Funding: The resources used in this study were from the hospital without any additional financing

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100852

Assessment of cellular proliferation, death and senescence in a model of steatosis in vitro

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Introduction and objectives: Hepatocyte cell culture in steatogenic medium is a useful, reproducible tool. During NAFLD, cells undergo processes in response to the steatogenic input. Here we aimed to study cellular proliferation, death, and senescence in an in vitro model of steatosis.

Materials and Methods: HepG2 hepatocytes were cultured in standard RPMI1640. Steatogenic medium was prepared by supplementing RPMI1640 with lipids in two levels: Mild steatosis (MS: 50 μM sodium oleate/sodium palmitate (OA/PA) at 2:1 ratio) and Severe steatosis (SS: 500 μM OA/PA 2:1). A control (C) group (RPMI1640) was included. 105 cells per well were allowed to attach for 24 h in RPMI1640 at 37°C and 5% CO2, then incubated in MS or SS medium for up to 96 h. Steatogenic medium was refreshed daily. Viability and mortality rates were assessed, and proliferation and senescence were analyzed by commercial kits, followed by a morphometric analysis. All assays were performed in triplicates. Data: Mean ± SD. 2-way ANOVA followed by Tukey. $P<0.05$.

Results: MS and SS showed significantly lower cell viability versus C. Mortality rates were increased in MS and SS. Proliferation was significantly decreased in MS and SS compared with C. MS showed a significantly increased senescence from 48 h versus C, whereas in SS decreased compared with C and MS.

Conclusion: MS showed an increment in senescence compared with C and might be considered a mechanism aimed at avoiding damaged-cell proliferation. In contrast, SS showed an increased mortality rate and decreased senescence, suggesting activation of death pathways as a response to lipid overexposure.

Funding: This work was funded by Conacyt (CB-221137)

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100853

The administration of pirfenidone modifies the expression of JMJD2B in a murine model of NASH

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Introduction and Objectives: This study aimed to evaluate how the administration of pirfenidone modifies the expression of JMJD2B in a murine model of NASH.

Materials and methods: 4–5-week-old male C57BL/6J mice fed a high-fat diet for 16 weeks. Follow-up was done at 4, 8, 12 and 16 weeks. Serum glucose, animal weight, caloric intake, AST, ALT, TAG, Chol and VLDL were measured. The liver was weighed, as was the epididymal adipose tissue. Masson's trichrome hematoxylin-eosin staining was performed. Dual-channel microarrays were hybridized to the 22,000-gene version of the Mus musculus genome. Analyzed with adjusted P-values of <0.05 and Z-score values of >1.5 and <1.5 considered significant. Quantitative variables were analyzed with ANOVA, Tukey for parametric data, and Kruskal-Wallis for non-parametric data. The trial was approved by the research ethics committee.

Results: The animals achieved the body and biochemical parameters that demonstrate the development of NASH. The genes involved in epigenetic processes responsible for the development of NASH (SIRT1, SIRT2, JMJD1B) and, in particular, in JMJD2B; which found to have significantly different between the HFD vs. HFP and HFD vs. ND groups.

Discussion: JMJD2B is a histone methylation modulating enzyme, implicated in the development of NASH. In our trial, pirfenidone modulates the expression of JMJD2B, helping the recovery of liver function through epigenetic regulation in a murine model of NASH.

Conclusion: Pirfenidone appears to modulate epigenetic factors, supporting recovery from the disease.

Funding: The resources used in this study were from the hospital without any additional financing

Declaration of interest: The authors declare no potential conflicts of interest.

https://doi.org/10.1016/j.aohep.2022.100854

Evaluation of deubiquiyinase USP15 expression during HCV replication

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Introduction and Objectives: This study aimed to evaluate the USP15 protein expression in HCV replication in vitro.

Material and methods: Huh-7 cell line was transfected with pFKI plasmid that encodes for the non-structural HCV proteins; this expression is regulated by the T7 RNA polymerase promoter; therefore, prior to transfection, the cells were infected with Vaccinia Virus for T7 RNA polymerase expression. After 24 h of transfection, at 37°C and 5% CO2, total protein was extracted and quantified using the Bradford method. USP15 expression was evaluated by Western Blot assay using the antibodies for USP15, NS3-HCV and actin as a control.

Results: The expression of NS3 was found in the transfected cells; in addition, a decrease in the expression of USP15 was observed compared to the control without viral proteins.

Discussion: USP15 expression was shown to be downregulated in cells expressing HCV nonstructural proteins compared to control cells. A lower effect on USP15 expression was detected in the transfected cells compared to the HCV-replicon cells (positive control); this may be due to the low expression of NS3 in transfected cells. Therefore, we observed a decrease in USP15 expression dependent on NS3 expression. USP15 is known to regulate pathways including TLR signaling, RIG-I signaling, NF-kB, and IRF3/IRF7-dependent transcription to produce pro-inflammatory cytokines and type I interferons. Therefore it is important to elucidate the mechanisms involved in this regulation by HCV.

Conclusion: USP15 expression is decreased in the presence of HCV nonstructural proteins.