation, correlates with evolution towards metastasis and poor prognosis in various studies [65,82,83]. Although p53 was reported to correlate with a poor prognosis [25,84], this finding was not confirmed by others [83,85,86]. Another IHC marker with prognosis value is the p63 protein, a member of the p53 family of transcription factors. Asioli et al [86], demonstrated an aggressive course, with poor overall survival in patients with p63 positive tumors. Survivin, an antiapoptosis protein, is expressed in most cancers, including MCC, while normal cells lack its expression. It was shown that nuclear expression was correlated with an aggressive course of MCC, whereas a predominantly cytoplasmic staining correlated with disease-free survival [87].A differential subcellular division of survivin was correlated with prognosis. The authors suggest that survivin staining can provide important information concerning the possibility for therapeutic intervention, contrasting with other prognostic markers [88].

As mentioned above, a significant percentage of MCCs express KIT, but it was not correlated with prognosis in most studies. [45,47,65,85] Nevertheless, Andea et al. demonstrated that a high KIT expression may contribute to poorer survival rates [89]. KIT expression was also associated with lymphovascular invasion and high mitotic rate, suggesting a more aggressive course [47].

Overexpression of a large number of other IHC markers have been associated with metastasis: vascular endothelial growth factor (VEGF), p38, matrix metalloproteinase (MMP) 7, MMP 10/2, tissue inhibitor of metalloproteinase 3, stromal NF-kappa B, synaptophysin, CD44, and CD151 [.90-92]. In contrast, CD9 expression was significantly correlated with better overall survival [92] (Table 2)

Table 2. IHC markers for poor prognosis and metastasis

Ki-67
p63
Nuclear expresion of survivin
high KIT expresion
VEGF
p38
MMP 7 and MMP 10/2
tissue inhibitor of metalloproteinase 3
stromal NF-kappa B
Synaptophysin
CD44
CD151

Conclusions

Increasing incidence and mortality of MCC make the early diagnosis and identification of prognostic factors mandatory. Viral and cytogenetic studies represent an exciting direction for future studies to determine the oncogenic events involved in promotion or maintenance of MCC. Furthermore, the understanding of characteristic signal transduction pathways will provide opportunities to explore new targeted therapies for this aggressive tumor.

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Conflict de interese
NEDECLARATE

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NONE DECLARED

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