IPD Project Details

Project ID: IPD8622

Project Title: Hepatitis C virus induces HuR relocalization by coordinating PKC-? and AMPK-? function

Description: Huh7.5 cells were transfected with HCV-JFH1 RNAand harvested after 48h of transfection. Total proteins were extracted in IP lysis buffer (Pierce, Thermo Scientific) containing 1× halt-protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, USA) with intermediate vortexing followed by mild sonication. The lysate was centrifuged at 14,000 × g for 30 min at 4 °C. The supernatant was quantified by the BCA method (Thermo Fisher Scientific, USA). An equivalent amount (4 mg) of proteins from each condition was incubated with 3?g of anti-HuR antibody for overnight at 4 °C. The immunocomplex was captured by using protein G Sepharose 4 fast flow beads (17-0618-01/ GE Healthcare). The immunoprecipitated complex was washed two times with IP lysis buffer and one time with mili-Q. Bound protein complexes were eluted in 50 µl SDS-PAGE 2X Laemmli sample buffer. The samples were resolved on 12% SDS–PAGE and stained with Coomassie stain (0.1% w/v). The gel was subjected to further in-gel mass spectrometry analysis.

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Sample Preparation: Gel slices corresponding to each lane were excised in individual eppendorf and destained using wash buffer (50 mM ammonium bicarbonate (ABC) and 50% ACN). Slices were hydrated with 10 mM DTT (56°C, 30 min), alkylated using 50 mM IAA (30 min, RT, dark), and further dehydrated using 100% ACN before digestion.

Peptide Separation: The available proteins in-gel pieces were digested with MS grade trypsin (Pierce™ Trypsin Protease MS grade, cat no. 1862743, USA) and incubated at 37 °C for 18 h. Digested peptides were extracted by adding 60% ACN with 0.1% FA and 100% ACN with 0.1% FA, followed by ultra-sonication. The digested peptides were collected, vacuum dried, and reconstituted in 40 ?l of solvent A (2% (v/v) ACN, 0.1% (v/v) FA in water) and subjected to LC?MS/MS experiments using Sciex 5600+ Triple-TOF mass spectrometer coupled with ChromXP reversed-phase 3 ?m C18-CL trap column (350 ?m × 0.5 mm, 120 Å, Eksigent, AB Sciex) and nanoViper C18 separation column (75 ?m × 250 mm, 3 ?m, 100 Å; Acclaim Pep Map, Thermo Scientific, USA) in

EksigentnanoLC (Ultra 2D plus) system. The binary mobile solvent system was consisted with solvent A (2% (v/v) ACN, 0.1% (v/v) FA in water) and solvent B (98% (v/v) ACN, 0.1% (v/v) FA). The peptides were analysed with 300 nl/min flow rate in a 60 min gradient with a total run time of 75 minutes. The acquisition was executed with conventional data-dependent IDA mode. Each cycle consisted of 250 and 100 ms acquisition time for MS1 (m/z 350?1250 Da) and MS/MS (100–1500 m/z) scans respectively with a total cycle time of 2.8 s. Each fraction was run in duplicate.

Protein Characterization: All raw files (.wiff) were processed to ProteinPilot software (version 4.5, SCIEX) using the Paragon algorithm (version 4. 5. 0. 0,1654). The phospho-peptides along with phospho-sites were identified against the complete sequence of Hepatitis C virus JFH-1 polyprotein. The identification settings were used as follows: (a) trypsin for proteolytic cleavage (b) Iodoacetic acid used for Cys alkylation and (c) phosphorylation emphasis used as a special factor. Peptides and proteins were validated at ?1% false discovery rate (FDR) and with unused ProtScore ? 0.05.

Experiment Type: Gel-based experiment, Affinity purification coupled with mass spectrometry proteomics

Species: Homo sapiens

Tissue: Hepatocyte (bto:0000575)

Cell Type: Hepatocyte (cl:0000182)

Disease: Unknown

Instrument Details: TripleTOF 5600 (MS:1000932)

Protein Modifications: phosphorylated residue

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