RNase-L protein expression from an mRNA that contained its 3′UTR, but not from an mRNA that lacked this sequence. Thus, the miR-17-92 cluster regulated RNase-L in a 3′UTR-dependent manner, consistent with the mechanism of miRNA action. Experiments are underway to evaluate the impact of miR-17 and other candidate miRNAs on the biological activities of RNase-L, and to assess the consequences of miRNA deregulation on RNase-L-dependent cell survival and apoptosis. Ultimately, understanding the details of RNase-L regulation will permit the manipulation of these mechanisms for therapeutic applications.

doi:10.1016/j.cyto.2009.07.317

PP1-195 Novel bioassays for mouse type I and type III interferons

Daniela Kugel, Julia Elisabeth Pulverer, Mario Köster, Hansjörg Hauser, Peter Staeheli, Poster Presentation I

Novel bioassays for mouse type I and type III interferons

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Quantification of type I (α/β) and type III (λ) interferons (IFN) of mice by ELISA or conventional virus inhibition assays is cost-intensive and cumbersome. We established rapid and highly sensitive nonviral bioassays that detect mouse type I and type III IFN, respectively. These assays based on cell cultures from Mx2p-LUC transgenic mice that express a firefly luciferase gene under control of the IFN-regulated mouse Mx2 promoter. We found that embryo fibroblasts from Mx2p-LUC mice which express only low levels of functional receptors for type III IFN are highly suitable for the preferential detection of type I IFN. The assay works even better if cells from Mx2p-LUC mutant mice are used which lack IFN-β genes and thus cannot trigger transgene expression via spontaneous release of IFN-β . When employed to measure type I IFN in either serum of Rift Valley fever virus-infected mice or extracts of lungs from influenza virus-infected mice, we found that this new bioassay was robust and substantially more sensitive than standard ELISAs. For the selective detection of type III IFN, we used embryo fibroblasts from Mx2p-LUC mutant mice that lack functional receptors for type I IFN. This assay readily detected recombinant mouse IFN-λ derived from cDNA-transfected human 293T cells. Surprisingly, however, this assay did not detect any IFN-λ in either serum of Rift Valley fever virus-infected mice or extracts of lungs from influenza virus-infected mice, suggesting that the levels of type III IFN in tissues of virus-infected mice are rather low.

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doi:10.1016/j.cyto.2009.07.318

PP1-196 Oct-6 is an interferon inducible protein and contributes to the transcriptional responses to poly(I:C)

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Oct-6 is an interferon inducible protein and contributes to the transcriptional responses to poly(I:C)

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The octamer binding factor 6 (Oct-6; also known as Pou3f1, SCIP or Tst-1) belongs to the family of Pit-Oct-Unc-(POU)-domain containing transcription factors and has been shown to have either positive or negative effects on target gene expression. Oct-6 is known to play a crucial role in the terminal differentiation of myelinating Schwann cells and squamous epithelia. Its expression is tightly controlled on the transcriptional level, but the factors and *cis*-acting elements involved are largely unknown. Tissue-specific and time-dependent expression is regulated by distal enhancer elements (e.g. the Schwann cell specific enhancer element 12 kb downstream of the transcription start site). We have found that Oct-6 is expressed in murine embryonic fibroblasts and bone marrow-derived macrophages after stimulation with type I or type II interferon (IFN). Oct-6 is also induced in response to poly(I:C) treatment and during viral infections, both in a strictly type I IFN dependent manner. The expression of Oct-6 is largely dependent on signal transducer and activator of transcription 1 (Stat1), partially dependent on tyrosine kinase 2 (Tyk2), but independent of IFN regulatory factor 1 (Irf1). We have identified a region in the Oct-6 promoter that contains two predicted IFNγ activated sites (GAS). Binding of Stat1 to this region in response to IFNβ and IFNγ treatment is shown by chromatin immunoprecipitation. In order to identify target genes of Oct-6 and to investigate its potential role in innate immunity, a microarray experiment was performed. Comparison of the transcriptome of wild type (WT) and Oct-6-deficient fetal liver-derived macrophages in response to poly(I:C) treatment showed involvement of Oct-6 in the transcriptional control of a subset of genes. One hundred and sixty genes displayed at least twofold differential expression levels ($p < 0.05$) between WT and Oct-6-deficient cells after poly(I:C) treatment. Validation of microarray data by RT-qPCR is currently in process.

doi:10.1016/j.cyto.2009.07.319

PP1-197 Inflammation in the liver is associated with decreased expression of immunohistochemically detected myxovirus resistance protein B

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Inflammation in the liver is associated with decreased expression of immunohistochemically detected myxovirus resistance protein B

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The IFN inducible myxovirus resistance protein B (MxB) has no detectable antiviral activity. MxB is expressed in absence of IFN stimulation and its role in the cellular physiology is currently not well defined. Previously we have shown that chronic liver disease evolution is associated with increased expression of immunohistochemically detected MxA in the liver. Here we report the findings of the study of MxB expression in liver diseases. We used retrospective diagnostic pre-treatment needle biopsies from chronic hepatitis and liver cirrhosis and surgical biopsies from patients with HCC. Group A consisted of 10 biopsies with minimal changes and histological activity score (HAI) 16 patients with chronic hepatitis HAI 3–18, Group C - 45 patients with liver Ci, Group D - 26 patients with HCC(BLCL stage 0-A). MxB protein was visualized using polyclonal rabbit antiserum (Julkunen, NPHI, Helsinki); DAKO Envison DAB+ kit was used for visualization. The Allred scoring system, validated in breast pathology, was used for semi-quantitative assessment of the expression. The MxB protein was expressed both in cytoplasm and nuclei of the hepatocytes. The corresponding cytoplasmic expression scores were as follows: Group A 6.18 +/− 2.04; Group B 4.2 +/− 1.34; Group C 6.02 +/− 1.45; Group D 6.5 +/− 1.04. The neoplastic cells in Group D showed lower mean score of MxB protein expression, as compared to the surrounding tissue. Paired comparison of the cytoplasmic MxB expression in hepatocytes (6.8 +/− 1.03) vs neoplastic cells (4.7 +/− 3.02) in the same biopsy showed lower expression in the neoplastic cells ($p = 0.049$). Group B showed significantly lower score as compared to group A - 0.006; C - 0.000; and D - 0,000072. There was no difference in the nuclear MxB expression. Biliary epithelial cells showed greater staining intensity than the hepatocytes. Our data suggest possible down regulation of MxB protein during chornic hepatitis with high HAI & cytolysis and in HCC.

doi:10.1016/j.cyto.2009.07.320

PP1-198 Expression of interferon-induced microRNAs in patients with chronic hepatitis C virus infection treated with pegylated interferon alpha

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Expression of interferon-induced microRNAs in patients with chronic hepatitis C virus infection treated with pegylated interferon alpha

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There have been recent reports of in vitro IFN-mediated antiviral activity against the hepatitis C virus (HCV) through microRNAs (miRNAs). The aim of this study was to evaluate the expression of several miRNAs (miR-1, miR-30, miR-128, miR-196, miR-296) in PBMCs from patients with chronic hepatitis C (CHC) before and 12 h after the first injection of pegylated IFN alpha. Gene expression analysis of MxA, a well-characterized IFN type I gene, was also performed. Expression of these miRNAs could