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**Microrna's regulating gene expression as novel markers and therapeutic markers**S. Gay, *Rheumatology, University Hospital, Zurich, Switzerland*

The epigenetic regulatory mechanisms involving all biological processes in health and disease include acetylation of histones, methylation of certain CPG islands in promoters, phosphorylation, sumoylation/ubiquitination and miRs or microRNAs. All these processes are intimately intertwined. In this regard, microRNAs can be methylated and regulated by specific histone marks. The best example was shown recently in a most comprehensive study performed by Stefan Kuchen et al characterizing the distinct sets of micro RNAs and their controlling histone marks regulating the immune response in mice (1).

Our laboratory studies the various epigenetic regulations in rheumatic diseases (2). With respect to microRNAs, which are short RNA sequences comprised of 20–22 nucleotides, bind to complementary mRNA sequences along with argonaute proteins resulting in translational repression and thereby silencing of gene expression. More than 1000 microRNAs are involved in the modulation of gene expression.

For example, we could demonstrate that microRNA 155 is regulated by TNF $\alpha$ , IL-6 and TLR signaling (3). Moreover, based on the fact that microRNA 155 is involved into B and T cell development, we could show that microRNA ko mice do not develop arthritis (4). On the other hand, we could report that microRNA 203 is regulating the expression of IL-6 and that this microRNA is induced by hypomethylation of its promoter (5). On the other hand, IL-6 regulates the microRNA cluster 17/92 in the down regulation of bone morphogenic protein receptor 2 (BMP2) in vascular cells during the development of pulmonary hypertension (6). In related studies Matthias Brock in our lab could show that microRNA-18a enhances the interleukin-6-mediated production of the acute-phase proteins fibrinogen and haptoglobin in human hepatocytes (7). These data reveal, for the first time, a microRNA-mediated positive feedback loop of IL-6 signal transduction leading to an enhanced acute-phase response in human hepatocytes.

MicroRNAs will be novel diagnostic biological markers and new therapeutic targets

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**Antiviral actions of the interferon-inducible IFIT proteins**G. Sen, *Cleveland Clinic, Cleveland, United States*

Antiviral actions of the interferon-inducible IFIT proteins

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The ISG56/IFIT genes are a multi-member family of interferon-inducible genes; there are four members in human and three in mouse. All IFIT proteins contain multiple TPR motifs which mediate protein-protein interactions. Some, but not all, members inhibit initiation of protein synthesis by binding to the translation initiation factor eIF-3 or by binding to the 5' end of the mRNA. The murine Ifit1 encodes P56, Ifit2 encodes P54 and Ifit3 encodes P49. For investigating the biological functions of the murine Ifit proteins, we have recently generated several knock-out mice. Challenging Ifit1<sup>-/-</sup> and Ifit2<sup>-/-</sup> mice with various viruses have revealed their strong, but selective, antiviral properties. Ifit1-mediated host restriction was shown to be evaded by 2'-O-methylation of viral mRNAs. Flavivirus (West Nile Virus), vaccinia virus and coronavirus mutants, that lack 2'-O-methylation of their mRNAs, were growth-restricted in Wt, but not in Ifit1<sup>-/-</sup> cells and mice. Further studies revealed that the action of Ifit1 on WNV replication is highly manifested in CNS infection, a deficiency of Ifit1 causing increased neuronal death in infected mice. Ifit2, on the other hand, blocked neuro-pathogenesis caused by intranasal infection with the rhabdovirus, VSV. All Ifit proteins were induced in the CNS of the infected mice, but Ifit1<sup>-/-</sup> mice were not more susceptible than Wt mice and most of the infected mice survived. In contrast, all Ifit2<sup>-/-</sup> mice died from neuro-pathogenesis; VSV replicated efficiently in the neurons of these mice. However, there was no effect on pathogenesis by another neuro-tropic virus, EMCV. Moreover, VSV did not replicate efficiently in the liver or the lung of infected Ifit2<sup>-/-</sup> mice and in vitro, in MEF or primary fetal neurons, the sensitivity of VSV replication to IFN-treatment was similar in Wt and Ifit2<sup>-/-</sup> cells. These results demonstrated the existence of tissue-, virus- and ISG-specific antiviral actions of interferon.

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**Linear positive and negative regulation of the interferon antiviral response**J. Hiscott, *Division of Infectious Diseases, Vaccine & Gene Therapy Institute of Florida, Port St. Lucie FL, United States*

The cytosolic RIG-I pathway is activated by many RNA viruses - including influenza - via viral RNA replicative intermediates that contain short hairpin dsRNA and 5' triphosphate structures. We characterized natural and synthetic RIG-I agonists based on sequences from the 5'UTR regions of distinct negative-strand viruses - vesicular stomatitis virus (VSV), Influenza, Rabies, Measles, and Sendai virus - and demonstrated potent stimulation of RIG-I antiviral responses at concentrations in the picomolar range. In human bronchial epithelial A549 cells, 5'pppRNA induced IRF3 phosphorylation and dimerization, STAT1 Tyr701 phosphorylation, as well as a >100-fold increase in the transcription of interferon stimulated genes (ISGs) and genes involved in inflammation. The magnitude and duration of ISG and inflammatory gene expression was evaluated by gene expression profiling, where 5'pppRNA triggered a sustained and diverse range of antiviral and inflammatory genes compared to treatment with IFN $\alpha$ , and bioinformatics analysis identified distinct nodes of IRF7, IRF1 and NF- $\kappa$ B gene activation. Pre-treatment of A549 cells with 5'pppRNA dramatically blocked H1N1 A/PR/8/34 Influenza virus replication; furthermore, intravenous delivery of 5'pppRNA to BALB/c mice generated an antiviral response in mouse lungs that protected animals from a lethal challenge with H1N1 A/PR/8/34. RNA agonist delivery inhibited virus replication in mouse lungs within the first 24h after H1N1 challenge and protected the lungs of infected animals from virus-induced pathology. Finally, 5'pppRNA pre-treatment also completely or partially blocked replication of Dengue, Vaccinia and HIV-1 replication. These results illustrate that naturally derived RIG-I agonists represent a potent stimulator of the innate antiviral response, with the capacity to block replication of multiple pathogenic human viruses.

Termination of IFN signalling is likewise crucial to the proper maintenance of the innate and adaptive immune response to virus infection. We also identified an essential role for LUBAC-mediated linear ubiquitination of NEMO in the negative regulation of the RIG-I antiviral pathway through sequestration of TRAF3 from the MAVS adapter. LUBAC and NEMO-Ub constructs inhibited RIG-I signaling downstream of MAVS and upstream of TBK1; linearly ubiquitinated NEMO interacted physically with TRAF3, and disrupted the MAVS-TRAF3 complex, thus providing a mechanistic explanation for the downregulation of RIG-I signaling. Using SHARPIN deficient *cpdm* MEFs, we observed on the one hand, an increased and prolonged antiviral response, while on the other hand, an impaired NF- $\kappa$ B activation, indicating that linear ubiquitination is required for NF- $\kappa$ B activation downstream of RIG-I. Interestingly, an increase in apoptotic cell death was also detected in SHARPIN-deficient *cpdm* MEFs after VSV infection, potentially attributable to the absence of the anti-apoptotic activity of NF- $\kappa$ B. These studies reveal a novel negative feedback mechanism used by host cells to regulate the IFN antiviral response.

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**Type 1 interferons in multiple sclerosis**F. Sellebjerg, *Copenhagen University Hospital Rigshospitalet, Danish Multiple Sclerosis Center, Copenhagen, Denmark*

The type 1 interferon (IFN) IFN-beta is used as immunomodulatory treatment for patients with relapsing forms of multiple sclerosis (MS). Recent studies do, however, indicate that endogenous type 1 interferons may also have immunoregulatory effects in MS. Patients with higher activity of an endogenous type 1 IFN gene expression signature have higher expression of interleukin (IL)-10, do not show evidence of myelin-reactive T cell activation, and were reported to have lower clinical and magnetic resonance imaging disease activity. Cell sorting experiments have indicated that monocytes is the main cell type expressing IL-10 in response to IFN-beta. Although IL-27 is also strongly induced by IFN-beta, ex vivo studies indicate that in contrast to what is the case in mice, IL-10 is more important than IL-27 for the immunoregulatory effect of IFN-beta in humans. The endogenous type 1 IFN gene expression signature is controlled by interferon response factors (IRF) such as IRF5 and IRF8, which show association with MS in genetic studies. Surprisingly, however, the alleles conferring an increased risk of developing MS are also alleles associated with increased endogenous IFN activity. We suspect that this may reflect that other genes induced by these IRFs are involved in the pathogenesis of MS.

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