Ubiquitous Merkel Cell Polyomavirus: Causative Agent of the Rare Merkel Cell Carcinoma

Naveed Shahzad, Usman Shah Gilani, Menahil Mahmood, Fareeda Tasneem, Muhammad Farhan Ul Haque, and Iqra Hussain

Abstract Merkel Cell polyomavirus (MCPyV) is the only member of the *Polyomaviridae* family that is directly linked to a type of human cancer, Merkel Cell Carcinoma (MCC). Predominantly, the clinical significance of MCPyV is due to the aggressive nature of MCC with a low survival rate and, presently, unavailability of a comprehensive and effective treatment regime. Secondly, the molecular mechanisms of MCPyV infection and oncogenic potential are currently poorly understood. Despite MCPyV ubiquitously infects humans, studies suggest that MCC is caused only after prolonged infection and integration of MCPyV DNA in the host genome. Mechanistically, transformation of normal cells into cancerous by MCPyV is mainly driven by truncated LT and sT antigens and the abnormal molecular modulation they carry out. Presently, conventional treatment options against MCC like surgery, radiotherapy and chemotherapy are on board, but, with limitations that pose them inadequate. However, modern treatment options are emerging which, in preliminary investigation, show great promise. However, extensive exploration needs to be carried out before their large scale acceptance and application as therapies against MCC.

1 Introduction

Polyoma viridae is a family of ubiquitous, icosahedral and non-enveloped viruses that contain small circular ds DNA genomes (DeCaprio and Garcea 2013). Till now, 14 different species of human polyoma viruses have been identified. Some of these polyomaviruses such as BKPyV, JCPyV, WUPyV, HPyV6, HPyV7 and TSPyV have been associated with human malignancies (Table 1). However, Merkel cell polyomavirus (MCPyV) is the only known human polyomavirus that is

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Table 1 List of human polyomaviruses and their associated diseases

Sr. no.	Virus (Abbreviations)	Discovery (Year)	Sample	Associated diseases	References
1.	BK polyomavirus (BKPyV)	1971	Urine	Polyomavirus-associated nephropathy (PVAN), haemorrhagic cystitis	Gardner et al. (1971)
2.	JC polyomavirus (JCPyV)	1971	Urine, Brain	Progressive multifocal leukoencephalopathy	Padgett et al. (1971)
3.	Karolinska Institute polyomavirus (KIPyV)	2007	Nasopharyngeal tissue	Unknown	Allander et al. (2007)
4.	Washington University polyomavirus (WUPyV)	2007	Nasopharyngeal tissue	WU-PyV-associated bronchitis,	Gaynor et al. (2007)
5.	Merkel cell polyomavirus (MCPyV)	2008	Lesion	Merkel cell carcinoma	Feng et al. (2008)
6.	Human polyomavirus 6 (HPyV6)	2010	Skin	Unknown	Schowalter et al. (2010)
7.	Human polyomavirus 7 (HPyV7)	2010	Skin	HPyV7-associated keratosis	Schowalter et al. (2010)
8.	Trichodysplasia spinulosa polyomavirus (TSPyV)	2010	Lesion	Trichodysplasia spinulosa	van der Meijden et al. (2010)
9.	Human polyomavirus 9 (HPyV9)	2011	Skin, Blood, Urine	Unknown	Scuda et al. (2011)
10.	Malawi polyomavirus (MWPyV)	2012	Stool, Wart	WHIM syndrome	Siebrasse et al. (2012)
11.	Human polyomavirus 12 (HPyV12)	2013	Stool	Unknown	Korup et al. (2013)
12.	St Louis polyomavirus (STLPyV)	2013	Stool	Unknown	Lim et al. (2013)
13.	New Jersey polyomavirus (NJPyV)	2014	Muscle Biopsy	Unknown	Mishra et al. (2014)
14	Lyon IARC polyomavirus (LIPyV)	2017	Skin	Unknown	Gheit et al. (2017)

convincingly linked with the development of any human cancer, Merkel cell carcinoma (MCC), a relatively rare but aggressive neuroendocrine skin cancer (Feng et al. 2008; Wollebo et al. 2015; Harms et al. 2018). The MCPyV sequences in humans were first reported in 2008 by Moore and Chang while they were identifying non-human stretches of DNA in human cancerous tissue using a technique named as Digital Transcriptome Subtraction (DTS) (Feng et al. 2008). They associated MCPyV infection with MCC on observing clonal integration of MCPyV DNA in cancerous Merkel cells in more than 80% of cases. The MCPyV infection and integration of MCPyV DNA was reported to occur before the clonal expansion of MCC cells which further strengthens the etiological role of MCPyV in MCC. Following that, MCPyV was classified as a 2A carcinogen (Bouvard et al. 2012), and numerous studies described various mechanisms by which MCPyV transforms normal cells cancerous. Interestingly, the presence of MCPyV DNA is not limited to Merkel cells alone, but is also detected in a variety of other tumor types. These include non-melanoma skin cancer (Kassem et al. 2009), chronic lymphocytic leukemia (Pantulu et al. 2010; Teman et al. 2011), cutaneous squamous cell carcinoma (Murakami et al. 2011), cervical cancer (Imajoh et al. 2012), non-small-cell lung cancer (Hashida et al. 2013), CNS tumors (Sadeghi et al. 2015), and breast cancer (Reza et al. 2015). Although these studies suggest the presence of MCPyV, however, its causal and mechanistic role in these cancers is still unclear. This chapter focuses on molecular features of MCPyV, its ubiquitous presence in human populations across the world, and most importantly the mechanisms utilized by the virus to induce MCC. It also tends to describe available therapeutic modalities against MCC.

2 Genomic Organization and Molecular Virology of Merkel Cell Polyomavirus

The genome of MCPyV is circular and measures approximately 5.4 kb for the prototype MCV350 strain and the majority of other sequenced strains. Like other polyomaviruses, the MCPyV genome is divided into early and late gene cassettes which encode T antigens (LT, sT, 57kT and ALTO), structural proteins (VP1, VP2), and a miRNA, respectively (Fig. 1a). The early and late coding regions are separated by a regulatory non-coding region known as non-coding control region (NCCR). It includes the origin of replication, clustered binding sites for LT which are required for DNA replication (Kwun et al. 2009), and bi-directional promoter elements which control transcription of early and late genes and allows temporal regulation of gene expression (Feng et al. 2011).

Structurally, MCPyV early region is transcribed into four mRNAs (T1–T4) that are alternatively spliced and encode LT (T1), sT (T2 and T3), and 57kT (T4) proteins. As a consequence of alternative splicing, all of these antigens have common 78 amino acid sequence at their N-terminal that contains epitopes of B

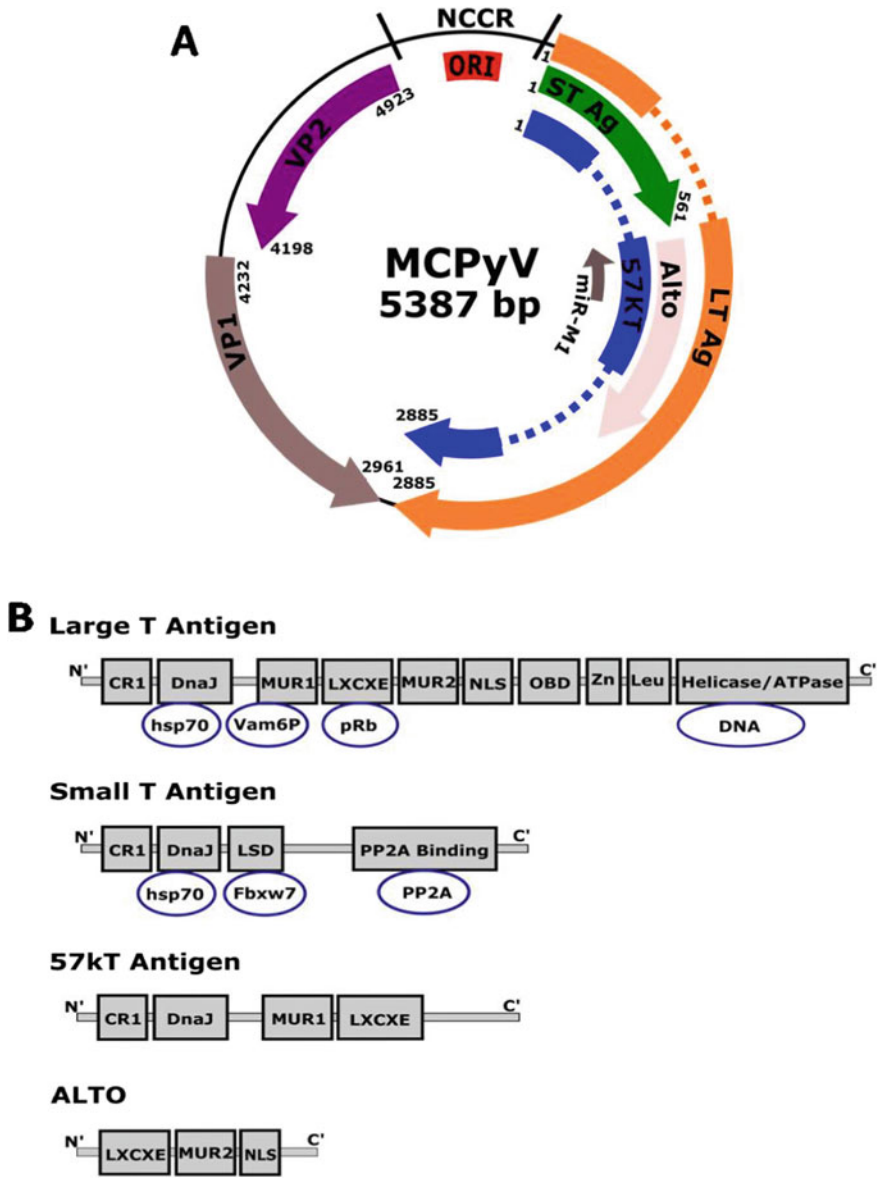

Fig. 1 Organization of MCPyV Genome. **a** The MCPyV early region encodes LT, sT and 57 kT whereas late region encodes VP1, VP2 and miRNA. **b** Interaction of MCPyV early gene products with various cellular host proteins

cells (Shuda et al. 2008). Through leaking scanning of early transcripts, an additional gene product of unknown function termed ALTO (Alternative Open Reading Frame) can be produced (Carter et al. 2013). Unlike early mRNAs, the late transcripts have not been described structurally. Although, it is known that the major and minor capsid proteins, VP1 and VP2, are encoded by open reading frames in this region. Moreover, in this open reading frame, despite the presence of an AUG codon that could initiate transcription of potential VP3, the Kozak consensus sequence is lacking, suggesting that MCPyV does not express a functional VP3 and it is also found to be absent in native MCPyV virions (Schowalter and Buck 2013). Additionally, mcv-miR-M1 is a miRNA encoded by MCPyV genome (Seo et al. 2009). It is located in antisense orientation to the early gene cassette and thus has the ability to direct cleavage of early transcripts (Seo et al. 2009; Grundhoff and Sullivan 2011; Kincaid and Sullivan 2012).

Functionally, immediately after infection, the early coding region, also called the T antigen locus, expresses its gene products which are mainly involved in the replication of viral genome. It has been described that MCPyV early gene products are expressed in an orderly manner where LT and 57kT are expressed first, followed by the expression of sT (Feng et al. 2011). After DNA replication, the late coding region becomes transcriptionally active and expresses viral structural components which assemble and result in the formation of progeny virions during the later stage of infection (Cole 2001).

Products of polyomavirus early regions are known to target cellular proteins involved in tumor suppression and cell cycle regulation (Fig. 1b). These gene products are called tumor antigens or T antigens and are important to initiate the synthesis of viral DNA. The LT antigen of MCPyV has certain conserved regions including the conserved region 1 (CR1) (LXXLL) and the heat shock protein binding DnaJ (HPDKGG) domains, an Origin Binding Domain (OBD), a pRb binding domain (LXCXE) and a helicase domain (Stakaitytė et al. 2014). In between the pRb binding domain and the OBD, there is a Nuclear Localization Signal (NLS) (Nakamura et al. 2010). Domains of the LT that are involved in viral DNA replication are mostly located at the C-terminal such as the OBD, helicase and ATPase domains. The C-terminal of LT is also where most of the tumor specific mutations occur (Feng et al. 2008). A mechanism unique for MCPyV LT is redistribution of LT to the cell's nucleus by binding with Vam6p through the MCPyV Unique Region (MUR) which is of about 200 amino acids and located at the beginning of the second exon close to the pRb binding (LXCXE) domain. The pRb binding domain of the MCPyV LT also contains a unique spacer region which separates the LXCXE motif from the 'Psycho' domain and also includes the viral micro RNA complementary sequence (Johnson 2010). This spacer region is present in the MUR and is unique to MCPyV, but its effect on the function of LT has not yet been elucidated.

The small T antigen (ST), also encoded by the same T antigen locus, has the same N-terminal as that of LT and 57kT, and thus possesses the CR1 and DnaJ domains. However, transcription beyond the first exon splice site results in the formation of a smaller protein of 186 amino acids which includes a protein

phosphatase 2A (PP2A) binding site in its C-terminal. The ability of sT to bind to PP2A has been conserved in various polyomaviruses and is thought to be important in cellular transformation induced by polyomaviruses (Pallas et al. 1990). PP2A is an enzyme that removes phosphate group from the eukaryotic initiation factor 4E binding protein 1 (4E-BP1) and allows it to bind to the eukaryotic initiation factor 4E (eIF4E) to inhibit translation. Recent studies indicate that the polyomavirus sT binds to the PP2A Aα subunit and does not allow it to perform its phosphatase activity. Thus 4E-BP1 remains phosphorylated and is unable to sequester eIF4E, and translation continues to allow cells to proliferate (Shuda et al. 2011). Hence MCPyV sT is important for both viral DNA replication and cellular transformation.

Another T antigen expressed by the early coding region is the 57kT protein which is formed by the alternative splicing of a transcript to combine three exons that code for a 432 amino acid protein sharing many features with the LT and sT antigens. The 57kT N-terminal is the same as the other two T antigens with CR1 and DnaJ domains, and it also includes the pRb binding motif, MUR, and most of the C-terminal amino acids present in LT. Information about the role of 57kT in viral life cycle or virus induced tumorigenesis is still insufficient to depict a clear mechanistic picture. Certain studies consider 57kT as an analog of the SV40 17kT antigen which functions independently or with other T antigen to control cellular proliferation in vivo (Comerford et al. 2012).

The non-coding regulatory region (NCRR) includes a minimum 71 bps long origin of replication. Similar to other polyomaviruses, the origin of replication of MCPyV has an AT rich region, a large T binding domain and an early enhancer region. The central portion of the LT protein contains an Origin Binding Domain (OBD) that binds to the pentanucleotide consensus sequences G(A/G)GGC in the origin of replication. This binding then allows LT to perform its activity as a helicase and initiate the replication of viral DNA (Cole 2001). The origin of replication of MCPyV, however, includes more of these pentanucleotide sequences which also lie in closer proximity as compared to in other polyomaviruses (Johnson 2010). This arrangement allows OBD-OBD interactions between different LT proteins bound at the origin (Harrison et al. 2011). However, binding of LT to origin does not require these OBD-OBD interactions and these intermolecular interactions also do not influence the structure of viral DNA. No such interactions have been observed in SV40 as its pentanucleotide sequences are more spatially arranged, thus it indicates that MCPyV replication and LT seeding is more complex than other polyomaviruses (Meinke et al. 2007).

Major capsid protein named the viral protein 1 (VP1) and the minor viral capsid protein 2 (VP2), encoded by the late region, have the function to form the capsid of the viral particle. These proteins also have the ability to self-assemble into 45–55 nm diameter virus-like particles (VLPs) when expressed in mammalian or insect cells. Majority of the serological assays being developed for MCPyV are based on these VLPs (Tolstov et al. 2009, 2011; Touzé et al. 2010, 2011; Viscidi et al. 2011). The size of MCPyV virion is comparable to the size of other polyomavirus particles (Neumann et al. 2011).

The MCPyV also express a 22-nucleotide long miRNA called mcv-miR-M1 that negatively regulates the early gene expression during late phases of virion encapsidation. It also causes cleavage of early mRNA leading to the reduced expression of LT. In addition, it interacts with several cellular targets including PIK3CD and PSME3. This could potentially mediate the host immune response against MCPyV through down-regulation of PSME3-dependent antigen presentation by the host cell. The mcv-miR-M1 also plays an important role in viral replication as its expression level correlates with the viral genome copy number in MCC tumor. The MCPyV miRNA may decrease viral replication to increase viral persistence, as observed in case of BKPyV (Broekema and Imperiale 2013).

3 Frequency of MCPyV Infection in Human

The prevalence of MCPyV in healthy individuals is usually evaluated at two levels; seroprevalence and the presence of MCPyV DNA in host cells. Regarding seroprevalence, it is interesting to note that in healthy individuals, MCPyV seroprevalence is very high. This is mainly attributed to the primary, asymptomatic MCPyV infection thought to occur in early childhood (Carter et al. 2009; Kean et al. 2009; Pastrana et al. 2009; Tolstov et al. 2009, 2011; Sadeghi et al. 2010; Touzé et al. 2010; Chen et al. 2011; Faust et al. 2011; Touzé et al. 2011; Viscidi et al. 2011). The idea of early age infection is acquired by observing the seroprevalence of MCPyV to be age-specific. Among children of the age group 1–5 years, 20% are positive (Kean et al. 2009), and of the age group <10–15 years, 35–50% are positive (Tolstov et al. 2009; Chen et al. 2011; Viscidi et al. 2011). In adults, seroprevalence of 46–87.5% has been reported (Carter et al. 2009; Kean et al. 2009; Pastrana et al. 2009; Tolstov et al. 2009, 2011; Touzé et al. 2010, 2011; Viscidi et al. 2011). This huge variation in prevalence is found in adults because MCPyV antibodies are found to increase with age (Viscidi et al. 2011). In case of MCC, higher antibody titers are observed in patients suffering from MCPyV-positive MCC than those with tumors negative for the virus (Pastrana et al. 2012). Moreover, unlike VP1 specific antibodies that are commonly used for detection and are prevalent in general population, seroreactivity against early gene products is only detected in rare cases and at very low levels. Whereas, in patients with MCPyV-positive MCC, high T-Antigen titers are detected (Paulson et al. 2010). This suggests that during asymptomatic MCPyV infection LT levels are tightly regulated to evade immune recognition. Despite MCPyV sero-positivity is found to be associated with MCC, till now, no association is found with other chronic viral infections (Tolstov et al. 2011).

Healthy human skin is believed to harbors transient MCPyV infections. MCPyV DNA has been detected in 0–100% samples taken from different skin samples through PCR or nested PCR, qPCR or rolling circle amplification (Feng et al. 2008; Dworkin et al. 2009; Andres et al. 2010a, b; Mangana et al. 2010; Mertz et al. 2010a, b; Mogha et al. 2010; Wieland et al. 2011). However, DNA based detection

greatly depends on the sensitivity of the technique, the sampling methods, and preparatory steps (Garneski et al. 2009; Foulongne et al. 2010; Schowalter et al. 2010; Faust et al. 2011; Wieland et al. 2011). Apart from skin, MCPyV DNA has been detected in various other anatomical sites; for instance, in a study MCPyV DNA has also been detected in anal and penile swabs with frequency 30% and 50% respectively (Wieland et al. 2009). Additionally, MCPyV DNA is detected at high level in the oral cavity, with detection rates ranging from 8.3 to 39–60% (Dworkin et al. 2009; Wieland et al. 2009; Loyo et al. 2010). MCPyV is also detected in whole blood (Shahzad et al. 2019) as well as other blood products i.e. peripheral blood mononuclear cells (PBMCs), serum, plasma, and buffy coats of healthy individuals with varying frequencies (Pastrana et al. 2009; Mertz et al. 2010a, b; Pancaldi et al. 2011). Interestingly, being able to amplify low levels of MCPyV through PCR based techniques from various human tissues (Feng et al. 2008; Kantola et al. 2009; Bergallo et al. 2010; Loyo et al. 2010), it is inferred that that MCPyV is distributed systemically, but in most tissues where it may undergo low-level replication, persistence, or latency, it is not pathogenic. Taken together, MCPyV infection is believed to be ubiquitous with frequent detection in different anatomical sites of healthy subjects.

4 Merkel Cells and Merkel Cell Carcinoma

In 1875, Friedrich Sigmund Merkel described Merkel cells as 'touch cells' considering their presence in the touch sensitive areas of the skin. These cells are identified to be present around hair follicles, some mucosal tissues and various skin sites, especially in area that is involved in the sensation of touch such as finger pads. In the skin, Merkel cells are located at the basal layer of epidermis connected with the ending of sensory nerves (Fig. 2a). Structurally, Merkel cells are round or oval shaped cells of around 10 μm diameter, with lobulated nucleus and certain cytoplasmic projections, mainly formed by microfilaments. Merkel cells also contain granules similar to those of packages hormones which are used to transmit information (Fig. 2b). The cytoplasmic projections anchor Merkel cells to neighboring cells by desmosome formation which enables them to communicate with surrounding cells by transferring and gathering information (Fig. 2c). Merkel cells express both neuroendocrine such as chromogranin-A, synaptophysin and epithelial, for instance, cytokeratins 8, 18, 19, and 20 markers. Among these markers, CK20 is considered as most sensitive and reliable (Lucarz and Brand 2007).

Despite their omnipresence in the skin, exact origin and function of Merkel cells is still unclear. However, it is established that they possess both epithelial and neuroendocrine phenotypes. Merkel cells possess the ability to synthesize and secrete neuropeptides located in the neurosecretory granules within their cytoplasm. These neuropeptides can be released into the intercellular space in response to specific stimuli. Once released, the neuropeptides may act as neuromodulators, mediators or neurotransmitters that may act on sensory nerve endings and perform

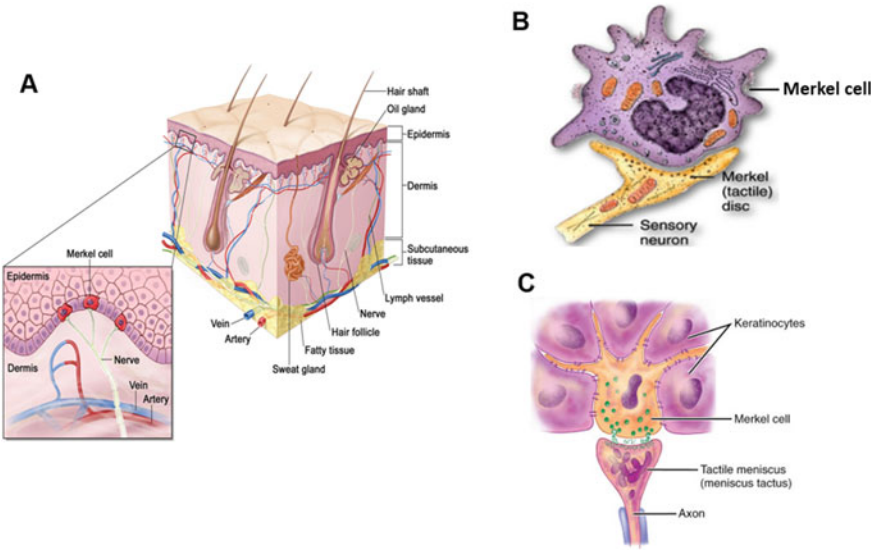

Fig. 2 Morphology and localization of Merkel Cells. **a** Location of Merkel cells in the skin (Image courtesy of the NIH, National Cancer Institute, USA). **b** Figure shows typical morphology of a Merkel cell having lobulated nucleus and certain cytoplasmic projections. **c** Desmosomes (shown as paired purple lines below) anchoring the Merkel cell to its neighboring keratinocytes

trophic roles on keratinocytes or some other endocrine function (Tachibana 1995). On the other hand, their presence in the epidermis and expression of epithelial cytokeratins (CKs) show that they might have an epidermal origin (Moll et al. 1996). Localization of these cells in the touch sensitive areas and the formation of Merkel cell neurites suggest that their role as mechanoreceptors (Ogawa 1996). Considering their well noted and characterized association with termini of somatosensory afferent nerve fibers in the epidermal basal layer adjacent to the dermis, their role of transducing mechanical stimuli from the skin to the central nervous system is hypothesized. The specialized junctions between unmyelinated dermal nerve fibers and Merkel cells are categorized as desmosome-like and synapse-like structures on their electron microscopic appearance. Innervation of Merkel cell clusters by slow adapting nerve fibers to form bulges known as the 'touch domes' in the epidermis is also noticed (Nakafusa et al. 2006).

Merkel cell carcinoma (MCC) was first observed as unusual tumors in the skin and named as trabecular carcinoma of the skin (Toker 1972). The MCC is typically represented as a rapidly growing, dome-shaped red or bluish nodule (Hodgson et al. 2005) (Fig. 3). However, sometimes it may have manifestation of a plaque-like appearance with small satellite lesions (Pectasides et al. 2006). The common primary sites of MCC are head and neck (40%), and then the upper extremities (19%) (Voog et al. 1999). Less than 10% of the cases affect the trunk region. Certain unusual primary lesion presentations of Merkel cell carcinoma have also been observed such as subcutaneous nodules in the inguinal region (Balaton et al. 1989),

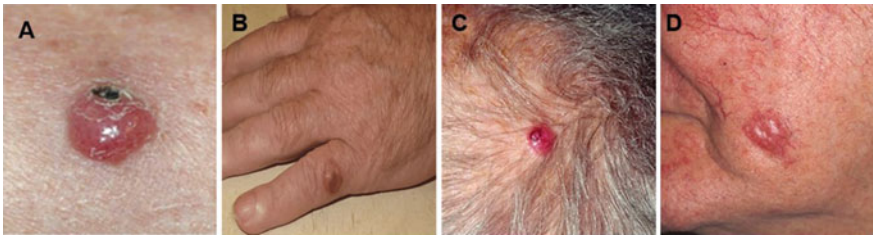

Fig. 3 Clinical presentation of Markel cell carcinoma. Rapidly growing flesh-colored or bluish red nodules on forehead (**a**), finger (**b**), head (**c**) and face (**d**). Figure opted from Mayo clinic (https://www.mayoclinic.org/) and merkelcell.org (https://merkelcell.org/)

an enlarged painless mass in the calvarium or ulceration of the vulva (Bottles et al. 1984).

The initial diagnosis of MCC is mostly based on histopathology and confirmed diagnosis can be made on the basis of both histological features and immunological markers' expression profiles of the lesions. Histologically, MCC shows an asymmetric dermal growth having irregular margins formed of tumor cells arranged in the form of strands (Plaza and Suster 2006). Atypical mitosis is usually observed in the tumors and the mitotic index remains high (Fig. 4). Usually the tumor occupies the entire thickness of the dermis and also commonly extends into the adjacent

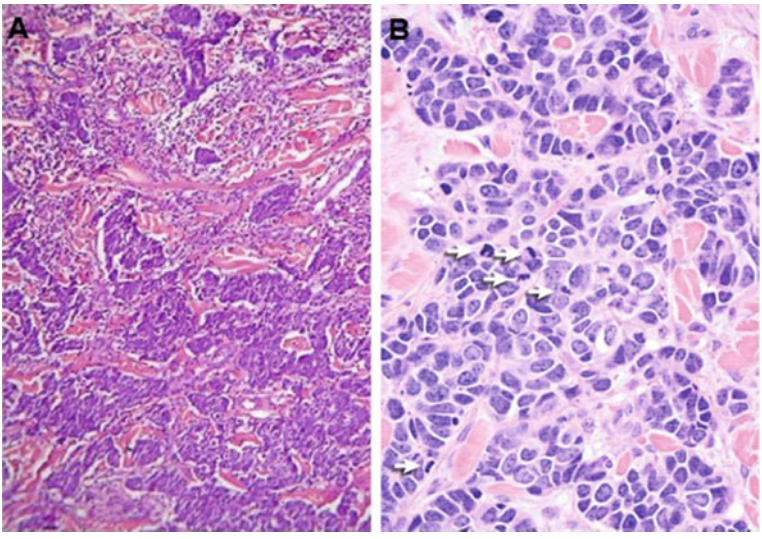

Fig. 4 Histological appearance of MCC. Hematoxylin and eosin (H&E) stained MCC tissues (A&B) contain scanty cytoplasm, round nucleus, dusty chromatin and high mitotic activity. **a** Figure shows sheets of small monotonous round cells extending throughout the dermis (Figure courtesy, Rafael et al. 2018) **b** 400X magnification of H&E stained tissue. Arrows show mitotic figures. Figure opted from Harms et al. (2018)

skeletal muscles and the subcutaneous fat layer (Silva et al. 1984). The tumor and the epidermis are generally separated by a dermal Grenz zone but epidermal changes such as ulceration have also been observed. The spread of tumor might also stimulate melanoma and rarely the tumor is seen to be exclusively intraepidermal.

Currently, three histological patterns have been recognized on the basis of arrangement and appearance of tumor cells in MCC: the trabecular, the intermediate and the small cell type. In between these three types, mixed and transitional forms are also common. The trabecular type of MCC is the least common. Mostly, the cells appear as diffused sheets having irregular borders with infiltrating growth pattern that intersect through the collagen fibers of the adjacent dermis. The trabecular type of MCC has tumor cells growing as narrow strands or ribbons. These ribbons are one to two cells-thick, and the cells have scanty amphophilic cytoplasm with oval or round nuclei. These nuclei stain pale, are vesicular and have delicate chromatin. In the small cell type, the nuclei may be hyperchromatic and spindle formed. Nucleoli are also commonly present but are not very prominent. The cells undergo numerous mitoses and thus result in atypical forms. Apoptosis is often marked and geographical areas of necrosis are seen. Small squamous foci and/or ductal differentiation may also be observed occasionally (Gould et al. 1988). Invasion of the lymphatic vessels is commonly present; rarely perineural infiltration is also seen. Usually the tumor infiltrate occurs together with lymphocytic infiltrate and sometimes the plasma cells are also present. The epidermis occasionally has in situ squamous cell carcinoma coexisting in it, but the merging of the two populations is rarely observed. The intermediate variant of MCC, namely the primary cutaneous neuroendocrine carcinoma, might at times coexist with the invasive squamous cell carcinoma with the two populations often blending together. However, they are distinguishable through immunocytochemistry (Iacocco et al. 1998). It is yet unclear if this coexistence implies origin from a common stem cell. Coexistence of neuroendocrine carcinoma and basal cell carcinoma is rarely seen (Cerroni and Kerl 1997).

A Characteristic immunohistological profile is usually observed for MCC. The cells of MCC express cytoskeletal keratins of both type I and II including CK8, CK18, CK19, and CK20 most importantly which is observed in the form of a paranuclear dot. Furthermore, neuroendocrine specific markers such as enolase, chromogranin, and synaptophysin are also expressed by MCC cells (Schmidt et al. 1998; Scott and Helm 1999). Major fraction of MCC (80%) express MCPyV T antigens and are denoted as MCPyV +ve MCC; whereas, a small subset of MCC cannot express viral oncoproteins and are named as MCPyV-ve MCC. It must be noted that two types of MCC (MCPyV +ve or MCPyV −ve) cannot be differentially diagnosed on the basis of immunohistochemistry alone since no immunological marker has been associated specifically with any type. However, both types can be distinguished by some characteristic features. For instance, MCPyV-ve MCCs have high mutational burden and do not express thyroid transcription factor 1 (TTF1), mammalian achaete-scute homologue 1 (ASH1), vimentin, S100B and CK7. In addition to that, a small subset of these MCCs (<10%) are negative for CK20 (Becker et al. 2017) (Fig. 5).

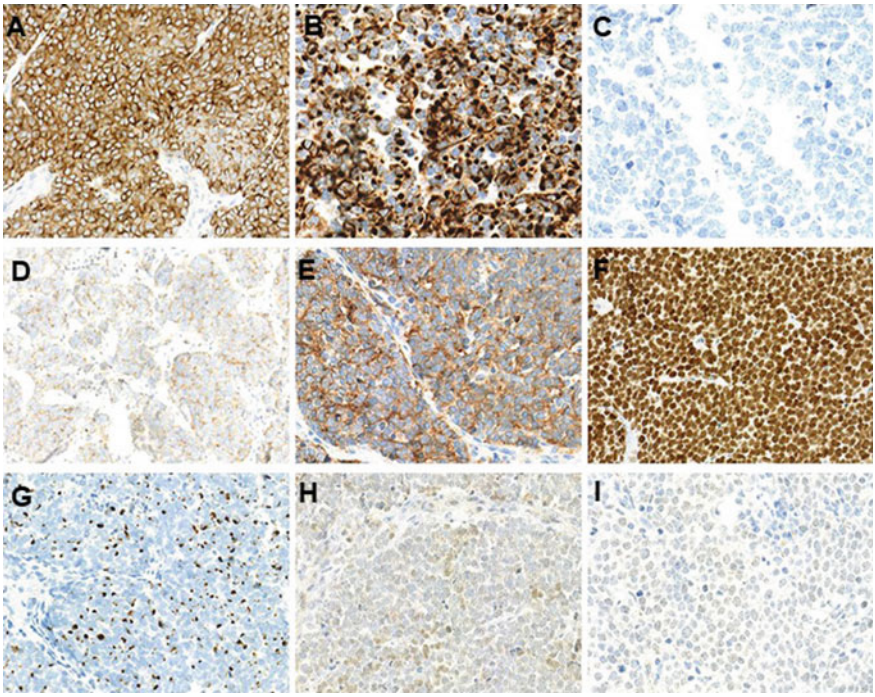

Fig. 5 Immunohistochemical features of MCC. **a** Chromogranin A cytoplasmic positivity, **b** cytokeratin 20 expression with paranuclear dot-pattern, **c** thyroid transcription factor-1 negativity, **d** membranous synaptophysin expression, **e** Membranous CD56 expression, **f** special AT-rich sequence-binding protein 2 (SATB2) nuclear expression, **g** neurofilament expression with a dot-pattern, **h** Terminal deoxy nucleotidyl transferase weak/moderate expression, **i** paired box 5 weak expression in tumor cells in comparison with intratumor lymphocytes. Images are taken from Kervarrec et al. (2019)

Several risk factors have been linked with the occurrence of MCC. These include prolonged exposure to UV and sunlight, old age and immune suppression. The individuals suffering from hematological neoplasms or having history of cutaneous tumors are also at higher risk of MCC (Reviewed in Becker et al. 2017). The disease is more prevalent among white population than the non-white (94.9% vs. 4.1%), and mean diagnosis in males is 73.6 and in females is 76.2 years (Albores-Saavedra et al. 2010). Although MCC has also been diagnosed in younger patients but in these cases it is most often related to immunosuppression due to organ transplantation (Lanoy et al. 2010). It has been indicated that patients undergoing organ transplantation have a 23.8-fold higher risk of MCC development than the immunocompetent patients (Clarke et al. 2015). If following transplantation, the immunosuppression is maintained for long periods of time the risk increases even further (Lanoy et al. 2010).

5 MCPyV: An Etiological Agent of MCC

Higher incidence of MCC in HIV-1 AIDS patients and immunocompromised (medically induced or in autoimmune diseases like rheumatoid arthritis) individuals, instigated the researchers to look for the pathogenic cause of MCC (Gooptu et al. 1997; Engels et al. 2002; Lanoy et al. 2010). Feng et al. identified some non-human stretches of DNA in human MCC tissue using a technique named as Digital Transcriptome Subtraction (DTS) (Feng et al. 2008). They detected MCPyV in 8 out of 10 studied MCC tumors by PCR and Southern blotting, indicating that a large subset of MCC is caused by MCPyV. Upon studying the primary tumor and a metastatic lymph node, they described that MCPyV is clonally integrated into the genome of MCC tumor cells. Later, several studies based on histopathology (Shuda et al. 2009; Busam et al. 2009; Bhatia et al. 2010; Shuda et al. 2011; Arora et al. 2019) and various molecular techniques (Feng et al. 2008; Busam et al. 2009; Duncavage et al. 2009; Fischer et al. 2010) confirmed the causal role of MCPyV in MCC. However, the percentage of MCPyV positive and negative MCC was found to be variable in different studies with an overall 80% MCPyV positivity.

6 How Does MCPyV Cause MCC?

MCPyV mediated cellular transformation and tumor development is exclusively linked with the activities of virus encoded oncoproteins, LT and sT. Both of these proteins have been immensely implicated in the initiation and progression of MCPyV-related carcinogenesis. This fact was first described by the finding of Houben et al. (2010) who observed decrease in proliferation and survival of MCC cells after knocking out T antigen locus, showing that continuous expression of LT and sT is required for the existence of MCPyV positive MCC cells (Houben et al. 2010). The expression of T antigens is found only restricted to the tumor cells lacking expression in surrounding healthy cells (Rodig et al. 2012). Despite the expression of both LT and sT was observed in majority of MCPyV induced MCC tumors suggesting the dependence of MCC cells on MCPyV T antigen proteins (Becker et al. 2017; Harms et al. 2018), their independent role in cellular transformation remained obscure. The knock down of sT in MCPyV positive MCC cell line by Shuda et al. (2011) resulted in halting cell growth but did not prime cell death, indicating other proteins might play a role in MCC survival. Whereas, sT alone was able to induce transformation in rodent fibroblasts by increasing phosphorylation and inactivating the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), ultimately resulting in the deregulation of cap-dependent translation that enhance proliferation and malignant transformation of cell (Shuda et al. 2011). The ability of sT to enhance phsopho-4EBP1 was linked to the Large T stabilization domain (LSD) of sT interaction with cell division cycle protein 20

(CDC20), rather than binding with protein phosphatase, PP2A (Shuda et al. 2015). The transforming activity of MCPyV sT was also confirmed in transgenic mice (Verhaegen et al. 2015). Furthermore, sT was described to play role in viral DNA replication, protection of MCPyV LT proteosomal degradation and of other cell-cycle regulators including c-Myc and cyclin E by targeting cellular ubiquitin ligase SCFFbw7 (Kwun et al. 2013). In fact, MCPyV sT has been shown to increase the level of LT protein via the activity of its LSD (Dye et al. 2019). Recently, the role of sT in inducing DNA damage response (DDR) pathway has been indicated in human MCC cells by observing sT over expression causing hyperphosphorylation of histone H2AX, a marker for DNA damage, along with the activation of ataxia telangiectasia mutant (ATM), an upstream kinase important for H2AX phosphorylation. Moreover, sT expression was also observed to induce hyperphosphorylation of other ATM downstream molecules (including 53BP1 and CHK2) as well as the hypermethylation of histone 3 and histone 4, indicating a novel link between sT and the DDR pathway in MCC (Wu et al. 2019). All these observations reinforce the role of MCPyV sT as a main driver of MCPyV mediated cellular transformation and oncogenesis. However, predominant role of sT is negated in study by Angermeyer et al. (2013) who showed that LT is more relevant in maintaining the proliferation and survival of MCC cell lines. Nevertheless, both LT and sT have been found essential for MCPyV medicated carcinogenesis.

Shuda et al. (2008) described that MCPyV genome is clonally integrated into the genome of MCC cells and this integration leads to mutations that generate premature stop codons resulting in the truncation of LT. The truncated LT retains N-terminal J-domain and RB binding motif, but lose the C-terminal regions. On the other hand, MCPyV strains isolated from healthy individuals were reported not to possess these signature mutations and encode full length LT. These observations suggested that truncation of LT plays an essential role in MCPyV mediated carcinogenesis. Owing to antitumor activities, there is a strong selective pressure to remove C-terminal during development of MCC. It was demonstrated that deletion of LT helicase domain eliminates the viral replication ability which is crucial in preventing the MCPyV cell death (Shuda et al. 2008). The truncated LT has been shown more efficient in promoting the growth and transformation of human and mouse fibroblasts when compared with full length LT and 57kT (Cheng et al. 2013). Similarly, truncated LT showed stronger binding affinity for pRb than full length LT (Borchert et al. 2014). It was found that the C-terminal of LT causes DNA damage stimulating host DNA damage responses which ultimately activate p53 and stop proliferation of cells that is not favorable for their malignant transformation (Li et al. 2013). Moreover, the C-terminal of full length LT was reported to halt the growth of several cell types (Cheng et al. 2013). It was also demonstrated that ATM cellular kinase may induce phosphorylation of MCPyV LT C-terminal, induce apoptosis and halt cell proliferation (Li et al. 2015). These studies altogether elucidate that deletion of the MCPyV LT C-terminus is not only necessary for the disruption of MCPyV replication but also important to overcome the growth-inhibitory properties of full length LT, henceforth, very crucial for oncogenic progression of MCPyV-associated carcinogenesis. Notably, the integration of

MCPyV does not induce any mutation in the sT leaving its expression in MCC in the native form.

Uncontrolled cell proliferation of cells is generally inhibited via one of two fundamental tumor suppression mechanisms mediated by retinoblastoma (pRb) and p53 proteins. The pRb restricts the progression of cell cycle by binding and sequestering the function of E2F transcription factor which transactivates the transcription of genes essential for G1 to S phase transition (Fisher et al. 2016). The MCPyV oncogenic potential is largely attributed to the binding and inactivation pRB by LT (Borchert et al. 2014). The LT binding to pRb is achieved via LXCXE motif of LT that is also retained in truncated LT. The truncated LT also preserves the ability to bind and inactivate the pRb as it retains the Dna J region and pRb binding pocket. The LT has been shown to bind with all proteins of pRb family (pRb, p107, p130). The inactivation of pRB by LT is crucial for the sustained growth of MCPyV positive MCC cells. In fact, it was described that pRb-binding motif is required by LT for promoting the growth of MCC cell (Houben et al. 2012). The LT also contain another 200 amino acids long domain named as Merkel unique region (MUR) which extends to LXCD domain, binds to cytoplasmic vacuolar sorting protein Vam6p and antagonizes its role in lysosomal clustering (Liu et al. 2011). However, this interaction does not seem to be critical in MCPyV mediated cellular transformation. The p53 is expressed under different stress conditions such as DNA damage and acts as a transcription factor which induces the expression of genes involved in DNA damage repair, apoptosis and senescence. Notably, no direct interaction between MCPyV LT and p53 has been described so far. However, full length LT was shown to significantly reduce p53-dependent transcription (Borchert et al. 2014). In most of the cancers, p53 pathway is inactivated through mutations in the p53 gene. However, p53 mutation seems to occur only occasionally in MCPyV positive MCC. It was suggested that p53 expression is negatively correlated with MCPyV DNA copy number and p53 mutations are only detected in MCPyV negative MCC (Sihto et al. 2011). These data suggest that p53 is most likely not a major driver of MCPyV-associated tumorigenesis. Recently, in a study, single-cell RNA-seq revealed that knockdown of T antigen directly inhibits the expression of Atoh1 by LT by up-regulating Sox2 through its retinoblastoma protein-inhibition domain (Harold et al. 2019). ATOH1 is a transcription factor that is a master regulator of Merkel cell development, with a controversial role in Merkel cell carcinoma (MCC) (Fan et al. 2020a, b).

Understanding the alteration in cellular protein network induced by MCPyV oncoproteins have also significantly contributed to our understanding of virus mediated cellular transformation and oncogenesis. In order to grab deep understanding of MCPyV mediated carcinogenesis, Gupta et al. 2019 performed expression profiling of NIKS cells expressing early genes of MCPyV. The MCPyV early genes down regulated the expression of tumor suppressor gene N-myc downstream-regulated gene 1 (NDRG1) which was found to exert its function in Merkel cell lines by regulating the expression of the cyclin-dependent kinase 2 (CDK2) and cyclin D1 proteins (Gupta et al. 2020). Likewise, the MCPyV small T

antigen was described to activate the expression of LSD1 which was shown to antagonize the tumor suppressor non-canonical BAF (ncBAF) (Park et al. 2020).

In terms of the various cellular pathways, the expression of T antigens in MCC can lead to oncogenesis by altering various mitogenic pathways. One of important pathway downstream of the growth factor receptors that is commonly deregulated in cancers is the MAPK (mitogen activated protein kinase) pathway. This pathway carries growth signals from the plasma membrane to the nucleus and involves three consecutive kinases i.e. RAF, MEK and ERK. However, no activating mutations of these kinases have been observed in MCC samples. In addition, immunohistochemical analysis has revealed that the ERK kinase is present in the inactive non-phosphorylated form in MCC and thus the MAPK pathway is considered inactive (Houben et al. 2006). The cell survival pathway also involves another branch of signaling from the growth factor receptors which relies on the generation of a second messenger, phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 is dephosphorylated to PIP2 by phosphatase and tensin homologue (PTEN), a tumor suppressor. PTEN thus inactivates growth signaling through PIP3. PTEN encoding gene is located on the long arm of chromosome 10, part of which is found to be frequently lost in case of MCC in a heterozygous pattern (van Gele et al. 1998). However, the inactivation of other alleles of PTEN through deletion or mutation occurs rarely in MCC, and other tumor suppressor genes might be the actual targets of loss of 10q (van Gele et al. 2001). Microarray analysis of MCC tissue, however, recently showed that the PTEN expression is very low in MCC tissues which hint toward epigenetic silencing of the second allele of PTEN (Fernandez-Figueras et al. 2007).

The comprehensive understanding of MCPyV mediated tumorigenesis is not possible without deciphering its interaction with the immune system of host. The evasion of the immune response is an important factor in determining the persistence and outcome of oncogenic viral infection. Owing to LT and sT oncoproteins, MCPyV has devised various strategies to escape the immune attack. One of these mechanisms is down- regulation of major histocompatibility complex class 1 (MHC-1) which prevents MCPyV from being recognized from the host immune system. MHC-1 has been found down regulated in 84% of MCC and this down-regulation was more pronounced in MCPyV MCC than in MCPyV negative tumors (Paulson et al. 2014). Another evasion mechanism was described by Shahzad et al. (2013) where MCPyV T antigen locus has been demonstrated to downregulate the expression of Toll-like Receptor 9 (TLR9) which are meant for the sensing of viral and bacterial dsDNA (Shahzad et al. 2013). It was also shown that TLR9 targeting plays a vital role in viral persistence, which is a prerequisite for cellular transformation in MCPyV mediated MCC. In another study, MCPyV sT has been shown to inhibit the transcription of NF-κB pathway associated genes, ultimately enabling MCPyV to undermine the host immune response in infected cells (Griffiths et al. 2013). MCPyV also encodes micro RNA (MCV-miR-M1) which facilitates the virus in establishing persistent infection and evasion of immune response. In fact, MCV-miR-M1 induces expression of several immune evasion related genes ultimately resulting in the attenuation of neutrophil chemotaxis

toward MCPyV harboring Merkel cells (Akhbari et al. 2018). The MCPyV T-antigens also revealed to induce expression of several host micro RNAs including miR-375 which suppresses autophagy related genes ultimately protecting MCC cells from death (Kumar et al. 2020). Due to these mechanisms, several MCC patients having normal immune system probably fail to clear the MCPyV and ultimately become victim of MCC (Heath et al. 2008). However, better prognosis and survival rate has been shown in MCC patients being capable of generating vigorous immune system (Sihto and Joensuu 2012; Paulson et al. 2011).

Inhibition of apoptosis is another critical step in the development of tumorigenesis. Majority of cancers usually have elevated levels of anti-apoptotic members of the Bcl-2 family in order to control mitochondrial apoptosis by binding pro-apoptotic proteins (Bender et al. 2013). Bcl-2 family profiling and functional studies showed that these proteins are overexpressed in 94% of the MCC tumors and all 11 MCC cell lines (Brunner et al. 2008; Verhaegen et al. 2014). Furthermore, downregulation of Bcl-2 family proteins induced cell death in majority of MCC cell lines (Verhaegen et al. 2014). However, no correlation has been observed between Bcl-2 expression and aggressive behavior of MCC (Feinmesser et al. 2004). Many cancers also show high expression of another anti-apoptotic protein named survivin which is undetectable in differentiated human tissues. In a study all MCC cases have shown strong survivin staining which was localized either in the nucleus or the cytoplasm. Nuclear localization corresponded to more aggressive clinical outcomes with patients developing distant metastasis (Kim and McNiff 2008).

Another important driver of MCC particularly in case of MCPyV negative MCC is exposure to UV radiation. Striking differences have been observed in the genome of MCPyV positive and negative MCC tumor by recent studies. Notably, MCPyV containing MCC cells harbor very little somatic mutations, whereas, MCPyV lacking MCC cell contain high number of mutations (25–90-folds higher) most probably because of UV radiation (Wong et al. 2015; Starrett et al. 2017; González-Vela et al. 2017). These UV induced mutations in MCC cells are very similar to mutations in other sun exposed tumors including basal and squamous cell carcinoma (South et al. 2015; Martincorena et al. 2014). The mutation caused by UV radiation in MCPyV negative MCC cells impairs the functionality of *pRb, p53, NOTCH1, FAT1* and *HRAS* genes (Wong et al. 2015). The UV radiation is known to play a crucial role in MCPyV mediated MCC as both MCPyV negative and positive MCC occur in sun exposed area (Arora et al. 2019). The UV radiation is also known to modulate the expression of inflammatory mediators and alter the functionality of antigen-presenting dendritic cells, ultimately causing induction of immunosuppression, which is a prerequisite for MCPyV mediated carcinogenesis (Prasad and Katiyar 2017). The MCPyV positive MCC was also elaborated to harbor additional mutations that are known to activate PI3K pathway (Reviewed in DeCaprio et al. 2017). The UV exposure is linked with the increase in the activity of MCPyV non-coding regulatory region and transcription level of sT (Mogha et al. 2010). The presence of MCPyV also negatively impact the DNA damage repair mechanism in cells exposed to UV (Demetriou et al. 2012). It was shown that UV

radiation induces certain growth factors (EGF and FGF) and activates WNT/β-catenin signaling pathway ultimately inducing expression of MMPs. These UV mediated activations may stimulate MCPyV infection and its potential to cause MCC, ultimately driving it towards MCC (Liu et al. 2016).

Epigenetic alterations in addition to genetic changes are also known to play critical role in the development of tumor. In fact, silencing of tumor suppressor genes (TSGs) by promoter hypermethylation has been established as an important mechanism in tumorigenesis (James et al. 2003). Like other tumors, several tumor suppressor genes have been described to be epigenetically silenced in MCC with varying frequencies. For instance, p16INK4a and p14ARFTSGs, which are important for cell cycle control, are found frequently hypermethylated in MCC (Horn et al. 2007). The epigenetic silencing ofp14ARF was observed in about half of the MCC cases, however, hypermethylation of the p16INK4a has not been significantly observed in MCC (Lassacher et al.,2008). Since p14ARF represses the permanent degradation of p53, it is possible that the p53 pathway is inactivated in case of MCC through the suppression of expression of p53 inducer p14ARF. Likewise, methylation of other cell cycle regulator such as CDKN2A was reported in MCC (Helmbold et al. 2009). Certain members of RASSFs tumor suppressors' family are also found aberrantly hypermethylated in MCC (Richter et al. 2013). In another study, the promoter of RB1 gene was found methylated in MCPyV positive as well negative MCC tumor (Sahi et al. 2014).

7 Treatment of MCC

Devising a comprehensive and effective therapy for MCC is currently a challenge. However, various treatment options are available which are recommended on the basis of different characteristics of MCC in a patient including location of the tumor, stage, regional lymph node involvement, co-morbidities and performance status of the patient (National Comprehensive Cancer Network 2016; Lebbe et al. 2015). An overview of therapeutics modalities for MCC is present in Fig. 6.

The preferred treatment option for primary MCC is surgery; however, this mode of treatment is limited to those patients who suffer only from locoregional primary MCC. Usually, it involves resection of a wide area (1–2 cm) of clinically free margins (Tai 2013). There are cases where surgery results in a positive margin which is then followed by re-excision (National Comprehensive Cancer Network 2016). Considering a wider area is resected, in cases where tissue sparing is crucial, a technique named as Mohs microsurgery is used (National Comprehensive Cancer Network 2016). However, its utility is debated as in few cases development of in-transit metastases is observed (Tai 2013; Hughes et al. 2014). In node-positive disease cases, complete lymph node dissection are conducted along with radiotherapy in a few cases (National Comprehensive Cancer Network 2016). Whereas, in patients that clinically appear node-negative, sentinel lymph node biopsy (SLNB) is mostly advised along with excision of primary MCC, which allows regional

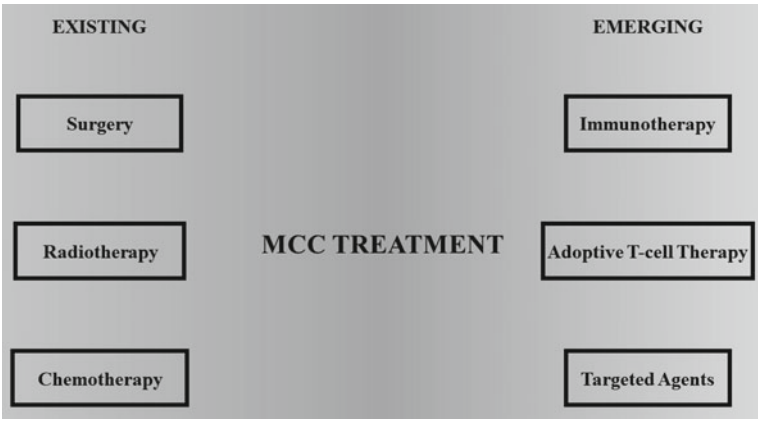

Fig. 6 Schematic representation of existing and emerging therapies against MCC

lymph node status at microscopic level (Paulson et al. 2013; Servy et al. 2016). If results of SLNB are positive, a complete lymph node dissection is conducted with or without radiotherapy to the nodal basin depending on the case (Cassler et al. 2016). However, further studies are required to determine the better suitability of both these surgical options; complete node dissection or radiotherapy, in patients with a positive SLNB result.

Radiotherapy could be considered at different disease stages whether as palliative treatment for cases of MCC where surgery is not possible or as an adjuvant treatment to surgery (Mortier et al. 2003; Veness et al. 2010; Poulsen et al. 2003). In numerous cases, adjuvant radiotherapy is observed to decrease recurrence in comparison to surgery alone (Lewis et al. 2006; Jabbour et al. 2007; Jouary et al. 2012; Chen et al. 2015; Hasan et al. 2013; Mojica et al. 2007; Veness et al. 2005). In patients where surgery in not possible due to poor performance status of the patient, radiotherapy can lead to long-term tumor control (Mortier et al. 2003). Despite surgery and/or radiotherapy may cure patients that suffer from local and regional MCC, recurrences are observed even if both are combined (Allen et al. 2005; Santamaria-Barria et al. 2013; Bichakjian et al. 2007). The benefit of adjuvant radiotherapy is also associated with the stage of MCC, as in an analysis of almost 7000 patients, an improved overall survival was observed in patients with stage I/II MCC as compared to surgery alone, but not in the case of stage III MCC (Bhatia et al. 2016).

Chemotherapy is recommended as standard treatment when MCC is at advanced-stage or becomes metastatic. However, responses are not durable and adjuvant radiotherapy is associated with better outcomes in comparison to adjuvant chemotherapy (Garneski et al. 2007). Survival benefit of chemotherapy is showed by only a limited number of studies, whereas, largely recurrences are seen to develop within 4–15 months after chemotherapy (Allen et al. 2005; Bichakjian et al. 2007; Poulsen et al. 2003, 2006; Saini et al. 2015). The complications

associated with chemotherapy include increased morbidity, resistance to chemotherapy on recurrence, decreased quality of life and immune suppression. On the other hand, benefits are insignificant and it is not recommended in clinical guidelines (National Comprehensive Cancer Network 2016; Cassler et al. 2016; Bhatia et al. 2016; Desch and Kunstfeld 2013; Iyer et al. 2016), except for stage IV metastatic MCC in which it is now gradually being believed to palliate symptoms (National Comprehensive Cancer Network 2016; Lebbe et al. 2015). Various natural compounds and derivatives including etoposide, taxanes and anthracyclines, either alone or in various combinations are being used as chemotherapeutic agents for MCC. Very recently, Liu et al. (2020) screened a library of natural compounds and found that a natural product, glaucarubin, reduces viability of the MCPyV-positive MCC cell line MKL-1. The anti MCC effect of glaucarubin was enhanced when combined with a FDA-approved BCL-2 inhibitor, ABT-199 (Liu et al. 2020).

In this view, there is a pressing need to develop treatments which are able to induce durable responses in patients with metastatic or recurrent disease and simultaneously possess a good safety and tolerability profile. Among the other emerging treatments, immunotherapy has recently been implicated in the case of MCC, especially immune checkpoint inhibitors against members of the CD28 family such as CTLA-4, PD-1 and PD-L1 that is up-regulated in the tumor microenvironment and correlate with poor prognosis (Topalian et al. 2015; Postow et al. 2015). In a study, in patients who were treatment-naive with stage IIIB/IV MCC, pembrolizumab, an anti-PD-1 antibody, showed significant clinical activity. Similarly, in another study, durable responses with the use of avelumab which is a PD-L1 antibody were seen in 88 patients suffering from stage IV MCC and had failed first-line chemotherapy (Kaufman et al. 2016). These responses were observed in both MCPyV positive and negative patients. Despite the above mentioned drugs are currently under clinical trials and not available for treatment, immunotherapy is a promising treatment option for treating advanced stage MCC.

Adoptive T-cell therapy is another novel avenue currently being explored with regard to MCC treatment. In preclinical studies, effective killing of MCPyV positive MCC was observed by MCPyV-specific T-cells (Lyngaa et al. 2014). In this view, a phase 1/2 study is ongoing (NCT02584829) which is exploring the efficacy and safety of the use of avelumab combined with MHC-Iup regulation (via IFN-b administration) and autologous T-cell transfer. Moreover, phase 1 studies are being carried out to gauge the feasibility of glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE). This adjuvant is an agonist of TLR-4 which plays a role in inducing a T helper 1 immune response through the stimulation of dendritic cells, in patients with MCC (NCT02035657). Presently, obtained results of these trials show that G100 (GLA-SE delivered intratumorally) has the ability to facilitate inflammatory changes in the tumor microenvironment, thereby activating T cells (Bhatia et al. 2016).

Another novel approach is to apply targeted agents, which in the case of MCC are mainly kinase inhibitors, considering that in MCC oncogenes and tumor suppressors are rarely mutated, whereas numerous receptor kinases and/or ligands are

expressed, such as PI3K/Akt, c-kit, VEGFA, VEGFC, VEGFR-2, PDGF-a and PDGF-b (Nardi et al. 2012; Brunner et al. 2008; Hafner et al. 2012). Treatment with imatinib, a tyrosine kinase inhibitor, showed a complete response in a patient (Loader et al. 2013), however, due to lack of its efficacy observed in further studies, a phase 2 clinical trial was prematurely discontinued (Samlowski et al. 2010). Cabozantinib is a c-met and VEGFR-2 inhibitor and its clinical trials against recurrent and/or metastatic MCC is ongoing (NCT02036476). In comparison, pazopanib which is another multi targeted kinase inhibitor, a limited clinical activity in advanced MCC was observed (Davids et al. 2009; Nathan et al. 2016). Keeping in view that PI3K activating mutations are detected in MCC, idelalisib provided a complete clinical response in a single patient with stage IV MCC (Nardi et al. 2012; Shiver et al. 2015). As well as, mammalian target of rapamycin (mTOR) inhibitors MLN0128 and everolimus are currently being investigated in patients with advanced MCC as it is a regulator of the PI3K and MAPK pathways (NCT02514824 and NCT00655655) (Lin et al. 2014; Kannan et al. 2016). Similarly, somatostatin receptor type 2 (sst2) is expressed in MCC tumors and is therefore a potential imaging and treatment target (Buder et al. 2014). Treatment with lanreotide has shown response in MCC tumor expressing sst2 (Fakiha et al. 2010). Moreover, to evaluate the activity of lanreotide in patients with locally advanced or metastatic MCC, a phase 2 trial is currently being carried out (NCT02351128).

8 Conclusion

Merkel Cell polyomavirus (MCPyV) is the 7th known oncogenic virus in human, being responsible for Merkel Cell Carcinoma (MCC), a rare but aggressive neuroendocrine cancer of skin having low survival rate. The MCPyV causes ubiquitous infection in human with higher incidence in elderly individuals. The MCC is caused only after prolonged infection and integration of MCPyV in host genome which is followed by mutation at 3′ end of MCPyV LT gene leading to the truncation of its carboxyl terminus having helicase domain. Oncogenic potential of MCPyV is largely attributed to truncated LT and sT antigens which drive the transformation of normal cells into cancerous cells and induce cell proliferation by various mechanisms. Several treatment options like surgery, radiotherapy and chemotherapy are available for MCC but have some limitations like lower efficacy, relapse and dangerous side effects. Recently, other treatment options like immunotherapy, adoptive T-cell therapy and the application of targeted agents have been extensively explored.

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