

Non-coding RNAs that regulate the cellular response to infection

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Summary

Non-coding (nc)RNAs are rapidly emerging as a fundamental mechanism through which changes in cell state are regulated. Recently, long intergenic RNAs (lincRNAs) have been found to regulate embryonic stem cell states through their effects on transcription and chromatin structure. Different sets of lincRNAs are also expressed in other cell types. Whether lincRNAs play a role in the response of cells to infection is unknown.

Aims of the project

1. Discover ncRNAs that form part of the cellular response to interferon.
2. Verify that these RNAs are regulated in response to viral infection.
3. Identify ncRNAs that are necessary for the anti-viral response.

Background

Non-coding RNAs

Recently, a great deal of excitement has been centred around emerging evidence that non-coding RNAs, that do not code for any proteins, play an important role in the regulation of transcription and chromatin structure. Long intergenic RNAs (lincRNAs) are transcribed from between genes or antisense to genes, generally in a cell type specific manner (Cabili et al., 2011). Around 8000 RNAs have been identified so far. A number of these have been investigated in more detail and these RNAs directly interact with transcription factors or chromatin regulators, leading to changes in the expression of protein-coding genes (Kanhere and Jenner, 2011). A number of RNAs have been found to interact with polycomb proteins, which act during development to maintain genes in a repressed state. The 2.2kb RNA HOTAIR binds to polycomb and targets it to the HOXD developmental transcription factor locus (Rinn et al., 2007) while the RNA Xist functions in X-inactivation (Zhao et al., 2008) - the process by which one of the two X-chromosomes is silenced in female cells. We have also identified a class of small ncRNAs transcribed from otherwise silent genes in T-cells and ES cells that also interact with polycomb proteins (Kanhere et al., 2010). Other ncRNAs interact with and modulate the activity of transcription factors, such as NRON, that modulates the activity of the immune cell regulator NFAT (Willingham et al., 2005) and Gas5 RNA that prevents the glucocorticoid receptor from binding to its target sites in the genome (Kino et al., 2010).

The cellular response to infection

Upon infection by bacteria and viruses, mammalian cells respond by initiating a complex transcriptional program targeted at disabling the pathogen and preventing its replication (Jenner and Young, 2005). This response is particularly important in cells of the innate immune system for control of pathogen replication and to activate the adaptive immune response. A significant part of this innate response is regulated by type I interferon, principally associated with host defence against viral infection, but also implicated in their pathogenesis. These cytokines are produced as the result of a signalling cascade triggered by one of a number of pathogen recognition receptors. Interferons then signal through cell surface receptors to activate IRF and STAT transcription factors that

upregulate the expression of hundreds of genes. These then act to create a cellular environment restrictive to pathogen replication, to process and present antigen and to activate the immune response. We have used microarrays to catalogue these interferon-induced genes and to identify how cells respond differentially to different pathogen types (Jenner and Young, 2005; Noursadeghi et al., 2009; Tsang et al., 2009). Whether this response includes regulation of ncRNAs and whether these RNAs play a role in the inhibition of pathogen replication is not known but is likely given the role for these RNAs in other cell types.

Aims and Techniques

1. Discover ncRNAs that form part of the cellular response to interferon.

The student will differentiate macrophages, treat them with interferon beta and collect RNA over a timecourse. The RNA will then be analysed by DNA microarrays and by next-generation sequencing. For sequencing, RNA will be separated into poly-adenylated RNA, total long RNA and total short RNA and then amplified by ligation-mediated RT-PCR. Sequencing with Illumina Hi-Seq sequencers will provide around 50 million sequence reads. RNAs up and downregulated during the response to interferon will then be identified by bioinformatics tools.

2. Verify that these RNAs are regulated in response to viral infection

Macrophages will be infected with a panel of innate immune stimuli and important human pathogens including HIV-1 and Herpes viruses. RNA will be purified and the expression of interferon-responsive ncRNAs identified in Aim 1 will be measured using custom microarrays and quantitative PCR and RNAs to identify shared or stimulus/pathogen specific responses. The expression of selected ncRNA will then be evaluated in clinical samples from patients with acute infectious diseases in order to establish that they exist in a physiological setting in vivo.

3. Identify ncRNAs that are necessary for the anti-viral response.

Once RNAs regulated by interferon and responsive to virus infection have been identified, experiments will be performed to assess their functional importance. RNA levels will be knocked down by RNA interference or increased by overexpression from vectors and the effects on the replication of HIV-1 and Herpes viruses measured.

4. Investigate the mechanism through which the RNAs function.

Efforts will then be made to determine how one or two functionally important RNAs act. To test whether the RNA causes changes in gene expression, the mRNA from cells in which the RNA is knocked down and overexpressed will be measured using DNA microarrays. To assess whether the RNA causes changes in histone modifications, these will be measured across the genome by ChIP-Seq. To determine whether the RNA interacts with specific cellular proteins, it will be biotinylated and proteins interacting in cell lysates identified by mass spectrophotometry.

Bench to Bedside

Our division is closely affiliated to Clinical Infectious Diseases at UCLH through the Infection Theme of the NIHR Biomedical Research Centre. Extensive interactions between laboratory and clinical researchers provide invaluable opportunity to obtain greater insight into the challenges of translating basic science knowledge into clinical benefits and the opportunities to focus scientific efforts on important medical problems.

References

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