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The human cytomegalovirus non-coding Beta2.7 RNA as a novel therapeutic for Parkinson's disease – Translational research with no translation

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ABSTRACT

Human cytomegalovirus (HCMV) encodes abundant numbers of microRNAs (miRNAs) and other non-coding RNAs (ncRNAs) whose functions are presently under intense investigation. In this chapter, we discuss the function of one of the more well characterised virus-encoded ncRNAs, derived from the viral major early gene (Beta2.7). This RNA plays an anti-apoptotic role during infection by directly interacting with mitochondrial complex I to help maintain high levels of ATP production and by preventing the stress induced re-localisation of retinoid/interferon-induced mortality-19 protein, GRIM-19. We then go on to describe how an 800 nucleotide sub-domain of the Beta2.7 transcript, p137, has been exploited in the development of a novel therapeutic for the treatment of Parkinson's disease.

1. Introduction

Human cytomegalovirus (HCMV) is member of the betaherpevirus sub-family which can cause significant morbidity and mortality in the immune compromised or immune naive. However, primary infection of the healthy immune competent is generally subclinical. Like all herpesviruses, the ability of HCMV to enter a latent life cycle, after primary infection, helps the virus persist for the life time of the host and this persistence likely includes periodic reactivation events which, again, rarely result in disease. HCMV has co-evolved over millions of years with its human host which has led to the adaptation of the virus to co-exist in human populations with extreme efficiency – the seroprevalance of HCMV can range from 60% to 90% in the developed world.

HCMV is the largest known herpesvirus with a double stranded DNA genome encoding approximately 250 open reading frames (ORFs) which encode functions to facilitate efficient latent and lytic infection cycles. The lytic lifecycle can be observed after primary infection in vivo in a variety of cells types; such as fibroblasts, macrophages and endothelial cells. In these cells, lytic infection is associated with a temporal cascade of, so-called, immediate early (IE), early (E) and late gene expression which culminates in the production of infectious virions. A substantial number of genes expressed during the virus lytic cycle have immune-modulatory functions which are believed to enable the virus to evade the host immune system and establish latency in cells such as CD34+ myeloid progenitor cells. During latency, the lytic transcription programme is suppressed, resulting in the expression of a much more restricted spectrum of latency-associated genes and this is characterised by the absence of production of infectious virions. Only following terminal differentiation of the latently infected progenitor CD34+ cells to macrophages or dendritic cells, does virus reactivation occur resulting in activation of IE gene expression and re-entry into the lytic lifecycle.

2. HCMV non-coding RNAs

It is now becoming clear that, in addition to ORFs which result in expression of mRNAs encoding proteins, HCMV also encodes a substantial number of ncRNAs. Although the functions of many of these ncRNAs are currently not well defined, roles for some of them have been identified.

2.1. HCMV-encoded microRNAs (miRNAs)

miRNAs are short single stranded RNA molecules which down-regulate expression of transcripts which contain complementary nucleotide sequences. Since their first identification as regulators of larval development in nematodes (Lee et al., 1993), they have been described to play key roles in the regulation of almost every

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important biological process in eukaryotes across the metazoan kingdom (Lu and Cullen, 2004).

Biochemical and bioinformatic analysis, as well as next generation sequencing, has now identified at least 20 mature HCMV encoded miRNAs derived from 13 pre-miRNAs (Fu et al., 2014; Meshesha et al., 2012; Stark et al., 2012). Whilst the functions of a number of these virally encoded miRNAs are still unclear, some targets, which include both viral and cellular mRNAs, have been identified and these are believed to be involved in orchestrating changes in the infected cell to optimise lytic infection. Perhaps unsurprisingly, a good number of viral miRNAs have been demonstrated to modulate the expression of cellular mRNAs which encode proteins that play a role in cell death or immune evasion. For instance, HCMV miR-UL170-3p and miR-UL148D have been reported to play a role in the regulation of apoptosis by targeting the pro-apoptotic proteins MOAP1, PHAP, and ERN1 (Babu et al., 2014). In addition to targeting anti-apoptotic proteins, a separate study demonstrated that UL148-D also targets CCL5 (RANTES) (Kim et al., 2012) which could contribute to a reduction in leukocyte chemoattraction to the infected cell. Another immune evasion strategy is the ability of viral miR-US4-1 to target ERAP-1. ERAP-1 is an amino peptidase which functions to regulate MHC class I presentation, thus perturbation of this gene contributes to the evasion of CD8+ T cell responses (Kim et al., 2012). The viral miR-UL112-1 targets IL-32 (Huang et al., 2013). Since IL-32 is a pro-inflammatory cytokine which can induce the expression of a number of pro-inflammatory cytokines in myeloid cells such as monocytes and macrophages (a site of latency and reactivation, respectively), targeting of this gene could potentially enhance carriage of the virus.

In addition to modulation of immune responses, HCMV miRNAs can also fine tune the intracellular environment by a number of mechanisms. There are a number of viral miRNAs that regulate the expression of cellular genes. For example, HCMV miR-UL148D targets the cellular immediate early gene X-1 (Wang et al., 2013) and the viral miR-US28-2-3p targets eIF41a (Qi et al., 2013). Viral miRNAs have also been reported to modulate the expression of HCMV genes. Viral miR-UL-112-1 targets the HCMV viral IE1 gene (Grey et al., 2007), viral US7 is targeted synergistically by two viral miRNAs, miR-US5-1 and miR-US5-2 (Tirabassi et al., 2011). HCMV viral miRNAs have also been implicated to play specific lifecycle-specific roles. For example, HCMV miR-UL36 targets the latency- associated viral gene, UL138, during lytic infection and thus decreases expression of this gene during the lytic cycle (Huang et al., 2013).

Although viral miRNAs have been shown to play important roles during latency in other herpesviruses (Pfeffer, 2007; Pfeffer et al., 2005; Samols et al., 2007), there are, as yet, no definitive reports of latency-associated functions of HCMV miRNAs. Although, it is likely that HCMV encoded miRNAs are also likely to be involved in the modulation of viral and cellular functions during latent infection. Indeed, viral miRNAs are well-suited to the demands of the latent life cycle; they are small non-immunogenic molecules that will minimise detection and eradication of the latently infected cells by the immune system. This is a particularly important consideration during latency as the majority of the immune evasion genes encoded by the virus are likely not expressed during this part of the viral life cycle. Furthermore, as individual miRNAs are able to concurrently modulate the expression of multiple targets (Zhu et al., 2013), their expression enables the alteration of gene expression at a global level. Thus, the known profound effects of viral miRNAs on cellular and viral gene expression are also likely to be mirrored during latency and could have significant roles in the establishment and maintenance of latency and reactivation. For example, the ability of HCMV miR-UL112-1 to target the viral major immediate early gene could serve to enhance the maintenance of latency by silencing any 'leaky' transcription from the major immediate early promoter of lytic IE gene expression (Murphy et al., 2008; O'Connor et al., 2014). Similarly, as mentioned above, HCMV miR-UL36 targets the latency- associated viral gene, UL138, during lytic infection and thus decreases expression of this gene during the lytic cycle (Huang et al., 2013).

2.2. Long non-coding RNAs

In addition to miRNAs, HCMV is also known to encode other RNAs which are polyadenylated but do not appear to function as protein coding RNAs. These include long non-coding RNAs (lncRNAs) such as RNA2.7, RNA1.2, RNA4.9 and RNA5.0 which can be expressed at extraordinarily high levels during lytic infection (Gatherer et al., 2011; Kulesza and Shenk, 2004) although deletion of RNA5.0 did not result in any growth defects of the virus in fibroblast cells (Kulesza and Shenk, 2004). Although none of these overlap with established annotated protein coding regions, they may produce polypeptides as a result of non-canonical translation of these RNAs (Ingolia et al., 2014).

Although the functions of many of these lncRNAs during HCMV infection is far from clear, recently, some have been shown to target specific gene promoters mediating transcriptional silencing, often by epigenetic targeting. For instance, one of the HCMV encoded lncRNAs, RNA4.9, has been proposed to play a role in transcriptional repression of viral IE gene expression during latency (Noriega et al., 2014; Rossetto et al., 2013a). Identified during both experimental and natural latent infection (Rossetto et al., 2013a), the RNA4.9 transcript has been shown to associate directly with the polycomb repressor complex 2 (PRC2) in experimentally latently infected CD34+ cells and this has been suggested to enhance silencing of the viral MIEP during latent infection by recruitment of histone modifiers so resulting in histone H3 tri-methylation at lysine 27 around the MIEP (Rossetto et al., 2013a). Viral lncRNAs are not specific to HCMV, other herpesviruses also encode them. Interestingly, the KSHV encoded PAN RNA also binds to PRC2 and is proposed to play a similar role to that of HCMV RNA4.9 (Rossetto et al., 2013b) during KSHV latency (Rossetto and Pari, 2014).

Arguably the most enigmatic HCMV lncRNA, is expressed from the viral Beta2.7 gene. Present as two copies in clinical isolates of HCMV (McSharry et al., 2003), it is the most abundant viral RNA produced during lytic infection – some 20% of all viral RNAs expressed at early times of lytic infection derive from the Beta2.7 genes (Gatherer et al., 2011). A potential coding frame within Beta 2.7 was identified in the highly passaged strain AD169 and this potential mRNA was termed RL4 (Bergamini et al., 1998). Similarly, the coding potential of Beta2.7 has been recently been re-assessed (Stern-Ginossar et al., 2012). However, sequencing of all other strains of virus demonstrated that this potential coding region is disrupted (McSharry et al., 2003). Further to this, although in vitro studies demonstrated that a short 24 kDa protein could be expressed from the RL4 region of Beta2.7, intracellularly the RNA was found to be nucleolar and its expression was post-transcriptionally inhibited by the 5′ sequence of the transcript (Bergamini et al., 1998). Taken together, therefore, it seems unlikely that the Beta2.7 encodes a polypeptide.

3. Anti-apoptotic functions of the non-coding HCMV RNA p137

By early times (12–24 h) of the HCMV lytic cycle, the transcript, represents approximately 20% of the total viral RNA expressed in the infected cell and is known to be associated with mitochondria (Gawn and Greaves, 2002). Since the Beta2.7 RNA does not encode any protein, potential cellular protein partners which may interact directly with this RNA were identified by screening a human cell cDNA expression library in lambda phage with an RNA probe