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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.

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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.

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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.

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Pirfenidone regulates antioxidant response via NRF2 in an experimental model of hepatocellular carcinoma

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Introduction and Objectives: 1) Determinate if pirfenidone (PFD) modifies oxidative stress markers. 2) Evaluate PFD effects on transcription factor Nrf2 signaling pathway.

Materials and methods: Eighteen Fischer-344 rats divided into three groups were used: 1) untreated (NT), 2) carcinogenic damage (HCC) generated by weekly administration of diethylnitrosamine (50mg/kg/week;i.p.) and 2-Acetylaminofluorene (25mg/ kg/wk, p.o) and 3) HCC treated with PFD (300 mg/kg, p.o.) (HCC/PFD) for 18 weeks. Histopathological analyzes of the liver were performed, MDA and GSH levels were quantified and SOD, CAT, GSTP1 and Nrf2 expression was evaluated by Western-Blot. Data were analyzed using ANOVA and Tukey's test as post hoc. The trial was approved by the research ethics committee.

Results: In the HCC group, Nrf2, SOD, CAT, and GSTP1 expression was increased. PFD treatment was effective in preventing the increase in MDA levels and allowed GSH increase; in addition, PFD was effective in modulating the expression of Nrf2 and antioxidant response proteins.

Discussion: Oxidative stress is key in the genesis of HCC and the mechanisms leading to antioxidant response are modulated by Nrf2. PFD is an antioxidant evaluated in several liver fibrosis models. Additionally, in this work, we have demonstrated that the antioxidant response of PFD in an HCC experimental model is mediated by Nrf2.

Conclusion: PFD delays the HCC development by regulating Nrf2 signaling pathway. Clinical studies with PFD are being devised to evaluate the safety.

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Dietary supplementation with methyl donors improves physiopathological conditions of NAFLD in a murine model

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Introduction and Objective: This study aimed to evaluate the benefits of supplementation with methyl donors of a diet rich in fat and sugars in a model of NAFLD.

Material and methods: Male mice of the C57BL/6J strain with an initial weight of 20-25g were fed with a conventional diet (ND n=8) or a diet high in fats and sugars (HF n=8) for 18 weeks; or with a diet rich in fats and sugars for 10 weeks, plus eight weeks of HF diet + supplementation with methyl group donors (HFMS n=8). At 18 weeks, ITT was performed; it was collected at sacrifice: liver, fat, and serum. Histological and biochemical analyzes were performed and global hepatic DNA methylation was quantified. The trial was approved by the research ethics committee.

Results: The supplemented animals (HFMS) showed a decrease in body weight, liver weight and epididymal and visceral fat ($p<0.001$). The area of the adipocytes in the HFMS group decreased significantly compared to the HF group. The HFMS group presented reduced serum levels of triglycerides and glucose and greater sensitivity to insulin. Histological analysis of livers from ND and HFMS animals showed no damage characteristic of NAFLD, such as lipid infiltration and inflammation. Global methylation increased in HFMS animals.

Discussion: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver damage worldwide. The results in this work reinforce the evidence that supplementation with methyl group donor molecules could work as a therapeutic strategy to prevent the progression of the disease.

Conclusion: Supplementation with methyl donors of a diet high in fats and sugars has beneficial effects in a murine model of NALFD.

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Effects of in vitro lipid overload on LX-2 hepatic stellate cells

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Introduction and Objectives: Studying lipid overload repercussions on hepatic stellate cells (HSC) is of great importance due to their role in fibrosis during NAFLD. Steatogenic cell culture of HSC is intended to elucidate pathogenic mechanisms in NAFLD.

Materials and Methods: LX-2 HSC were cultured in standard DMEM. Steatogenic medium was prepared: mild steatosis (MS:50μM sodium oleate/sodium palmitate (OA/PA) at 2:1 ratio), severe steatosis (SS:500μM 2OA:1PA). Control (C) was cultured in DMEM. Cells were pre-incubated in DMEM at standard conditions for 24h the incubated in MS or SS medium. Cells were incubated for up to 72h. Viability and mortality ratios were assessed; cellular proliferation and senescence were assessed. Data: Mean ± SD, two-way ANOVA followed by Tukey. $P<0.05$.

Results: Cell viability in MS significantly diminished by 13.6% at 72h, whereas SS showed 49.6 % lower viability from 48h compared with C. Regarding mortality rate, it was increased by 16.0% in MS from 72h and by 50.0% in SS from 48h compared with C. Proliferation was increased in both MS and SS at 24h and significantly decreased by 72h compared with C. Cellular senescence in both steatogenic conditions was diminished among 1.8-22.4% compared with C at 24 and 48h.

Conclusion: Steatogenic conditions induced an increased proliferation and lower senescence in LX-2 HSC at 24h in both MS and SS groups. These findings suggest that HSC might turn into an activated state. Our results agree with other reports showing that HSC activation and transdifferentiation increase their proliferation, avoiding