Declaration of interests
None

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Pirfenidone slows the development of fibrosis and malignant neoplasms by modulating inflammation in an experimental model of hepatocarcinoma.

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Introduction and objectives: Hepatocellular carcinoma (HCC) is the most common liver neoplasm in the world. Inflammatory, oxidative and fibrogenic processes are key in tumor development and propagation. Pirfenidone (PFD) has been shown to have hepatoprotective, anti-fibrogenic and immunomodulatory properties during hepatocarcinogenesis. However, its effect on established HCC is unknown. Our aim is to evaluate the effect of PFD administration on the tumor and inflammatory microenvironment in an experimental hepatocellular carcinoma model.

Materials and Patients: Fischer-344 rats (n=18) protocolized into three groups: CTL: control, HCC: damage group, (induced by diethylnitrosamine (DEN) 50 mg/kg and 2-acetaminofluorene (2AAF) 25mg/kg/weekly for 16 weeks), HCC/PFD: damage group + administration of PFD 300 mg/kg/daily. Subsequently, immunoassays and histological analyzes were performed to assess inflammatory patterns, fibrosis, and malignancy.

All animals received human care, and all the experiments were performed according to the Guide for the Care and Use of Laboratory Animals, under the approval of the Research, Ethics, and Biosafety committees of the CUCS whit approval number CI-03020.

Results: In the HCC/PFD group, the observed nodules were smaller in number, size, and protrusion compared to the HCC group. Additionally, there was a decrease in fibrosis development, extracellular matrix synthesis, as well as collagen and α-SMA expression. The loss of hepatic architecture was restored, and there was a decrease in the percentage of transformed hepatocytes positive for Glypican-3 expression, in contrast to the HCC group. Furthermore, there was a restoration of p53 expression. Moreover, the local secretome showed a decrease in IL-10 and an increase in IL-6 and IL-1β compared to the HCC group. Finally, the expression and localization of CD45 and CD161 were observed to be increased within the tumor niches compared to the HCC group.

Conclusions: Treatment with PFD slows the development of both macroscopic and microscopic patterns of malignancy and fibrosis, decreases the activation of hepatic stellate cells, the local inflammatory secretome, and modulates the components of the tumor microenvironment, thus improving the conditions and progression of the neoplasia. Therefore, pirfenidone could mean an improvement in the quality of life and an increase in the survival of patients with HCC in advanced stages.

Ethical statement
The protocol was registered and approved by the Ethics Committee.

Declaration of interests
None

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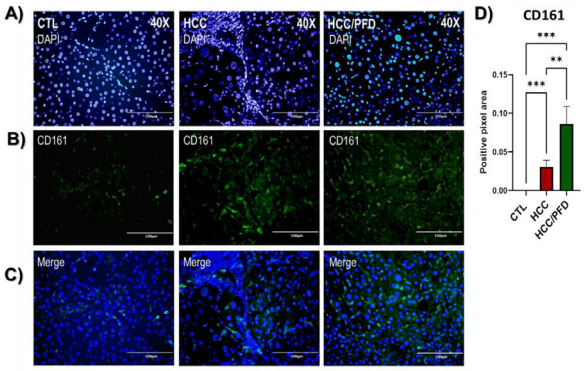

Figure 2. Immunofluorescence for CD161 detection. In the blue channel (A), cell nuclei stained whit DAPI. In the green channel (B), Alexa-green for CD161. (C) Merge. It is observed that the PFD treatment increased the infiltration of CD161-positive cells, whit co-localization in tumor nice centers, suggesting enhanced cytotoxic activity by these cells. (D) Quantification of positive pixel areas, showing a higher positive area in the PFD-treated group. One-way ANOVA analysis considered statistically significant at $p<0.05$, $^{*}p<0.5$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$.

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Pirfenidone induces translocation of sirt1 to nucleus and deacetylation of histone 3 slows down the development of fibrosis and tumorigenicity in a experimental model of hepatocarcinoma.

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Introduction and objectives: Hepatocellular carcinoma (HCC) is the most common liver neoplasm worldwide. Pro-inflammatory and

pro-fibrogenic processes are key in tumor development. On the other hand, Pirfenidone (PFD) has anti-inflammatory and antifibrogenic properties useful to counteract hepatocarcinogenesis; however, the effects of this drug on SIRT1, and histone H3 regulation in this disease are unknown.

The objective this work is evaluate PFD effects on SIRT1 translocation, and histone H3 lysines 9 and 14 (H3K9 and H3K14) deacetylation in an experimental model of HCC.

Materials and Patients: Fischer-344 rats were divided into three groups: CTL: control group, HCC: group damaged with diethylnitrosamine (DEN), 50 mg/kg and 2-aminofluorene (2AAF), 25 mg/kg/p.o. HCC/PFD group: damage group and with PFD (300 mg/kg/day) for 16 weeks. Histological and molecular analyzes were performed evaluating patterns of protein acetylation, fibrosis, and malignancy.

Results: Normal liver architecture is disturbed by dysplastic nodules formation surrounded by extracellular matrix and fibrosis, also an increase in cells with anaplasia and steatotic foci was observed in liver tissues of HCC group. PFD administration was effective to prevent these changes. Immunohistochemistry reveals an overexpression of GPC3 and α-SMA in damage group, which is correlated with malignant degeneration, these responses was prevented by PFD too. Finally, western blots evidence a SIRT1 overexpression in nuclear fraction of PFD group, triggering H3K9 and H3K14 deacetylation, in addition, a decrease in p300 acetylase expression in nuclear fractions. Notably, c-Myc was reduced and p53 increased significantly.

Conclusions: PFD treatment reduces fibrotic and malignant patterns development. Likewise, PFD induces SIRT1 expression and nuclear translocation, and H3K9 and H3K14 deacetylation, decompacting chromatin and possibly increasing tumor suppressor genes expression for example c-MYC. These results demonstrate for the first time the capability of PFD to regulate epigenetic hallmarks on histones.

Ethical statement

All the experiments were carried out in accordance with the guidelines approved by the Ethics, Research and Biosafety committees of the CUCS with approval number CI-03020.

Declaration of interests

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Bovine matrix scaffold implanted in rat liver improve regeneration in a partial hepatectomy model.

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Introduction and objectives: Liver transplant is the recommended therapeutic option for advanced liver damage in chronic liver disease. However, the biocompatibility and low donation rates significantly reduce the chances of performing a successful transplant. The use of inert biomaterials such as collagen matrix scaffolds (CMS) has been suggested as a promising option to restore the function of organs, including liver. The objective was to evaluate the biocompatibility and liver restoration after partial hepatectomy and the implant of bovine matrix scaffold in a rat model.

Materials and Patients: Three groups of Wistar rats were evaluated: Sham, Partial hepatectomy (PH) (40%, left lobe) and PH + Collagen Matrix (CM). After surgical procedure the animals were monitored and the exploratory laparoscopy and histological analysis at 14 and 30 days was performed. The liver function was also compared in the three animal groups. The study was approved by the ethics committee of the School of Medicine at the *Universidad Nacional Autónoma de México* (*UNAM*). All procedures were performed according to official Mexican policy (*SAGARPA*, 1999). Our institution fulfills all technical specifications for the production, care, and use of laboratory animals and is certified by national law (*NOM*-062-ZOO-1999).

Results: The biomaterial showed evidence of reabsorption, the animals did not display signs of infection or systemic alterations. Moreover, the histopathological evaluation showed abundant hepatocyte proliferation and angiogenesis near to the site of CM implantation. An incipient inflammation or exacerbate macrophages, Langhans-type, and foreign-body giant cells were observed; these findings strongly suggest not rejection at 30 days. Furthermore, no statistical differences in albumin, bilirubin, cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were observed at day 14 in sham, PH and PH + CM.

Conclusions: Bovine collagen matrix showed great compatibility with the liver and was bioabsorbable. The incorporation of this biomaterial does not interfere with liver function and promotes the proliferation of hepatocytes and vessels, showing typical arrangement of the hepatic parenchyma. The use of the biomaterial could be beneficial to reduce the current limitation of organ transplant.

Ethical statement

This study was approved by the ethics committee of the School of Medicine at the Universidad Nacional Autónoma de México (UNAM). All procedures were performed according to official Mexican policy SAGARPA, 1999). Our institution fulfills all technical specifications for the production, care, and use of laboratory animals and is certified by national law (NOM-062-ZOO-1999).

Declaration of interests

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