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Transcriptome analysis of rat liver regeneration in a model of oval hepatic stem cells

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Abstract

We have performed serial analysis of gene expression of the regenerating liver. In the rat model of partial hepatectomy and 2-acetamidofluorene treatment liver regeneration recruits hepatic stem cells referred to as oval cells. We analyzed a total of 153,057 tags in livers from normal control (52,343 tags), from sham 2-acetamidofluorene-treated control (50,502 tags), and from the early stage of oval cell proliferation (50,212 tags). Comparative analysis of the three transcriptomes identified 27 up-regulated and 18 down-regulated genes. Real-time PCR analysis confirmed 11 temporally regulated genes that correlate with oval cell development. Interestingly, we found by Western blot protein analysis of regenerating livers that the cell cycle gene Cdc42 was induced concomitant with the proliferation marker cyclin D1 and the oval cell marker alpha-fetoprotein. Our studies provide new insights into the molecular mechanism of liver regeneration through oval cells.

Keywords: Rat liver; Liver regeneration; Partial hepatectomy; Oval cells; Serial analysis of gene expression; Transcriptome

Proliferation of mature hepatocytes restores liver mass after partial hepatectomy (PH) and after transplantation [1,2]. In other situations, like severe liver injury, adult liver stem cells, called "oval cells," can regenerate the hepatic lineages [3,4]. The oval cells define a facultative stem cell population, activated only when the replicative and functional capacity of the mature hepatocytes is impaired because the insult to the organ is too massive [5,6]. Oval cells are epithelial stem cells originating from the distal part of the biliary tree, the canals of Hering, which are understood to constitute the stem cell niche in the adult liver [7]. The oval cell compartment is a heterogeneous cell population that expresses different combinations of phenotypic markers from both hepatocytic and biliary lineages [8,9]. Furthermore, this stem cell progenitor compartment expresses the oncofetoal marker gene of the liver, alpha-fetoprotein (Afp) [10], and also hematopoietic stem cell markers like c-kit, CD34, and Thy1 [11,12]. Thus, the oval cells are considered as bipotential progenitor cells, capable of generating mature hepatocytes and biliary cells [13,14]. Interestingly, oval cells were also described as differentiating into intestinal epithelium [15] or pancreatic acinar cells [16] under certain conditions.

Several models of liver injury and regeneration in rodents were established for triggering oval cell proliferation [17]. In the model of 2-acetamidofluorene (2AAF) treatment and PH operation (2-AAF/PH), the proliferating oval cells form irregular duct-like structures (ductular reaction) that are connected with preexisting bile ducts [18]. The oval cells proliferate and migrate from the portal area and infiltrate into the lobular parenchyma through the bile canaliculi between hepatic cords [19]. After a peak of oval cell proliferation, basophilic foci of new hepatocytes and new bile ductules appear to arise from differentiating oval cells [20]. The new hepatocytes reconstitute the

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original mass of the liver, while the oval cells gradually disappear.

Employing serial analysis of gene expression (SAGE), we analyzed the rat liver transcriptome and identified 45 genes differentially expressed in oval cell regeneration. Most of them represent new genes in the context of liver regeneration. We applied real-time PCR to confirm the differential expression and to characterize the temporal expression of the identified genes during the oval cell regeneration process. We found that 11 genes were kinetically regulated in the regenerating liver after 2-AAF/PH treatment. We studied in particular the protein expression of the cell cycle gene Cdc42 by Western blot of hepatic protein extracts. Cdc42 was coexpressed temporally with the oval cell marker Afp and the proliferation marker cyclin D1. Our report suggests a possible role for the Cdc42 gene in cell proliferation in the oval cell model of liver regeneration.

Results

Induction of oval cell proliferation

We sacrificed 2-AAF/PH-treated rats at different time points after the operation (1, 3, 7, 11, 16 days). Control sham-operated rats were treated with 2-AAF and sacrificed according to the same protocol. We found that in the rat model of 2-AAF/PH, the liver mass does not increase significantly until 11 days after PH (from 33 to 57%). Upon normal regeneration after PH only, the liver mass is almost completely recovered 7 days after the operation. These observations suggest that hepatocyte proliferation is efficiently impaired by the 2-AAF treatment.

To determine the oval cell response in the regenerating liver samples on a molecular level, we analyzed the expression of Afp as a typical marker of the oval cell population by Northern blot [21] (Fig. 1A). According to the literature [22], we found two Afp transcript variants of 2.1 and 1.7 kb (Fig. 1A). The Afp product of 2.1 kb appeared to be the strongest induced in the regeneration process. Indeed, such transcript was described in a previous report to be specifically expressed by ductular cells [22]. Anyway, the Afp transcripts started to become detectable at 3 days after the PH operation as reported earlier [18]. Afp gene expression peaked at 7 days, declined at 11 days after the PH, and was hardly detectable in the later stages after PH. No induction of Afp transcripts was observed in the corresponding sham-operated control livers. By in situ hybridization we characterized the spatial distribution of the Afp transcript in liver tissue (Figs. 1B, 1C, and 1D). Afp-positive oval cells were detected from 3 to 7 days after the PH operation; they proliferated from the portal field and migrated inside of the liver lobules, infiltrating the liver parenchyma toward the central veins. Based on the Afp expression kinetic, we decided to analyze the transcriptome at the early time point of oval cell regeneration at 3 days after PH.

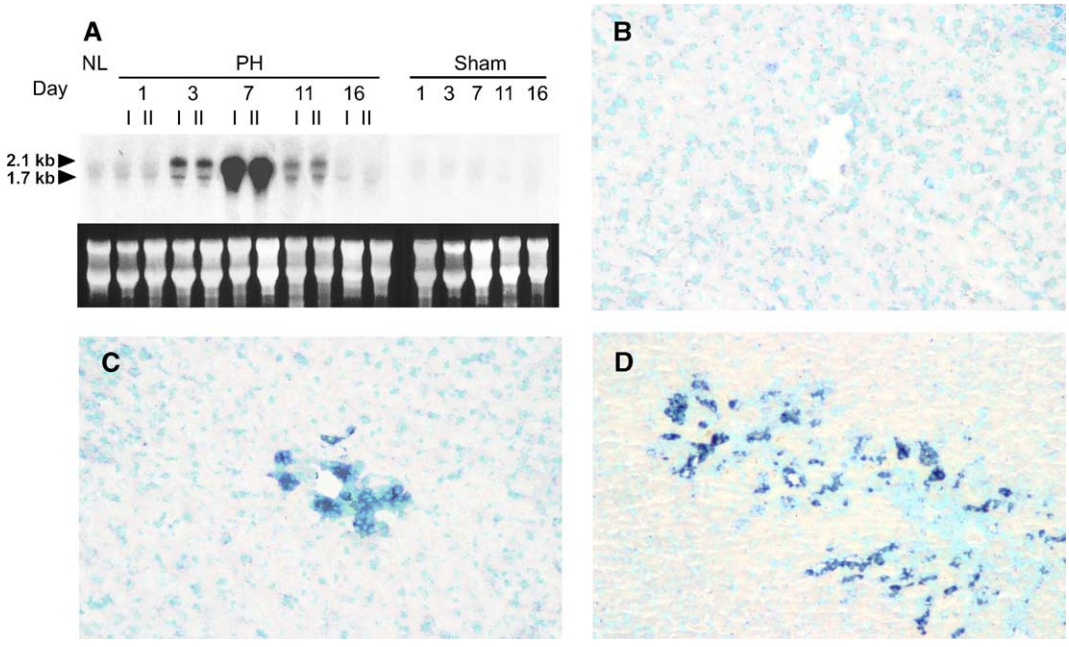

Fig. 1. Afp transcript expression in oval cell liver regeneration. (A) Northern blot analysis of the Afp gene shows the induction of its transcripts (2.1 and 1.7 kb) concomitant with the onset of oval cell development at 3 days after PH (NL, normal liver; PH, partial hepatectomy operation; Sham, sham operation; Day, day after operation; I and II indicate two different animals). (B–D) In situ hybridization of Afp of oval cell regenerating livers. Oval cells expressing the Afp transcripts proliferate from the portal field area and invade the liver lobule: (B) normal liver is negatively stained for Afp, (C) at 3 days after PH AfP-positive oval cells appear in the portal field, (D) at 7days after PH Afp-positive oval cells have proliferated and migrated into the liver lobule.