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Viral Factors Involved in Marek's Disease Virus (MDV) Pathogenesis

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Abstract

Purpose of Review Marek's disease virus (MDV) is a highly oncogenic alphaherpesvirus that causes various clinical symptoms including fatal lymphomas in chickens. The virus encodes several MDV-specific genes that play a major role in viral pathogenesis. This review will focus on the recent advances in our understanding of how these viral factors contribute to pathogenesis and tumor formation.

Recent Findings Several viral factors involved in MDV pathogenesis have been identified including the major oncoprotein Meq, the viral chemokine vIL-8, MDV-encoded microRNAs, RLORF4, RLORF5a, pp14, pp38, a virus-encoded telomerase RNA (vTR), and viral telomeric repeats (TMRs). Our current knowledge of the role of these viral factors in MDV pathogenesis has immensely increased over the last few years; however, more work needs to be done to completely understand the mechanisms for most of them.

Summary MDV pathogenesis and tumor formation is a complex process. Deciphering the mechanisms of viral factors involved in MDV pathogenesis and lymphomagenesis will not only improve our understanding of this neoplastic disease but will also provide new strategies for vaccine development against this deadly pathogen.

Keywords Marek's disease virus (MDV) · pp14 · pp38 · vIL-8 · TMR · vTR · miRNA · Meq · Splice variants · RLORF4 - RLORF5a · Pathogenesis · Tumorigenesis

Abbreviations

MDV	Marek's disease virus
MD	Marek's disease
vTR	Viral telomerase RNA
vIL-8	Viral interleukin-8
RL	Repeat long regions
RLORF4	Repeat long open reading frame 4
TMRs	Telomeric repeats

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Introduction

Marek's disease virus (MDV, Gallid herpesvirus 2) is a lymphotropic alphaherpesvirus and the causative agent of Marek's disease (MD) [1]. MD is characterized by generalized nerve inflammation, immunosuppression, and T cell lymphomas [1]. These MDV-induced lymphomas are considered to be the most clinically diagnosed cancer in the animal kingdom [2]. In addition, MDV serves as a natural virus-host animal model for herpesvirus-induced tumorigenesis and pathogenesis [3]. MD is one of the most important infectious diseases in chickens and causes dramatic losses in poultry industry worldwide of up to 1–2 billion US-dollars annually [4]. To date, vaccines are the only option to protect chickens from this deadly disease [5].

Upon infection of the host, MDV infects and replicates efficiently in B cells. However, recent data revealed that

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MDV can also productively infect a plethora of cells including macrophages, dendritic cells [6•], natural killer (NK) cells (Christine Jansen, unpublished data) and T cells [7••, 8, 9]. In addition to lytic replication, MDV establishes latency in T cells. Intriguingly, the virus integrates its genome into the telomeres of host cell chromosomes in latently infected cells [10, 11]. This ensures replication of the virus genome with the host chromosomes and maintenance in the host for life. This integration event is also crucial for transformation of mostly CD4+ T cells and a prerequisite for lymphoma formation [11].

MDV pathogenesis is characterized by four overlapping phases: (i) the early cytolytic phase with an initial amplification of the virus in the infected animal, (ii) the latent phase with latency establishment predominantly in CD4+ T cells, (iii) the late cytolytic phase, and (iv) the transformation phase with a rapid lymphoma development and dissemination of these tumors preferentially into visceral organs and skeletal muscles [3, 12].

MDV encodes more than 100 proteins that play a role in various processes of the virus lifecycle [13]. This brief overview highlights the current advances in our understanding of the key viral factors that contribute to MDV pathogenesis and lymphomagenesis.

The Major Oncogene *meq*

The major oncogene of MDV is *meq* (MDV Eco Q-encoded protein, MDV005 and MDV076) that encodes a 339 amino acid nuclear basic leucine zipper protein (bZIP). The Meq protein has a DNA-binding domain and can form homodimers as well as heterodimers with cellular bZIP proteins such as c-Jun and Fos [14, 15], allowing it to regulate cellular and viral gene expression [16, 17]. Two copies are present in the MDV genome, one in the internal and one in the terminal repeat long region (IR_L and TR_L) (Fig. 1) [15]. Meq is constitutively expressed in both lytic and latent stages of MDV infection as well as in lymphoblastoid cell lines derived from MDV tumors [9]. In infected chickens, the percentage of Meq expressing peripheral blood mononuclear cells (PBMCs) increases in the early latent phase, but decreases thereafter [18]. A recent publication indicated that also some infected lymphocytes do not express Meq [18]. *meq* itself is dispensable for MDV replication in vitro [19]; however, deletion of both copies of the *meq* gene or parts of it completely abrogates tumor formation [20–23].

Several mechanistic pathways have been discovered for this viral oncogene. Meq represses p53-mediated transcriptional activity and apoptosis through its direct interaction with p53 [24]. It thereby plays a pivotal role in maintaining MDV latency in CD4+ T cells by blocking apoptosis [25]. Other Meq interaction partners that contribute to MDV pathogenesis include the heat shock protein 70 (hsp70) [26] and the retinoblastoma tumor suppressor protein (pRB) [4, 27], as well as Par-4 and SKP-2 [2]. Meq also was shown to trans-activate latent gene expression [28] and to suppress the promoters of the lytic MDV genes ICP4, pp38, and pp14 [29].

Repeat Long Open Reading Frame 4 and 5a

Repeat long open reading frame 4 (RLORF4) is located within the TR_L/IR_L regions of the MDV genome in the same orientation as *meq* (Fig. 1) [2, 30]. Comparative sequence analysis revealed that four out of six attenuated MDV-strains lack RLORF4, suggesting that RLORF4 plays a role in MDV pathogenesis [31]. To investigate the role of RLORF4 in MDV pathogenesis, Jarosinski and colleagues generated recombinant viruses that lack RLORF4 based on the very virulent MDV RB-1B strain [30]. Deletion of RLORF4 resulted in an increased virus replication and spread in vitro [30]; however, virus load was severely reduced in vivo. Furthermore, tumor development was severely impaired in the absence of RLORF4 compared to wild-type virus [30]. The exact mechanism that allows RLORF4 to contribute to MDV pathogenesis remains unknown. In addition, MDV encodes repeat long open reading frame 5a (RLORF5a) that is located upstream of RLORF4 (Fig. 1). Schat and colleagues initially demonstrated that deletion of RLORF5a in CVI988 did not alter virus replication [32]. This was confirmed by Jarosinski and colleagues for the very virulent RB-1B. In contrast to RLORF4, they observed that RLORF5a is dispensable for RB-1B pathogenesis and tumorigenesis [30].

Viral Interleukin-8

Viral interleukin-8 (vIL-8) (MDV003 and MDV078) is a secreted CXC chemokine that facilitates recruitment of target cells and plays a crucial role in MDV pathogenesis [33, 34]. It is the first CXC chemokine identified in an alphaherpesvirus and was initially named after interleukin-8 (cIL-8; cCXCL8), the first CXC chemokine identified in chickens [35, 36]. However, recent data demonstrated that vIL-8 is a functional orthologue to the chicken CXC ligand 13 (CXCL13) and binds to the cellular CXC receptor 5 (CXCR5) [37•]. It is expressed during lytic replication and has true late expression kinetics [36]. As mentioned above, vIL-8 plays an important role in pathogenesis as it allows recruitment of B cells, which serve as primary targets for lytic replication. In addition, it recruits CD4+ CD25+ T cells that could serve as a target for the establishment of latency and tumor formation [34]. Deletion of the vIL-8 gene or abrogation of vIL-8 expression decreases disease and tumor incidence in experimentally infected animals. In the case of a natural route of transmission, disease and tumor formation is completely abrogated [33, 34], underlining that the recruitment of target cells is crucial for MDV pathogenesis.

Splice Variants of *meq*, RLORF4/5a, and vIL-8

Multiple spliced transcripts have been identified in the region containing *meq*, RLORF4/5a, and vIL-8 [2, 38–40]. These splice variants include fusion proteins of Meq, RLORF4, and RLORF5a with exons II and III of vIL-8 [38]. The role of these splice variants in MDV pathogenesis remains poorly understood. Intriguingly, splice variants of *meq* and exons II and/or III of vIL-8 show differences regarding their localization and cellular dynamics compared to the full-length *meq* [41]. These splice forms are also expressed in MDV-induced tumor cells, suggesting that they might contribute to tumor formation. However, more work needs to be done to understand the contribution of these splice variants in MDV pathogenesis and tumorigenesis.

Neurovirulence Factor pp14

In addition to tumor formation, MDV can also cause various neurological symptoms. A viral protein associated with an increased neurovirulence in MDV-infected chickens is pp14 (MDV006 and MDV075), a 14 kilodalton (kDa) polypeptide (Fig. 1) [42–44]. pp14 is expressed with immediate early (IE) kinetics and is dispensable for virus replication and tumorigenesis [44]. Two splice variants of pp14 are expressed that differ in their N-terminal amino acid compositions and are expressed at different levels [45]. The pp14 transcript with a 5′ leader intronic internal ribosome entry site (IRES) is more abundant in MDV infected and transformed cells than its counterpart lacking this element. This is due to the ability of the 5′ IRES to mediate cap-independent translation initiation and may enable this mRNA to overcome translation inhibition [45–47]. In vivo, a pp14 deletion virus showed significantly less clinical MD signs compared to the wild-type virus. In addition, nerve lesions including cellular infiltration, proliferation of lymphoblastic cells, and edema in the nerve tissue were reduced in the absence of pp14 (wild type: 62.8%, pp14 deletion mutant 16.6%) [44]. However, the exact molecular mechanisms of MDV-mediated neurovirulence and how pp14 contributes to these symptoms remain elusive.

Phosphoprotein pp38

pp38 (MDV073) is a 38 kDa immediate early protein that is encoded in the junction of the U_L and the IR_L (Fig. 1). Deletion of the pp38 gene severely impaired tumor formation [48, 49], underlining its role in MDV pathogenesis. Besides the full-length protein, two splice variants of pp38 (Spl A and Spl B) were identified in vitro and in vivo [50]. The full-length pp38 is primarily expressed in early lytic replication, while the splice variants are present during the establishment of latency from 7 to 14 days post infection. This differential expression has been linked to an increased metabolic activity of infected cells that could contribute to the establishment of latency and/or to transformation [50, 51]. Like Meq, pp38 is also involved in the inhibition of apoptosis in MDV-infected and -transformed cells [51–53]. For pp38, it remains unclear if this is due to a direct block of apoptosis or the inhibition of a cytotoxic T cell response [52]. In contrast, there is evidence that full-length pp38 can induce apoptosis via the oxidative phosphorylation pathway [51, 54], a phenomenon that was not observed for the two splice variants Spl A and Spl B [51].

MDV MicroRNAs

A number of MDV-encoded microRNAs (miRNAs) have been discovered that are classified into three distinct miRNA clusters (Fig. 1). These clusters encode 14 precursor sequences and 26 mature miRNAs that are highly conserved between virus isolates [55]. They play an important role in MDV-induced pathogenesis and tumorigenesis [55]. The first cluster, termed the Meq-cluster, is located upstream of the *meq* oncogene and contains six pre-miRNAs (Fig. 1) [56, 57]. Deletion of this cluster severely impaired disease and tumor development, indicating that some miRNAs in this cluster play an important role in MDV-induced pathogenesis and tumorigenesis [58, 59]. The most highly expressed member of the Meq-cluster is mdv1-miR-M4-5p, a functional orthologue of the cellular gga-miR-155 [60]. gga-miR-155 is highly conserved from humans to chickens [61••] and is involved in virus-induced cancers such as Epstein-Barr virus-induced lymphomas in humans [62]. Similarly, miR-M4-5p of MDV plays a crucial role in MDV-lymphomagenesis as reviewed by Zhuang and colleagues [58, 59, 61••]. The mid-cluster is located downstream of *meq* and includes three pre-miRNAs (Fig. 1) [63]. This cluster is dispensable for MDV replication; however, deletion of one of the mid-cluster miRNAs, miR-M31, also leads to a decrease in MD incidence and tumorigenesis [64]. The last cluster is termed LAT-cluster as it is present within the latency-associated transcripts (LAT) and consists of five pre-miRNAs (Fig. 1) [56, 57]. The LAT-cluster encodes for at least one IE gene-specific miRNA, miR-M7-5p, that may contribute to the establishment and maintenance of latency [65].

Viral Telomerase RNA

Telomerase is a large ribonucleoprotein complex that is involved in the maintenance of telomeres at the end of eukaryotic chromosomes [66]. The telomerase complex contains two major components, the catalytic subunit telomerase reverse transcriptase (TERT) and a telomerase RNA (TR or TERC), which provides the template for the extension of the telomeres.

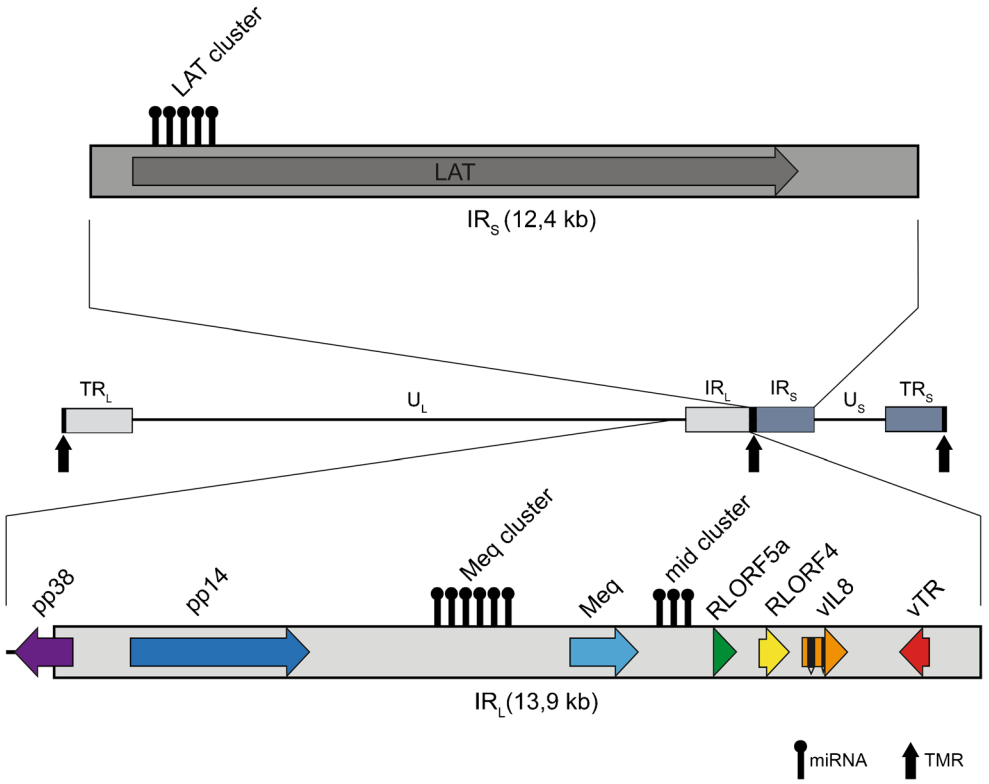

Fig. 1 Overview of the MDV genome. Schematic representation of the MDV genome with a focus on the viral factors involved in pathogenesis and tumorigenesis. The two unique regions, unique long (U_L) and short (U_S) are flanked by terminal (TR_L and TR_S) and internal (IR_L and IR_S) inverted repeat regions. The unique regions mainly harbor genes that are conserved among alphaherpesviruses and are involved in DNA replication and production of progeny virus. The repeat regions contain MDV-specific genes encoding for proteins or RNA that are important for pathogenesis, cellular tropism, tumorigenesis, and latency. The viral telomeric repeats (TMR) are crucial for integration of the virus genome into host telomeres and are highlighted with arrows. The position of the following genes is shown in the IR_L and IR_S: latency-associated transcripts (LAT), phosphoprotein 14 (pp14) and 38 (pp38), major oncogene *meq*, RLORF4 and 5a, viral chemokine vIL-8, viral telomerase RNA (vTR), miRNAs, and TMR

Beyond that, the complex contains a number of species-specific telomerase-associated proteins that regulate telomerase activity and biogenesis [67, 68]. MDV encodes a viral TR (vTR) homologue that is crucial for efficient MDV-induced lymphoma formation [69]. vTR has an 88% sequence identity to the cellular TR in chickens (chTR) [70] and is the most abundant viral transcript detected in MDV-induced tumor cells [69]. Chbab and colleagues demonstrated that these high expression levels are crucial for MDV-induced tumor formation [71].

vTR is incorporated into the telomerase complex and enhances its activity when compared to chTR [72]. To investigate whether the tumor-promoting functions of vTR are dependent on its role in telomerase activity, Kaufer and colleagues generated recombinant viruses with a mutation in vTR that abrogated incorporation into the telomerase complex [73]. Intriguingly, lymphoma formation was not altered in the absence of the vTR-induced telomerase activity and only the onset of disease was slightly delayed [73]. Furthermore, tumor dissemination was also comparable to wild-type virus [73], suggesting that the tumor-promoting functions of vTR are independent of its role in the telomerase complex [73]. We recently demonstrated that another viral RNA, the Epstein-Barr virus-encoded RNA-2 (EBER-2), can complement the loss of vTR in MDV-induced tumor formation [74], suggesting conserved mechanism(s) between these viral RNAs. Further investigations are needed to decipher the mechanism of vTR in MDV-induced transformation.

MDV Telomeric Repeats

MDV establishes latency in CD4+ T cells and integrates its genome into the telomeres of their chromosomes [10, 11]. Interestingly, the integrated virus genome is usually detected in multiple chromosomes of latently infected and tumor cells [11]. Integration is facilitated by telomeric repeat (TMR) arrays present within the a-like sequences at both ends of the virus genome and at the IR_L-IR_S junction [75, 76]. Each a-like sequence harbors two TMR arrays: short telomeric repeats (sTMR) with a fixed number of 6 repeats and multiple telomeric repeats (mTMR) with a variable number of repeats [11, 77, 78]. Deleting or mutating the mTMR severely impaired integration, pathogenesis, and tumor formation [11]. The sTMR have a dual function in the MDV life cycle. On the one hand,

the sTMR play a key role in the integration of MDV, as mutation of the sequence reduces integration frequency and decreases MDV pathogenesis and tumor formation. One the other hand, the sTMR serve as essential spacers between the packaging signal pac-1 and the DR-1 cleavage sites, as its deletion completely abrogates MDV replication [77]. Truncation analyses revealed that the exact length of the sTMR is crucial for virus replication [77]. Intriguingly, several other herpesviruses harbor TMR arrays at the ends of their genomes [79], which also contribute to the integration of the respective virus into host telomeres [78, 80]. While our understanding of the role of the TMR arrays has tremendously increased over the last years, it remains completely unknown which viral and/or cellular proteins facilitate this integration into host telomeres.

Conclusions

Most of the genes encoded in the MDV genome have homologues in other alphaherpesviruses. They play important roles in DNA replication, particle formation, egress, and many other processes essential for the virus lifecycle. In addition, MDV encodes several virus-specific genes that are not primarily involved in replication but play a key role in viral pathogenesis. The best characterized gene by far is the major oncogene *meq* that is crucial for tumor formation as it regulates gene expression and blocks apoptosis [81]. The viral chemokine vIL-8 ensures that the target cells are recruited to the site of infection, a prerequisite for the success of this highly cell-associated pathogen. RLORF4 and the two phosphoproteins pp14 and pp38 also drive MDV pathogenesis; however, more work needs to be done to understand how these proteins contribute to this process. In addition to these proteins, MDV also encodes several RNAs that are crucial for MDV pathogenesis such as vTR and the MDV-encoded miRNAs. Furthermore, the MDV genome also harbors sequence elements such as the viral telomeric repeats that facilitate integration into host telomeres and maintenance of its genetic material with the host chromosomes. This complex set of proteins, RNAs, and sequence elements in the virus genome contributes to MDV pathogenesis and makes it such a successful pathogen.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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