Mini-Review

Harnessing the Power of miRNAs in Influenza A Virus Research

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Abstract | The application and use of miRNA-based technology in influenza research is rapidly expanding. miRNAs are currently being used to manipulate pathogenicity and immunity and as biomarkers for disease severity.

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The biological interaction between a pathogen and its host is dynamic and complex, much of which is regulated by proteins associated with normal cell function, i.e. proliferation, differentiation and survival. As miRNAs are key regulators of gene and therefore protein expression, characterisation of miRNA profiles following infection is an area of current intense research as a way of informing our understanding of pathogenesis and host cell immunity. Unsurprisingly, these miRNA profiles are being investigated as potential biomarkers for lung, prostate and breast cancer (Chen et al., 2008; Mattie et al., 2006), chronic hepatitis (Xu et al., 2011), tuberculosis (Qi et al., 2012) and influenza A virus infection.

Viruses have also taken advantage of miRNAs and co-opted their gene silencing properties to manipulate the host cell environment. Viruses, including members of the Herpesviridae family, such as Epstein-Barr virus (EBV), herpes simplex viruses (HSV1 and HSV2) and cytomegalovirus (CMV) have been shown to produce miRNAs targeting numerous host cell pathways, including MAPK, PI3K and KEGG (Carl et al., 2013). Not all viruses however encode their own miRNAs. For example, while influenza A viruses have been shown to express high levels of small viral leader RNAs, the 5'-terminal triphosphates they express prevents them from functioning as bone-fide miRNAs (Umbach et al., 2010). Furthermore, next generation sequencing of RNA extracted from influenza A virus infected cells failed to identify classical virally encoded miRNAs (Cullen, 2010). This however, does not mean that influenza A viruses are incapable of expressing miRNAs. In 2010, Varble et al. modified the non-structural (NS) gene segment of influenza A virus to generate an artificial intron between the NS1 and NEP genes where a brain-specific miRNA was expressed (Varble et al., 2010). The purpose of this study was to demonstrate that RNA viruses were capable of expressing miRNAs. In 2014, our group further refined this method to allow targeted modulation of host immunity in response to influenza virus infection (Izzard et al., 2014). We concluded that this novel delivery system provided a valuable platform from which to dissect host cell biology and could be used to manipulate host gene expression to improve disease outcomes.

Naturally occurring host cell miRNAs that can directly target viral genes are still quite rare. A few known examples include; (i) direct targeting of the HIV Nef gene by human miR-29a, a gene critical to HIV-1 pro-

gression (Ahluwalia et al., 2008) (ii) inhibition of the human cytomegalovirus (HCMV) UL122 gene by human miR-200, a gene shown to assist in establishing and maintaining viral latency (O'Connor et al., 2014), (iii) direct targeting and mRNA degradation of the influenza virus PB1 gene by host miR-323, miR-491 and miR-654, a gene important for virus replication (Song et al., 2010) (iv) inhibition of H1N1 influenza A virus M1 protein expression by host let-7c (Ma et al., 2012) and (v) discovery of putative targets for hsa-miR-145 and hsa-miR-92a within the 2009 H1N1 influenza virus hemagglutinin (HA) gene segment of (He et al., 2009). Additional evidence that supports the interaction between influenza A viruses and host miRNAs comes from a study performed by Matskevich and Moelling and colleagues (2007) where they demonstrated that knocking out endoribonuclease Dicer activity, a vital component of the RNAi pathway, led to an increase in influenza A virus replication.

Characterisation of miRNA profiles and their downstream effects on influenza virus pathogenesis and immunity have been documented in the literature. In 2010, Li et al. discovered that the miRNA profile in mice infected with a recombinant 1918 pandemic H1N1 virus was altered when compared to a non-lethal seasonal control (A/Texas/36/91) (Li et al., 2010). In this study the authors also noted that the majority of differentially expressed miRNAs targeted mRNAs known to play a role in immune responses and cell death pathways. This led them to speculate that gene expression patterns observed in infected mice may be partly attributed to miRNA regulation and that this regulation could be a contributing factor to the high virulence associated with the recombinant 1918 pandemic H1N1 virus (Li et al., 2010). Furthermore, miRNA signatures and profiles are increasingly being developed as biomarkers of disease and have been used to differentiate between influenza a virus-infected and uninfected patients. Blood samples obtained from patients infected with the 2009 H1N1 pandemic virus differentially expressed 193 miRNAs when compared to healthy uninfected controls. Fourteen of the identified miRNAs were consistently altered in both *in vitro* and *in vivo* systems (Tambyah et al., 2013). More recently, a study characterising the serum of H7N9 influenza virus infected patients was able to differentiate between infected and healthy patients by analysing as few as 4 miRNAs (Zhu et al., 2014).

The identification of distinct miRNA profiles or signatures can also be used to differentiate infection between two influenza virus strains. Loveday and colleagues analysed the exosomal miRNA profile of A549 cells infected with swine-origin 2009 H1N1 or avian-origin H7N9 influenza viruses and showed not only a large number of commonly altered miRNAs, but also a significant number of miRNAs that were unique for each strain (Loveday et al., 2012). These unique miRNA signatures could potentially be used as biomarkers for strain-specific diagnosis of infected hosts.

Recombinant technology has also be used to generate modified viruses that contain miRNA target sequences within their genes. This is of particular interest given the fact many miRNAs are tissue specific (Lagos-Quintana et al., 2002). To this end, Langlois and colleagues generated a recombinant H5N1 influenza A virus that incorporated miR-192 target sites within the open reading frame of the HA gene segment (Langlois et al., 2013). It is important to note that miR-192 is only expressed in respiratory tract epithelial cells of the mouse but not the ferret. Infection studies with this modified virus demonstrated successful attenuation in mice without altering replication or transmissibility in ferrets. The ability to modify the host range of a virus without altering its pathogenicity in susceptible hosts offers scientists the possibility of added levels of protection when working with highly pathogenic strains. For example, an otherwise highly pathogenic virus such as H5N1 could be modified to contain target sequences for miRNAs highly expressed in human cells, so that it was incapable of infecting humans while still retaining highly pathogenic characteristics in other hosts that did not express these miRNAs.

RNAi is a rapidly advancing field, with its use documented in numerous fields including cancer, infectious disease and molecular biology. In the area of influenza A virus research, miRNAs are currently being used as biomarkers of disease and as tools for the modulation of pathogenicity and immunity. As our knowledge of miRNAs improves, their use in this and other fields will only continue to grow.

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