Review Article

# Targeting Cellular and Virus Encoded LncRNAs: Emerging Opportunities for Novel Anti-influenza Therapies

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**Abstract** | Long non coding RNAs (lncRNA) contribute to a large proportion of the cellular RNA and are involved in regulating a plethora of biological and pathological processes. There is a strong evidence to suggest the association of differential regulation of non-coding RNAs (ncRNAs) to a number of disease processes including viral infections. Cellular and virus encoded lncRNAs are emerging as novel regulatory factors which can escape the anti-protein environment in the virus infected cells and are likely to be conserved across the species. Here, we summarize our current knowledge about the role of lncRNAs in influenza virus infection and the exciting prospect of exploiting lncRNAs as targets to develop novel anti-viral therapies.

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It is interesting that only a small portion of the human genome (~2%) is transcribed into messenger RNA (mRNA), and the majority of the genome is transcribed into non-coding RNAs (ncRNAs) (Cawley et al., 2004) that do not code for any proteins. Long non coding RNAs (lncRNAs) are non-coding transcripts larger than 200 nucleotides located in various chromosomal regions and are recognized as the major class of RNA species encoded by the genome (Fitzgerald et al., 2014). Although our understanding of the specific functions of lncRNAs is still in its infancy, in the recent years, high throughput transcriptome analysis techniques have helped reveal more information about lncRNAs. LncRNAs regulate innate and adaptive immune responses through a number of mechanisms such as chromatic modification, interferon (IFN) pathway regulation, genomic imprinting, and transcriptional regulation.

A large proportion of cellular lncRNAs are differentially regulated during many viral infections, for example lncRNA B cell integration cluster (BIC) is upregulated in avian leukosis virus infection (Tam et al., 1997), expression of lncRNA nuclear enriched abundant transcript 1 (NEAT1) increases in mice infected with Japanese encephalitis virus or rabies virus (Saha et al., 2006). In addition to cellular lncRNAs, certain virus encoded lncRNAs are also expressed in infected cells. While cellular lncRNAs appear to regulate a range of biological function including the regulation of antiviral pathways, viral lncRNAs control transcription and translation of cellular and viral genes (Fortes et al., 2015).

Recent studies revealed differential regulation of a range of cellular lncRNAs during influenza A virus (IAV) infection and one lncRNA is supposedly re-

quired for virus multiplication in an IFN-independent pathway (Landeras-Bueno and Ortin., 2015), but the existence and functions of IAV encoded lncRNA is yet to be revealed. A human lncRNA designated as negative regulator of antiviral response (NRAV) in IAV infection was first demonstrated in a recent study (Ouyang et al., 2014). LncRNA NRAV is located within intron 1 overlapping the antisense strand of dynein light chain coding gene *dynll* and is a part of host's antiviral innate immune response which is significantly downregulated in various viral infections. Downregulation of NRAV is initiated as a self-defense mechanism by the host to initiate virus clearance as it helps in accumulation of a number of antiviral proteins by negatively regulating the transcription of multiple interferon stimulated genes (ISGs). LncRNA NRAV is downregulated during an infection with IAV subtype, A/WSN/33 in both human alveolar epithelial cells (A549) and in transgenic mice expressing human NRAV. LncRNA NRAV negatively regulates the transcription of multiple critical ISGs, by remodeling chromatin. Further, it appears that lncRNA NRAV is conserved across different species, although it might have evolved to be differentially regulated. More mechanistic studies exploring the precise regulatory role of lncRNA NRAV in the IFN and other cellular signaling pathways are required to see if it can be exploited as a potential target for therapeutic intervention.

Expression of lncRNA, BST2 IFN-stimulated positive regulator (BISPR) that is regulated by IFNα2 correlates to expression level of the neighboring protein coding gene bone marrow stromal cell antigen 2 (BST2) in a range of viral infections including Influenza (Barriocanal et al., 2014). BST2 also known as tetherin, is expressed following IFN pathway stimulation and is known to inhibit the release of various enveloped viruses without interacting with viral proteins (Perez-Caballero et al., 2009; Blanco-Melo et al., 2012). Post-transcriptional inhibition of lncRNA BSPR by RNA interference (RNAi) decreases the levels of BST2 mRNA which further impacts the antiviral effects of IFN negatively. Upregulation of BISPR is seen in human immunodeficiency virus (HIV), influenza virus, vesicular stomatitis virus (VSV) and hepatitis C virus (HCV) infections and its downregulation has no effect on other IFN related genes, based on which the authors suggested that interference with these factors may have therapeutic relevance in HIV, Influenza, HCV and VSV virus infections.

NEAT1 plays a role in interleukin 8 (IL8) regulation in IAV infected cells. NEAT1 is induced through the activation of TLR3-p38 pathway and is involved in splicing factor proline/glutamine-rich (SFPQ) mediated splicing regulation and causes relocation of SFPQ/PSF form the IL8 promoter region activating IL8 transcription (Imamura et al., 2014). Role of NEAT1 during viral infection is presumably evolutionarily conserved and hence is a good target for therapeutic intervention.

Peng et al., 2010 observed a similar lncRNA regulation in response to severe acute respiratory syndrome – Corona virus (SARS-CoV) and IAV infection in mice suggesting that differential expression of lncRNAs may be a common host response to respiratory viral infections. Another virus inducible lncRNA (VIN) is found to be differentially regulated as a response to intact IAV particles and not to the indirect stimulation by infected cells (Winterling *et al.*, 2014). LncRNAs are documented to have high tissue specificity than coding genes (Guttman et al., 2009) and they can function in both *cis* and *trans* manner. It is anticipated that cis-regulatory mechanisms should markedly increase the chances of finding targeted therapies (Wahlestedt, 2013). While most of these studies on the role of cellular lncRNA in IAV infections have been carried out using a single IAV subtype (for example A/WSN/33 or A/PR8/34), it is important to evaluate if the function of these lncRNAs is virus subtype dependent.

Several virus-encoded lncRNAs have been discovered (Greenaway et al., 1987; Urosevic et al., 1997). For example lncRNA PAN (polyadenylated nuclear) is found in Kaposi's sarcoma virus (KSHV) that controls viral gene expression (Rossetto and Pari, 2012), which silences the host immune genes and binds to LANA (latency associated nuclear antigen) helping the virus switch from latent to lytic infection. Similarly, Saayman et al. (2014) demonstrated that an antisense lncRNA is essential for the maintenance of latency in HIV. However, to date several virus encoded ncRNAs have not been studied in detail for their role in virus pathogenesis and multiplication. Viruses have evolved mechanisms to subvert host defense to replicate efficiently (Horner and Gale, 2013). There is a mounting evidence to support the central role of ncRNAs in controlling gene expression by targeting various stages of epigenetic remodeling and therefore it is highly likely that virus encoded ncRNAs could

play a key role in virus evasion of host cellular defense. Virus encoded ncRNAs can escape the protein-hostile environment established by the infected cells where the cell blocks translation of new proteins and the preexisting protein structures are affected in their stability (Fortes et al., 2015). To date there have been no reports about IAV encoded ncRNAs. Since ncRNAs can tolerate mutations better than the coding genes (Ulitsky et al., 2011; Ponjavic et al., 2007) and they are probably better conserved among various IAV strains, identification of IAV encoded ncRNAs present new opportunities.

The currently available therapeutic options for Influenza infection are constantly threatened by the emergence of resistant strains (Tomkins et al., 2004). Hence, there is an urgent need to explore novel anti-influenza therapeutic strategies. Targeting lncRNA has major advantages over targeting the protein coding part of the genome for therapeutic intervention. Nakagawa et al. (2011) demonstrated that nuclear lncRNA NEAT1 knocked down mice were viable and fertile showing no apparent phenotype. Similarly, Eibmann et al. (2012) observed that loss of abundant nuclear lncRNA metastasis-associated lung adenocarcinoma transcription 1 (MALAT1) is compatible with cell viability and normal development. Successful manipulation of the functions of regulatory lncRNAs via antago natural antisense transcript (NATs) (Wahlestedt, 2013) has led to a growing interest in therapeutically targeting lncRNA through this approach. Increasing number of functional lncRNAs in human cells is being identified and the understanding of the disparate roles of these transcripts in different disease systems is rapidly increasing. Efforts to identify IAV encoded ncRNAs and their functional evaluation could lead to the development of novel intervention strategies for this global problem of animal and human health importance.

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