OPEN ACCESS Freely available online

PLOS ONE

HCC Development Is Associated to Peripheral Insulin Resistance in a Mouse Model of NASH

Samuele De Minicis[1]*, Laura Agostinelli[1], Chiara Rychlicki[1], Gian Pio Sorice[2,3], Stefania Saccomanno[1], Cinzia Candelaresi[1], Andrea Giaccari[2,4], Luciano Trozzi[1], Irene Pierantonelli[1], Eleonora Mingarelli[1], Marco Marzioni[1], Giovanna Muscogiuri[2], Melania Gaggini[5], Antonio Benedetti[1], Amalia Gastaldelli[5], Maria Guido[6], Gianluca Svegliati-Baroni[1,7]*

1 Università Politecnica delle Marche, Department of Gastroenterology, Ancona, Italy, **2** Università Cattolica del Sacro Cuore, Policlinico Gemelli, Division of Endocrinology and Metabolic Disease, Rome, Italy, **3** Diabetic Care Clinics, ACISMOM, Rome, Italy, **4** Don Gnocchi Foundation Onlus, Milan, Italy, **5** Institute of Clinical Physiology, Head of Cardiometabolic Risk Unit, Pisa, Italy, **6** Università di Padova, Department of Medicine Anatomic Pathology Unit, Padova, Italy, **7** Università Politecnica delle Marche, Obesity Center, Ancona, Italy

Abstract

NAFLD is the most common liver disease worldwide but it is the potential evolution to NASH and eventually to hepatocellular carcinoma (HCC), even in the absence of cirrhosis, that makes NAFLD of such clinical importance. Aim: we aimed to create a mouse model reproducing the pathological spectrum of NAFLD and to investigate the role of possible co-factors in promoting HCC. Methods: mice were treated with a choline-deficient L-amino-acid-defined-diet (CDAA) or its control (CSAA diet) and subjected to a low-dose i.p. injection of CCl_4 or vehicle. Insulin resistance was measured by the euglycemic-hyperinsulinemic clamp method. Steatosis, fibrosis and HCC were evaluated by histological and molecular analysis. Results: CDAA-treated mice showed peripheral insulin resistance at 1 month. At 1–3 months, extensive steatosis and fibrosis were observed in CDAA and CDAA+CCl_4 groups. At 6 months, equal increase in steatosis and fibrosis was observed between the two groups, together with the appearance of tumor. At 9 months of treatment, the 100% of CDAA+CCl_4 treated mice revealed tumor versus 40% of CDAA mice. Insulin-like Growth Factor-2 (IGF-2) and Osteopontin (SPP-1) were increased in CDAA mice versus CSAA. Furthermore, Immunostaining for p-AKT, p-c-Myc and Glypican-3 revealed increased positivity in the tumors. Conclusions: the CDAA model promotes the development of HCC from NAFLD-NASH in the presence of insulin resistance but in the absence of cirrhosis. Since this condition is increasingly recognized in humans, our study provides a model that may help understanding mechanisms of carcinogenesis in NAFLD.

Citation: De Minicis S, Agostinelli L, Rychlicki C, Sorice GP, Saccomanno S, et al. (2014) HCC Development Is Associated to Peripheral Insulin Resistance in a Mouse Model of NASH. PLoS ONE 9(5): e97136. doi:10.1371/journal.pone.0097136

Editor: Birke Bartosch, Inserm, U1052, UMR 5286, France

Received October 10, 2013; **Accepted** April 15, 2014; **Published** May 22, 2014

Funding: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement n° HEALTH-F2-2009-241762 for the project FLIP, from MIUR grant PRIN 2009 - prot. 2009X84L84_003 and Ministero della Salute grant GR-2010-2306996 to MM, from MIUR grant PRIN 2009 - prot. 2009YNERCE_002, FIRB 2010 - prot RBAP10MY35_001 to GS-B. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: g.svegliati@univpm.it (GS-B); s.deminicis@yahoo.it (SDM)

Background

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide. [1,2]. Moreover, several studies have shown that 5% to 30% of patients with HCC lack a readily identifiable risk factor for their cancer [1]. Most of these "cryptogenic" HCC might be attributed to Non-Alcoholic Fatty Liver Disease (NAFLD) and the concomitant metabolic syndrome [2,3], nevertheless it is not yet clear what predisposes to the progression of the disease [4].

On this regard, NAFLD is a major health problem that integrates several liver conditions ranging from simple fatty liver to Non-Alcoholic Steatohepatitis (NASH), which is associated with fibrosis that may evolve into cirrhosis and results into HCC [5]. How HCC develops from NASH livers is still obscure. Some hypotheses suggest that obesity, insulin resistance, release of inflammatory cytokines and autophagy can contribute to the carcinogenic potential in NASH liver, where HCC can occur in 65% of patients without an over cirrhosis in the background liver [6]. However, no direct link has been provided yet. Studies that aim to link HCC and NAFLD are blunted by the lack of reliable animal models. The use of a choline-deficient L-aminoacid-defined diet (CDAA) in rats provided the most interesting results inducing steatohepatitis [7]. Concerning this issue, a still unresolved question is however related to the potential of CDAA diet in inducing insulin resistance [8,9]. The purpose of this study is to develop a mouse model of liver injury which mimics NASH features that lead to HCC.

Materials and Methods

Animals and Treatment

Male 6 to 8 weeks old C57BL/6 mice were purchased from Charles River Laboratories International, Inc. (Wilmington, MA). Animals were fed a CDAA diet or its control diet, CSAA diet (Laboratorio Dottori Piccioni, Milano, Italy). In parallel experi-