CREATING A CELL LINE SUITABLE FOR INVESTIGATION INTO THE ADAR1 ROLE IN HEPATITIS C VIRUS REPLICATION

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Adenosine deaminases acting on RNA (ADAR) perform the adenosine to inosine (A-to-I) type of editing. Out of the three human ADAR proteins, ADAR1 is responsible for the majority of A-to-I editing of dsRNA outside the brain. By introducing I into the RNA sequence, thereby altering the base pairing in the region, or by its sheer dsRNA binding activity, ADAR1 can influence miRNA processing, alternative splicing, nuclear export, degradation or protection of RNA molecules (as reviewed in 1). On top of the variety of effects ADAR1 can have on a particular RNA, ADAR1 editing itself has been shown to be influenced heavily by the cell type. In recent years, studies on particular ADAR1 effects have relied mainly on RNA-seq experiments and knock-down cell line assays.

Not only cellular RNAs, but also viral RNAs can be targeted by ADAR1, where the effect of ADAR1 protein binding or editing can have a proviral or antiviral effect (as reviewed in 2). Identifying the ADAR1 effect on a particular virus faces the same difficulties as in the case of cellular RNAs - cell tropism and possible artifacts of the knock-down method used. Hepatitis C virus (HCV) exhibits a high level of tropism for hepatocytes and is almost exclusively studied in cell lines derived from hepatocellular carcinoma, Huh7.5 (3).

In order to avoid knock-down effects in the identification of ADAR1 effect in further virological experiments, we decided to use CRIPSR/Cas9 system to establish an Huh7.5 ADAR1 KO cell line. While the Huh7.5 ADAR1 KO cell line exhibits similar morphology as the parental Huh7.5 wt, we observed an increase of cell area of Huh7.5 ADAR1 KO. The ADAR1 KO cell line also exhibited increased sensitivity to IFN-α and IFN-β treatment at concentrations as low as 0.1 nM. The interferon treatment induced a rapid growth and translation arrest in the Huh7.5 ADAR1 KO cell line.

For further characterization of the Huh7.5 ADAR1 KO cell line, we performed RNA sequencing of poly(A) RNA from total RNA and polysomal profile fractions originating from the Huh7.5 wt and Huh7.5 ADAR1 KO cell lines. The poly(A) RNA sequencing data from total RNA was used for transcriptome analysis, which revealed hundreds of genes with altered transcript abundance. The poly(A) RNA sequencing data from separate polysome profile RNA fractions was used for a new method of translatome analysis. We found that both transcriptome and translatome of the Huh7.5 ADAR1 KO cells were significantly changed. Among the RNAs and processes that were significantly changed in the Huh7.5 ADAR1 KO cell line, there were the deregulations of snoRNA and Y RNA levels and negatively affected transcription by RNA polymerase III. Furthermore, we observed that polysomal fraction in Huh7.5 ADAR1 KO was enriched in mRNAs coding for proteins pivotal role in a wide range of biological processes such as RNA processing and RNA localization, whereas the unbound fraction was enriched mainly in mRNAs coding for translational factors and ribosomal proteins. This indicates that ADAR1 possibly plays a more relevant role in small RNA metabolism and ribosome biogenesis.

With the cell line characterized, we are now ready to move onto the virological experiments. We already performed pilot experiments with HCV infection of Huh7.5 wt and Huh7.5 ADAR1 KO aimed at the investigation into ADAR1 role in HCV replication in hepatocytes.

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