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Contents

Introduction

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) form a spectrum of disease characterized by accumulation of excess fat within (steatosis) the liver variously associated with lobular inflammation parenchymal injury and fibrosis [1, 2]. They are considered as the hepatic manifestations of the so called 'Metabolic Syndrome' a cluster of closely related clinical features linked to visceral obesity that include insulin resistance, dyslipemia and cardiovascular diseases. NAFLD/NASH is now the most frequent hepatic lesion in western countries with prevalence in the general population ranging from 3-15% but reaching up to 70% among overweight individuals [1, 3]. While simple steatosis in NAFLD is most often benign, in about 20-30% of patients the disease can progress to NASH, a condition characterized by hepatocellular damage, intralobular inflammation and fibrosis that not rarely, leads to liver cirrhosis and in some case to hepatocellular carcinoma [4]. In fact, within 8 years, 15% of NASH patients develop clinically and histologically evident cirrhosis. Death rate ascribed to NASH-related cirrhosis accounts for 12-25%, while end-stage NASH is responsible for about 4-10% of liver transplants. A further matter of concern in NAFLD/NASH epidemiology is its increasing diffusion among children and adolescents as consequence of the growing of childhood obesity and overweight [5]. NAFLD prevalence among children and adolescents ranges between 2.6% and 9.8% respectively and is especially high among obese subjects, making it the most frequent pediatric chronic liver disease all over the world. The clinical and social relevance of NAFLD/NASH has stimulated a number of studies to clarify the mechanisms leading to the disease in an attempt to develop effective treatments. However, the mechanisms responsible for the progression of NAFLD to more severe liver injury remain poorly understood.

Studies in patients and in experimental models of NASH suggest that oxidative stress and metabolic alterations may play a role in the progression of disease. [6]. In turn, ROS-dependent lipid peroxidation promotes a self-sustaining loop that leads to further mitochondrial damage and causes mitochondrial DNA (mtDNA) mutations [7-9]. Increased production of reactive nitrogen species (RNS) generated as a consequence of nitric oxide synthase 2 (NOS2) inductions by pro-inflammatory cytokines also contributes to oxidative injury [10]. Accordingly, a significant increase of oxidative damage markers along with a concomitant reduction of the liver antioxidant content are evident in NAFLD/NASH patients. Excess liver uptake of FAA also promotes endoplasmic reticulum (ER) stress and lipotoxicity by activating a variety of stress-responsive kinases, including c-Jun N-terminal kinases 1/2 (JNK1/2) [11, 12]. On their turn, oxidative stress, ER stress, mitochondrial disfunctions and JNK1/2 activation may further promote hepatic insulin resistance, favour hepatocyte death. Inflammation, along with hepatocyte damage, is the main feature of the progression from simple steatosis to NASH. In fact, the molecular mechanisms able to promote inflammation cross-talk with those responsible for hepatocellular damage and fibrosis [13, 14]. In this context, the oxidative stress within parenchymal cells stimulates NF-κB-mediated production of TNF-α and IL-6 by hepatocytes [14, 15]. In turn, these cytokines stimulate Kupffer cells to secrete inflammatory mediators, which recruit to the liver phagocytic cells. Pattern-recognition receptors, including Toll-like receptors (TLRs), contribute to the pro-inflammatory responses in fatty livers. TLR-

responses can be activated by apoptotic cell death, hypoxia/HIF, and inflammation [16, 17]. TLR stimulation activates NF-κB, and thereby further amplifies and sustains inflammatory signals. Consistently, NASH patients show an increased hepatic expression of cytokine genes that correlates with the severity of liver lesions [16, 18]. Moreover, interference with NF-κB activation protects significantly from the development of steatohepatitis and reduces the expression of TNF-α and intercellular adhesion molecule-1 in rodent models of NASH [19, 20].

Unresolved inflammation promotes pathologic repair, thus progressive fibrosis and cirrhosis represent the final outcomes of NASH. NASH-related fibrosis develops primarily in the pericentral areas, where thin bundles of fibrotic tissue surround groups of hepatocytes and thicken the space of Disse, in a "chicken wire" fashion. The main cell type responsible for extracellular matrix deposition are hepatic stellate cells (HSCs), which under the local influence of transforming growth factor β1 (TGF-β1), platelet-derived growth factor (PDGF) and MCP-1 trans-differentiate into myofibroblast-like cells (HSC/MSs) producing collagen and extracellular matrix components [21, 22]. Furthermore, decreased hepatic matrix degradation due to a reduced production of matrix metalloproteases (MMPs) and/or an increased production of matrix metalloprotease inhibitors might also contribute to collagen accumulation [23, 24]. Kupffer cell activation in response to chronic inflammatory stimuli is mostly responsible for the secretion of pro-fibrogenic cytokines [25, 26].

One still unsolved problem in the pathogenesis of NASH concerns the changes occurring in the inflammatory responses during the progression toward fibrosis when active inflammation and healing processes are present at the same time in the tissue.

To explore these events we have taken advantage from an animal model of NASH involving mouse feeding with a methionine/choline deficient (MCD) diet that allow to obtain in a reproducible manner steatohepatitis similar to human NASH after 3-4 weeks of treatment or steatohepatitis plus fibrosis by extending the treatment up to 8-10 weeks.

Materials & Methods

***Animals and Experimental protocol*.** OPN knockout mice on C57BL/6 and wild-type C57BL/6 were breed on the same facility. Eight weeks old male were used for all the experiments. Methionine-choline deficient (MCD) and control diets were supplied by Laboratorio Dottori Piccioni (Gessate, Italy) and mice were fed for 4 or 8 weeks. Body weight was recorded weekly throughout the experiment. At the end of the study protocol, mice were anesthetized with sevofluorane and blood was collected by cardiac puncture. Livers were rapidly removed, weighed, and cut in pieces that were immediately frozen in liquid nitrogen and kept at -80° until analyzed. Two portions of each liver were, respectively, fixed in buffered pH 7.4 10% formalin or snap-frozen in OCT for histology. The experiments were approved by the Italian Ministry of Health and by the University Commission for Animal Care following the criteria of the Italian National Research Council.

Biochemical analysis. Plasma ALT and liver triglyceride were determined by spectrometric kits supplied by Radim S.p.A. (Pomezia, Italy) and Sigma Diagnostics (Milano, Italy), Liver osteopontin content was evaluated by a commercial ELISA kit supplied by R&D Systems (Abingdon, UK).

***mRNA extraction and Real time PCR*.** RNA was extracted from mouse livers with TRI reagent and retro-transcripted using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems Italia, Monza, Italy) according to the manifacturer's instruction. Real time PCR was performed in a Techne TC-312 termalcycler (TecneInc, Burlington NJ, USA), using TaqMan Gene Expression Master Mix and TaqMan Gene Expression probes for mouse TNF-α, IL-12p40, CCL2, CXCL10, iNOS, osteopontin, α-smooth muscle actin, α1-procollagen , and beta-actin (Bio-Rad, Italy). All samples were run blind in duplicate and the relative gene expression was calculated as $2^{-\Delta Ct}$ (ΔCt = Ct of the target gene minus Ct of beta-actin taken as housekeeping gene) using the 7000 System Software. The results were expressed as fold increase over control samples.

***Histology and immunohistochemistry*.** Liver pathology was assessed in hematoxylin-eosin and Masson's trichrome stained sections. Steatosis and lobular inflammation were scored blind by an experienced pathologist (C.B.) according to Kleiner et al. [21]. The number of necro-inflammatory foci were counted in ten different high magnification microscopic fields. Hepatic macrophages were evidenced in formalin-fixed sections using, respectively, anti-mouse F4/80 (eBioscience, San Diego CA, USA) in combination with peroxidase-linked goat anti-rat IgG and horse-radish peroxidase polymer kit (Biocare Medical, Concord, CA, USA).

Data analysis and statistical calculations. Statistical analyses were performed by SPSS statistical software (SPSS Inc. Chicago IL, USA) using one-way ANOVA test with Tukey's correction for multiple comparisons or Kruskal-Wallis test for non-parametric values. Significance was taken at the 5% level. Normality distribution was preliminary assessed by the Kolmogorov-Smirnov.

Results

For characterizing the changes occurring in inflammatory cells during the evolution of NASH we treated WT mice with MCD diet for 4 weeks and 8 weeks monitoring the extent of steatosis, inflammation and parenchymal injury by histology. The serum ALT levels and liver triglycerides were also measured as biochemical marker of liver damage. In 4 weeks treated mice, high degree of steatosis was observed together with lobular infiltration of leucocytes and signs of focal necrosis. The inflammation becomes more extensive after 8 weeks of MCD feeding with greater infiltration of macrophages and recruitment of lymphocytes. Furthermore, centrilobular collagen deposition was also evident at this stage indicating the onset of fibrosis (Fig. 1). The progression of liver injury was confirmed by a steadily increase of liver triglycerides and ALT from the 4^{th} to the 8^{th} week of treatment (Fig. 1). Similarly, the worsening of inflammation was supported by detecting an time-dependent increase in the hepatic m-RNA content of pro-inflammatory cytokines TNF-α and IL-12p40 as well as of CD11b, at the surface of activated leucocytes such as granulocytes, monocytes/macrophages and natural killer cells (Fig. 2). In accord with these findings, immunohistochemical staining of liver sections from MCD-treated mice with antibodies against F4/80, a marker of tissue macrophages, revealed a progressive increase in the number of macrophages infiltrating the hepatic parenchyma (Fig. 2). However, by extending our investigation to other inflammatory markers the inducible such NO synthase (iNOS) and chemokines such as CCL2, CCL5 and CXCL10, we obtained rather contradictory findings. iNOS is the main source of NO in activated leukocytes and is extensively expressed by activated macrophages. As expected, in mice treated with the MCD diet for 4 weeks, the onset of hepatic inflammation was reflected by increased expression of iNOS (Fig. 3). However, surprisingly extending the treatment up to 8 weeks resulted in dramatic decline in the hepatic iNOS mRNA content despite macrophage number and macrophage cytokines TNF-α and IL-12p40 were increased at this time point. On the same line, we also observed the m-RNA expression of CCL2, CCL5 and CXCL10 behaved as iNOS increasing at 4 weeks and then declining to almost control levels at 8 weeks (Fig. 3). CCL2 is known to recruit dendritic cells, memory T cells and monocytes at the site of inflammation, CCL5 is a chemoattractant for both T cells, and macrophages and CXCL10 is chemotactic for polymorph nuclear leukocytes and hematopoietic stem cells. The decline CCL2, CCL5 and CXCL10 expression along with that of iNOS suggest that important changes occurs in the pattern of activation of hepatic inflammatory cells during NASH progression to a more advanced disease.

Osteopontin (OPN) is a Th1 cytokine that plays an important role in the pathogenesis of various inflammatory and fibrotic diseases [3, 27-31]. It is synthesized and secreted by a variety of immune cells as well as epithelial, endothelial, and smooth muscle cells. It is present as a native 78-kDa protein in various cell systems, whereas the 66-kDa secreted form of OPN is the predominant active form of OPN involved in many pathophysiological processes [3, 29, 32-34]. OPN stimulates T cell proliferation and induces T cells and macrophages to express Th1 cytokines such as TNF-α and IL-12 [30, 31, 35-37]. Although the function of OPN is not completely defined, it is involved in macrophage recruitment during inflammation, acts as a survival or mitogenic factor for epithelial and vascular cells, and is associated with renal

extracellular matrix synthesis and fibrosis [38, 39]. The role of OPN in liver diseases is complex. Carbon tetrachloride administration in the rat has been shown to increase OPN expression in liver where it was localized mainly to Kupffer cells, macrophages, and stellate cells [38, 40, 41]. Recombinant OPN also stimulated hepatic macrophage migration in vitro and promoted collagen production by hepatic stellate cells. These data suggest that OPN could play an important role in modulating inflammation and fibrosis in the liver[42, 43]. As recent reports and ongoing studies in our lab have implicated OPN in NASH evolution, we examined the role of OPN in the evolution of steatohepatitis and liver fibrosis in our murine model of NASH.

As shown in figure 4 OPN expression is not significantly modified in the early phases of NASH after 4 weeks of feeding with the MCD diet, while it greatly increased in advanced NASH at 8 weeks. The increase in osteopontin production occurring in advanced NASH was confirmed by measuring liver osteopontin content by ELISA (Fig. 4). This suggested the possibility that OPN production might contribute in modulating the pattern of inflammatory responses during the disease progression. To investigate this point we analyzed MCD-induced NASH in OPN knockout (OPN-ko) mice obtained on the C57BL6 genetic background.

According to the capacity of OPN to stimulate macrophage production of TNF-α and IL-12 we observed that OPN depletion in mice receiving the MCD diet significantly reduced the up-regulation in liver TNF-α mRNA levels and almost abolished the expression of IL-12p40 mRNAs after both 4 and 8 weeks of treatment (Fig. 5). However, despite the lowering in the expression of these important pro-inflammatory cytokines we observed that liver injury as evaluated by measuring serum ALT release as well as by histology was not improved in OPN-ko mice (Fig. 5). In particular semi-quantitative evaluation of the extension of lobular inflammation showed similar scores in both WT and OPN-ko mice (Fig. 5). Consistently, both strains showed comparable mRNA levels for the leucocyte activation marker CD11b (Fig. 5). Furthermore, OPN inactivation did not interfered with the expression of genes related with the evolution to fibrosis such as pro-collagen 1α and α-smooth muscle actin and actually at the 8 weeks pro-collagen 1α and α-smooth muscle actin mRNAs were higher in OPN-ko than in WT mice (Fig. 6). At the moment, the mechanisms by which the lack of OPN does not affect the evolution of NASH despite it lowers TNF-α and IL-12 expression are still unclear. Preliminary data show that different from WT, mice lacking of OPN failed to down-modulate iNOS expression after 8 weeks of MCD feeding (Fig. 7). Furthermore, at the same time-point OPN-ko mice maintained high CCL2 mRNA levels (Fig. 7) and showed increased liver macrophage infiltration as compared to WT mice (Fig. 7). This suggests that OPN might actually contribute modulating the pattern of inflammatory responses during the evolution of NASH, but OPN ablation per se is not sufficient to bock the disease progression as in OPN-ko mice compensatory mechanisms likely maintains macrophage recruitment and activation.

Discussion

The growing prevalence of obesity and of the related inflammatory and metabolic disorders such as NAFLD and NASH demands novel therapeutic approaches.

The aim of my research project during my first year as PhD student was to investigate the changes occurring in the inflammatory responses during the progression of NASH toward fibrosis when active inflammation and healing processes are present at the same time in the liver. The data obtained mice treated with a NASH-inducing diet for 4 and 8 weeks show that while hepatic injury and inflammation progress there are dramatic changes in the expression of different cytokines. In fact, in parallel with an increase in the mRNAs for TNF-α and IL-12p40 it is evident an unexpected decrease in the expression of iNOS as wells as of several chemochines including CCL2, CCL5 and CXCL10 that are implicated in the recruitment of monocytes, lymphocytes an NK cells to the inflammatory sites. Little is known on the mechanisms responsible for these effects as far only few studies have investigated the changes in cyto/chemokine expression occurring during the evolution of NASH. However, the decline in the expression of iNOS, CCL2, CCL5 and CXCL10 can be interpreted as a re-modulation of the inflammatory reactions occurring when the healing process began. Nonetheless, my data demonstrate that even in the presence of a down-modulation of inflammation the expression of important pro-inflammatory cytokines such as TNF-α and IL-12 is instead further enhanced. It is known that the cytokine osteopontin (OPN) is among the factors that regulate TNF-α and IL-12 production by macrophages [44]. Osteopontin is an inflammatory Th-1 cytokine that is increasingly recognized to play important roles in inflammation and tissue healing [45]. In the liver OPN is produced T-lymphocytes, NKT cells macrophages as well as by [46, 47] and its hepatic content is up-regulated in various models of liver injury. Measuring the intrahepatic production of OPN demonstrated that OPN expression is not significantly modified in the early phases of NASH after for weeks of feeding with the MCD diet, while it greatly increase in advanced NASH at 8 weeks in concomitance with a further increase in TNF-α and IL-12 expression. To investigate the possible role of OPN in maintaining inflammatory activity during the evolution of NASH we have used OPN knockout mice that were fed the MCD diet for 4 and 8 weeks. The data obtained indicate that OPN deficiency markedly reduced macrophage expression of TNF-α and IL-12, supporting the view that OPN production might contribute to substain inflammation in the advanced NASH. This is consistent with the findings of different research groups including our that have recently reported that hepatic OPN production is significantly up-regulated in murine MCD treated wild type mice and correlates with the severity of hepatic steatohepatis and fibrosis [48, 49]. These studies also indicated that NKT cell recruitment was responsible for the increased production of OPN in advanced NASH [50-52]. However, differently from studies above, we failed to observe any protection from liver injury

and fibrosis in OPN-ko mice. Indeed, transaminase release, liver histology as well as the detection of markers of fibrosis were comparable in WT or OPN-ko fed the MCD diet. This difference is likely due to the fact that in those studies OPN-ko mice were generated in A/J background, while we used mice on C57BL/6 background. Indeed, differently from that reported by Sahai and co-workers using A/J mice [49, 53] we did not observe changes in hepatic osteopontin expression in wild-type C57BL/6 mice receiving the MCD diet for 4 weeks. Furthermore, we have reported that strain differences greatly influences the effects of the inactivation genes related to inflammation in relation to the evolution of NASH [54].

Thus it is possible that Opn-ko mice mechanisms other that OPN might support inflammatory process even in the absence of OPN. Indeed, we have observed that differently from WT OPN-ko mice failed to down-modulate iNOS and CCL2 expression in advanced NASH, Moreover, the number of liver infiltrating macrophages was also higher in OPN-ko than in WT mice after 8 weeks on the MCD diet.

In conclusion our present data suggest that OPN might have a role in the progression of NASH possibly contributing to substain the macrophage production of TNF-α and IL-12 even in a context of down-modulation of other pro-inflammatory signals, nonetheless more experiments are needed to better characterize such a role.

Future Prospects

Although previous studies have implicated Osteopontin is a key factor in stimulating hepatic inflammation and fibrosis in NASH, our finding indicate that the role of Osteopontin in modulating inflammation in the hepatic environment is not as straight forward as originally postulated. Furthermore, the use of OPN-ko mice has proved to be unsuitable to define the actual role of OPN in the progression of NASH. Thus we plan to address the role of OPN in regulating hepatic inflammatory process by blocking osteopontin action using injected anti-Osteopontin antibodies in the WT mice receiving the MCD diet. In this experiment the animals will receive the anti-OPN antibodies after 4 week on the MCD diet when the production of the cytokine began to increase in the liver. The effects on hepatic damage and inflammation will be monitored during the following 4 weeks of treatment.

Meanwhile, we will take advantage of the experiments performed in OPN-ko mice with NASH to better investigate the possible mechanisms leading to liver injury by characterizing the expression of chemokines possibly involved in causing the increase in liver macrophage in these animals. We will also investigate whether macrophage activation or the recruitment of other inflammatory cells might account for the lack of protection against liver injury and fibrosis. This will help elucidate the complexity of the signal network possible involved in driving inflammatory responses in NASH.

Figures

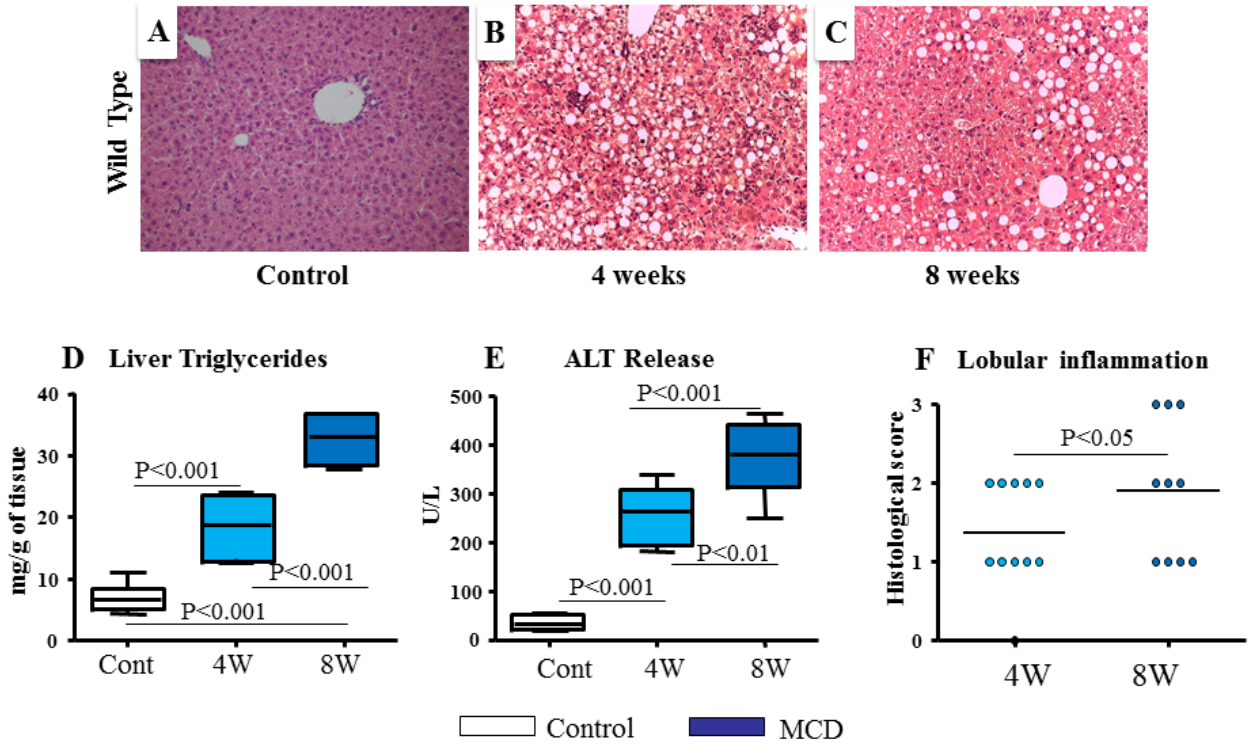

Figure 1 – MCD diet results in liver steatosis, liver injury and lobular inflammation

Liver histology was detected by hematoxilin/eosin (Panels A-C: magnification 100x) in WT mice fed either control or the MCD diet for 4 or 8 weeks. Liver triglyceride content, and alanine aminotransferase release (ALT) (Panel D, E) were determined at the same time points. Lobular inflammation (Panel F) was scored semi-quantitatively according to Kleiner et al. [55]

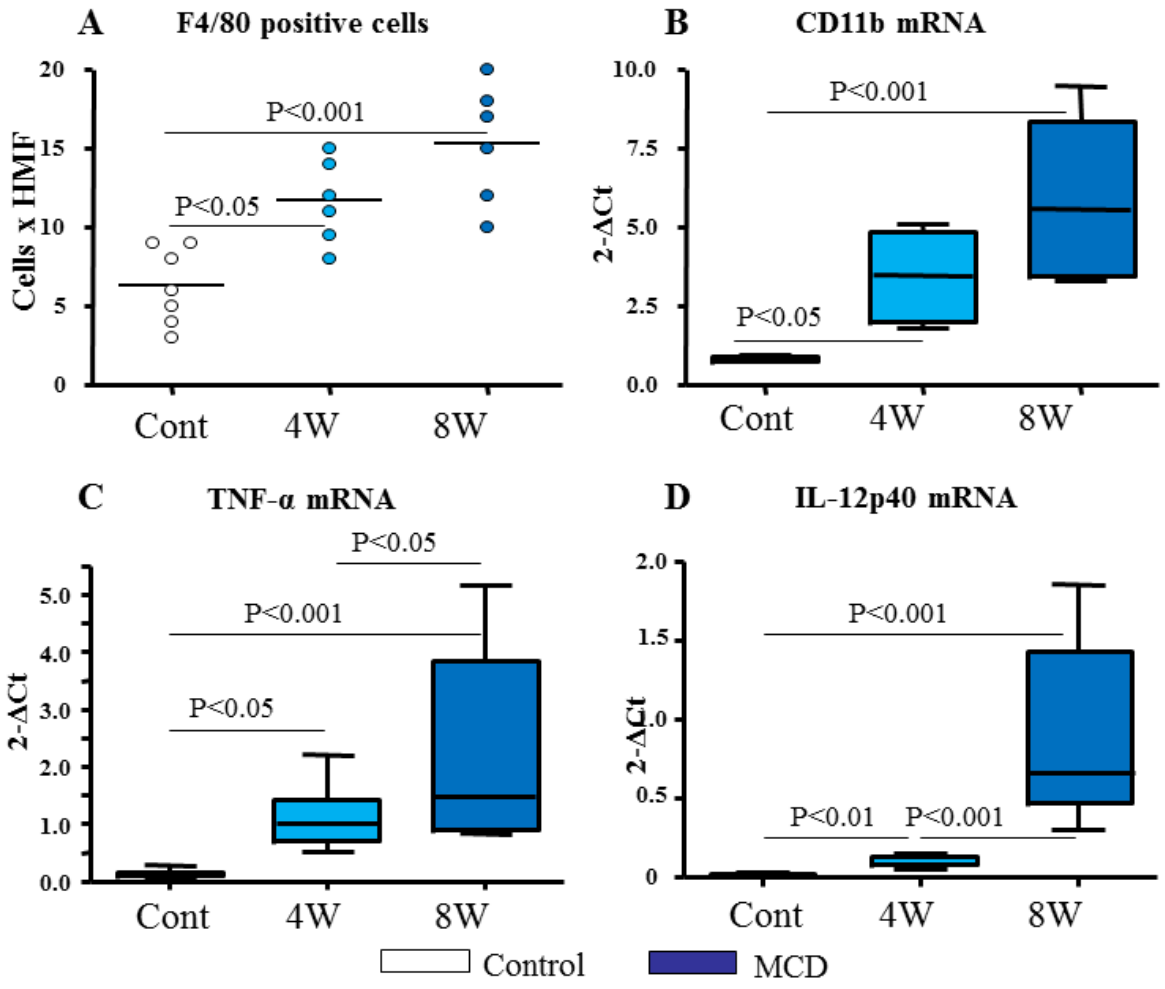

Figure 2 – MCD diet induces liver inflammation along with the recruitment of hepatic macrophages

Liver sections obtained from WT mice fed either control or the MCD diet for 4 or 8 weeks were stained for macrophage marker F4/80 and the F4/80 positive cells (Panel A) were counted in 10 high magnification field. The liver mRNA expression of CD11b, TNF-α, IL-12p40 (Panel B-D) was determined by quantitative real-time PCR and expressed as 2-ΔCT to the β-actin gene. The values refer to 8-10 animals in each group and the boxes include the values within 25th and 75th percentile, while the horizontal bars represent the medians. Eighty percent of the values are comprised between the extremes of the vertical bars (10th-90th percentile).

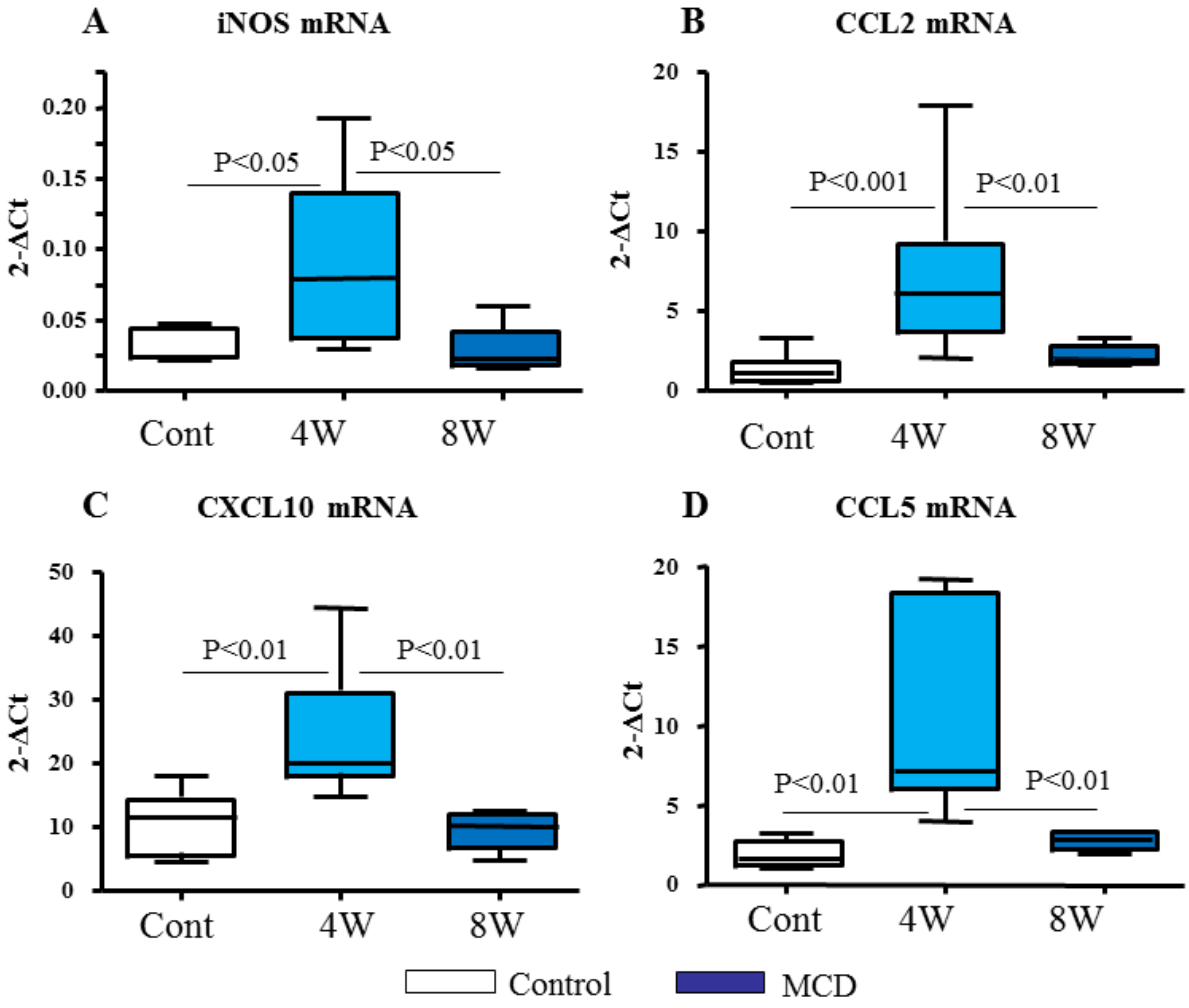

Figure 3 – Differential expression of inflammatory markers during MCD diet induced NASH

The liver m-RNA expression levels of iNOS and of inflammatory chemokines CCL2, CCL5 and CXCL10 (Panel A-D) was determined by quantitative real-time PCR in WT mice fed either control or the MCD diet for 4 or 8 weeks. The values are expressed as 2-ΔCT to the β-actin gene. The values refer to 8-10 animals in each group and the boxes include the values within 25th and 75th percentile, while the horizontal bars represent the medians. Eighty percent of the values are comprised between the extremes of the vertical bars (10th-90th percentile).

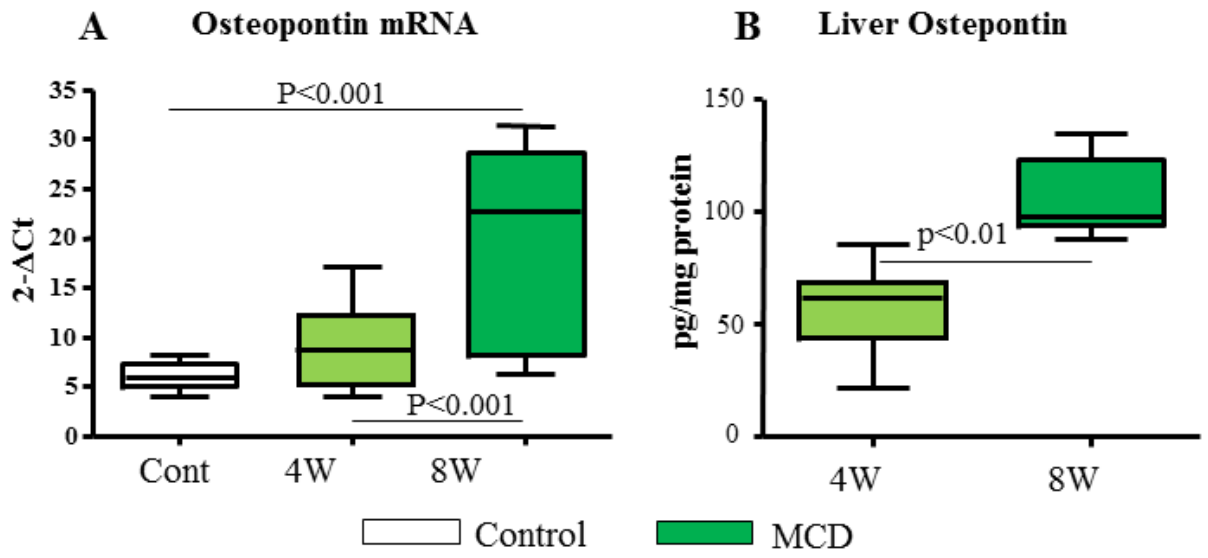

Figure 4 – Osteopontin plays a role in inflammation during MCD diet induced NASH in WT mice

The liver m-RNA expression levels of osteopontin (Panel A) were evaluated in WT mice fed either control or the MCD diet for 4 or 8 weeks by quantitative real-time PCR. The values are expressed as 2-ΔCT to the β-actin gene. Liver osteopontin content (Panel B) was evaluated by a commercial ELISA kit. The values refer to 8-10 animals in each group and the boxes include the values within 25th and 75th percentile, while the horizontal bars represent the medians. Eighty percent of the values are comprised between the extremes of the vertical bars (10th-90th percentile).

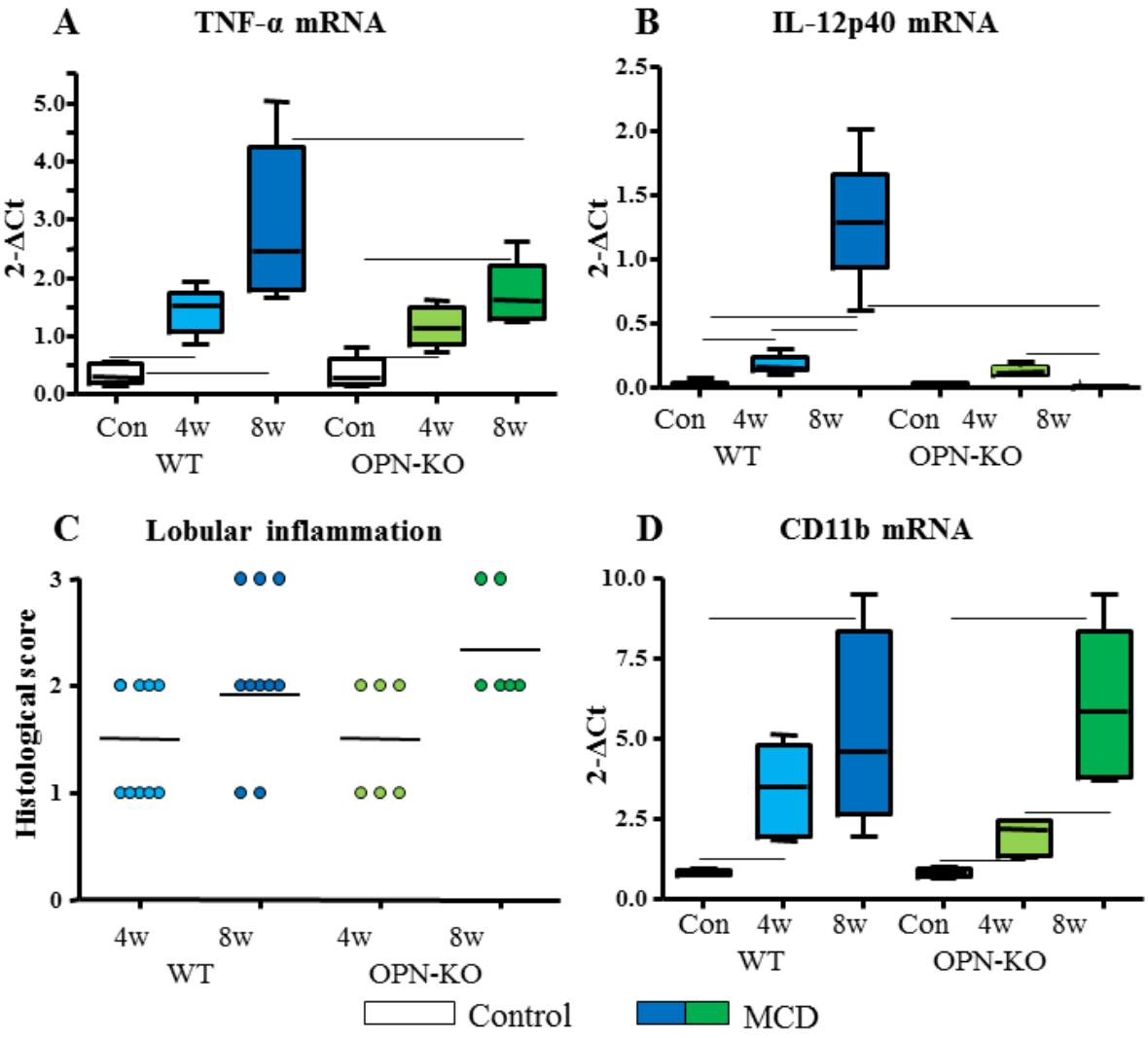

Figure 5 – Osteopontin knockout mice with MCD induced NASH shown decreased expression of TNF-α and IL-12, but no effect of liver inflammation

The liver mRNA expression of the inflammatory cytokines TNF-α and IL-12p40 (Panel A, B) and of the leukocyte activation marker CD11b (Panel D) was measured in WT and OPN-ko mice fed with either control and MCD diet for 4 and 8 weeks by quantitative real-time PCR. The values are expressed as 2-ΔCT to the β-actin gene. The values refer to 8-10 animals in each group and the boxes include the values within 25th and 75th percentile, while the horizontal bars represent the medians. Eighty percent of the values are comprised between the extremes of the vertical bars (10th-90th percentile). Lobular inflammation (Panel C) was scored semi-quantitatively according to Kleiner et al. [55]

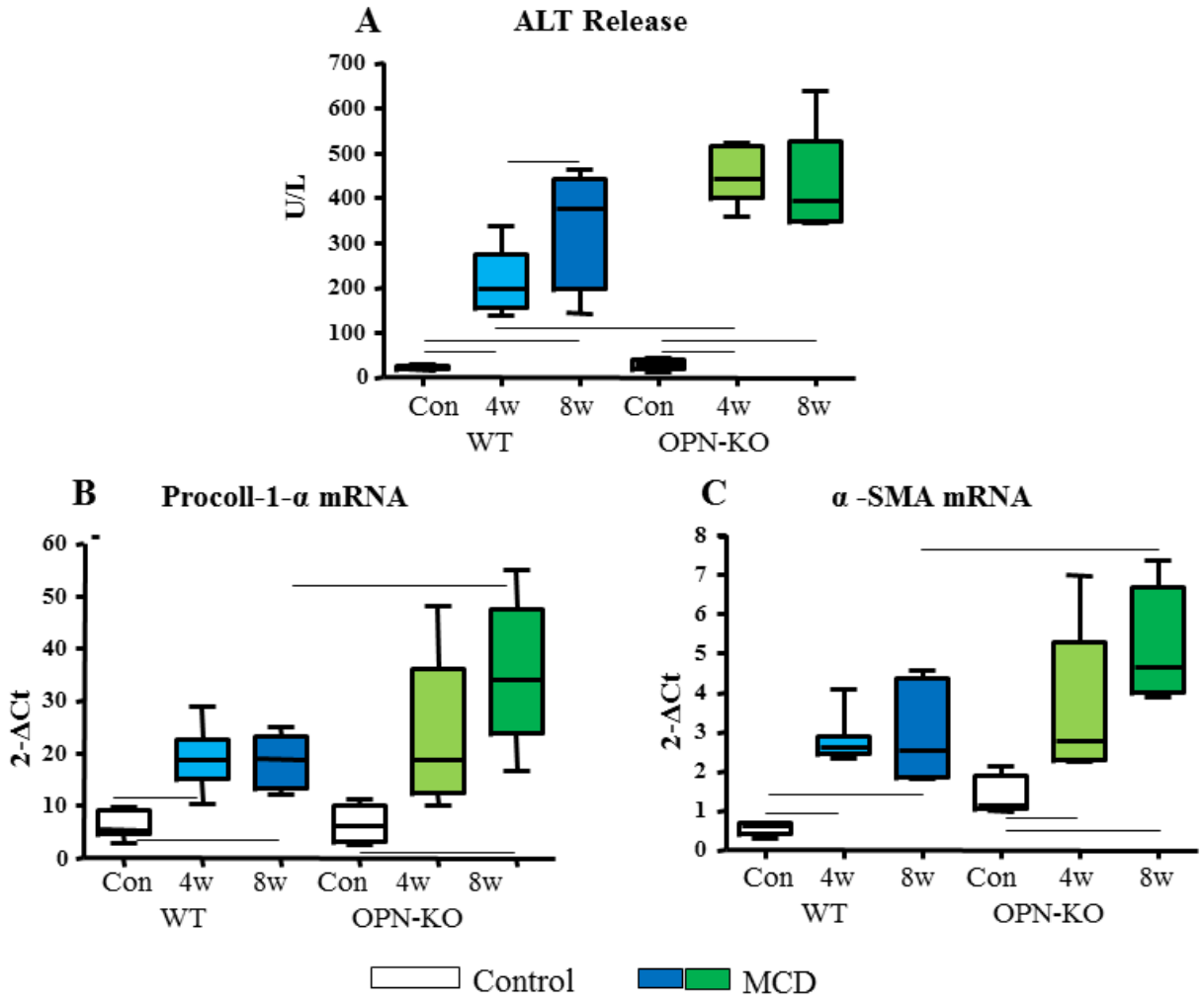

Figure 6 – Liver damage and fibrosis in OPN-ko mice with MCD diet

Serum alanine transaminase levels (ALT) and the liver mRNA expression of fibrosis marker procollagen 1α and smooth muscle actin (alpha-SMA) (Panel A, B, C) measured in WT and OPN-ko mice fed with either control and MCD diet for 4 and 8 weeks. Liver histology was detected by hematoxilin/eosin (Panels A-C: magnification 100x). The values refer to 8-10 animals in each group and the boxes include the values within 25th and 75th percentile, while the horizontal bars represent the medians. Eighty percent of the values are comprised between the extremes of the vertical bars (10th-90th percentile).

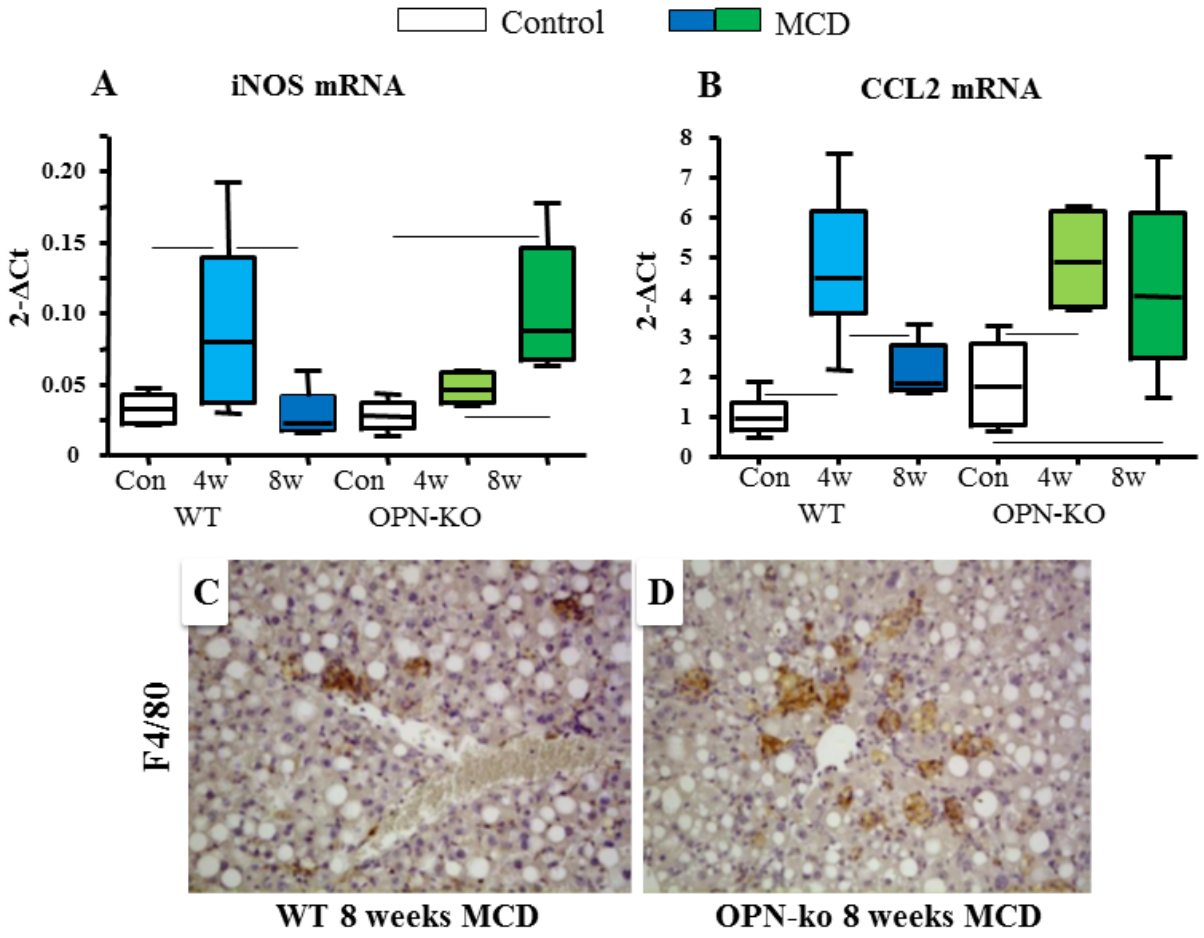

Figure 7 – Increased liver inflammation and macrophage recruitment in OPN-ko mice with MCD diet

Liver sections obtained from WT and OPN-ko mice fed either control or the MCD diet for 4 or 8 weeks (Panel C, D) were stained for macrophage marker F4/80 and the F4/80 positive cells were counted in 10 high magnification field. The liver m-RNA expression a level of iNOS and CCL2 (Panel A, B) was determined by quantitative real-time PCR in WT mice fed either control or the MCD diet for 4 or 8 weeks. The values are expressed as 2-ΔCT to the β-actin gene. The values refer to 8-10 animals in each group and the boxes include the values within 25th and 75th percentile, while the horizontal bars represent the medians. Eighty percent of the values are comprised between the extremes of the vertical bars (10th-90th percentile).

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Seminars Attended

1. “Next-generation DNA sequencing and target arrays in the clinics” – Dr. Paolo Fortina – 25 Gennaio 2012 – Department of Cancer Biology, Jefferson Genomics Laboratory, Kimmel Cancer Center, Thomas Jefferson University Jefferson Medical College, Philadelphia, PA, USA

2. “Signalling pathways controlling integrin trafficking during invasion” – Dr.ssa Elena Rainero – 8 Marzo 2012 – Beatson Insitute for Cancer Research, Glasgow, UK

3. “Role of Diacylglycerol kinases in the control of T cell activation and differentiation programs”– Prof.ssa Isabel Merida – 23 Marzo 2012 - Centro Nacional de Biotecnologia, Madrid

4. “Developing strategies for tissue specific targeting” – Prof. Costantino Pitzalis – 28 Marzo 2012 – Barts and the London School of Medicine, London

5. “The role of cutaneous HPV in skin cancer” – Prof. Ingo Nindl – 29 Marzo 2012 - DKFZ - Charité Cooperation, Viral Skin Carcinogenesis, Berlin

6. “Microparticles as novel effectors in inflammation” – Prof. Mauro Perretti – 15 Maggio 2012 – William Harvey Research Institute, Barts and The London School of Medicine, Queen Mary University of London, London, UK

7. “Resolvins and Omega-3 in inflammation” – Prof. Mauro Perretti – 16 Maggio 2012 – William Harvey Research Institute, Barts and The London School of Medicine, Queen Mary University of London, London, UK

8. " High-throughput Biochemical Target Investigation Unveils a Novel Function of miR-21 as a Negative Modulator of Signal Transduction in T-lymphocytes" – Prof. Pino Macino – 15 Giugno 2012 - University "La Sapienza", Rome

9. "Recent Advances in Hematopoietic Stem Cell Gene Therapy: from microRNA Regulation to Targeted Gene Transfer" – Prof. Luigi Naldini – 21 Giugno 2012 – San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Milan, ITALY

Congress/Conferences Attended

OXYGEN CLUB OF CALIFORNIA WORLD CONGRESS 2012 – Alba, 20-23 June 2012

“Oxidative Stress Driven Immune Responses Contributes To Hepatic Inflammation In Nonalcoholic Steatohepatitis (NASH)”

Aastha Jindal, Irene Locatelli, Salvatore Sutti, Marco Vacchiano, Cristina Bozzola, Emanuele Albano

Abstract

XXXI MEETING OF THE ITALIAN SOCIETY OF PATHOLOGY AND TRANSLATIONAL MEDICINE – Udine, 12-15 September 2012

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