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Review

What are the mechanisms of regeneration inhibition in alcoholic hepatitis?

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Contents

1. Introduction

When human biopsies taken from patients with alcoholic hepatitis (AH) are stained immunohistologically for the expression of different proteins involved in the cell cycle, 2 proteins which inhibit the progression of the cell cycle, p21 and p27, stained positive in numerous hepatocyte nuclei. PCNA and Cyclin D1 positive nuclei were numerous in AH but Ki67 positive nuclei were reduced in number The Mallory-Denk bodies (MDBs) present stained positive for Ki67 (Fig 1) (French et al., 2012). This suggested that the increase in the expression of p21 and p27 inhibitors had caused cell cycle arrest, producing a reduction in the expression of Ki67. Koteish et al. (2002) showed that in partial hepatectomy in alcohol fed mice, regeneration of liver cells was inhibited and the expression of p21 and p27 was increased.

A study of liver explants from patients with AH, with Mallory-Denk bodies (MDB) and balloon cell change, showed virtual absence of the positive markers for hepatic proliferation, compared to alcoholic cirrhosis and normal livers. Only a few hepatocytes stained positive for Ki67, compared to the alcoholic cirrhosis livers. Instead, liver cells had changed into hepatic progenitor cells and bile ducts (bile duct metaplasia) (Dubuquoy et al., 2015).

Singh et al. (2014) showed that patients who had severe alcoholic hepatitis and were treated with G-CSF for 0–6 days showed an increase in CD-34 positive cells in their peripheral blood at 6 days. This indicated that CD34 hematopoietic stem cells had led to a marked increase in survival at 90 days (78.3%) compared to those patients who did not receive G-CSF, where only 30.4% survived 90 days. The increase in CD-34 cell response leads to increased levels of hepatocyte growth factors and induced hepatic progenitor cells to proliferate within 7 days (Spahr et al., 2008). The exact mechanism as to how G-CSF treatment works is not known (Singh et al., 2014) but in their study no deaths occurred after 6 days of treatment with G-CSF.

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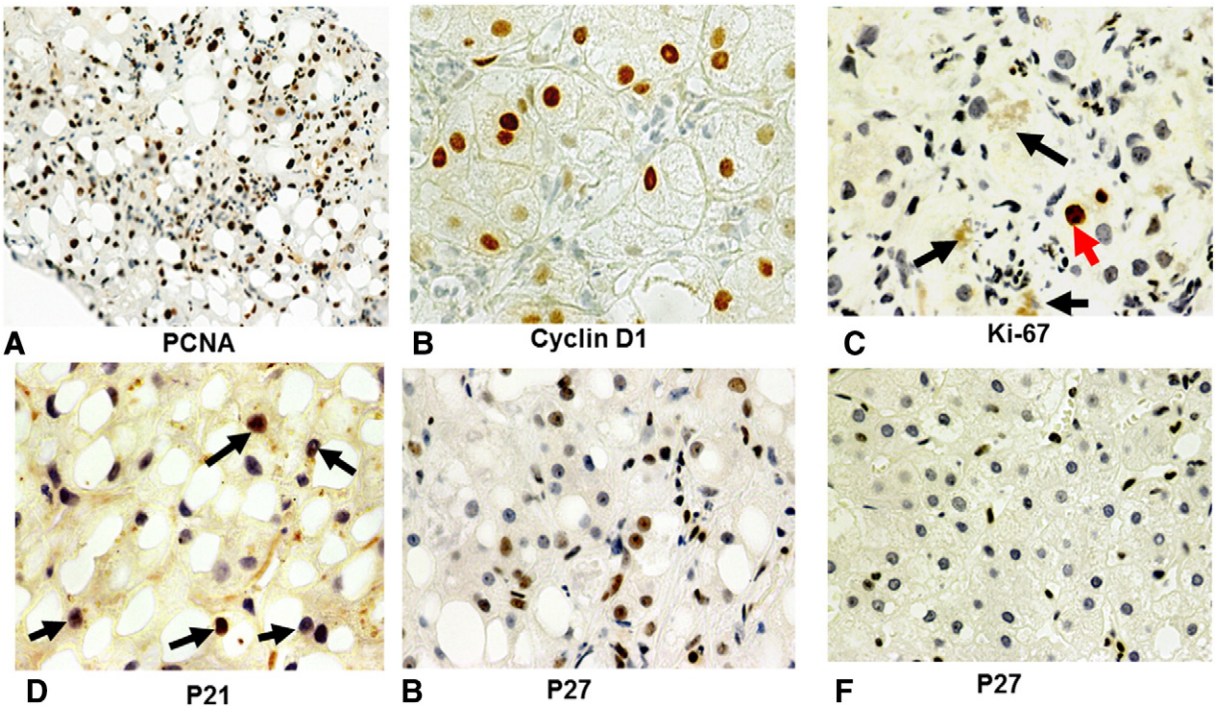

Fig. 1. The liver biopsy slides from patients with alcoholic hepatitis were stained with antibodies to PCNA (A), Cyclin D1 (B), Ki-67 (C), p21 (D) and p27 (E). Only a few scattered nuclei were positive for Ki67 (arrow). In one of the alcoholic hepatitis biopsies no nuclei stained positive. (F) The arrows point to Mallory Denk bodies staining yellow orange (C). Magnification (A ×218), (B ×654), (C ×654), (D ×684), (E ×436), F ×436).
This figure was previously published in Exp. Mol. Pathol. 92:318–326 (2012).

2. Clinical data identifies the inhibitors that cause cell cycle arrest in AH

In a study of liver biopsies from patient with AH, where global RNA sequencing was performed, an increase in the expression of p21 and p27, both cell cycle inhibitors, was found (Liu et al., 2015a,b). Other cell cycle inhibitors, up regulated, were ATM, and p15 (Liu et al., 2015a).

p21 results in CDK inhibition and cell cycle arrest, preventing the replication of damaged DNA (Ko and Prives, 1996). It specifically inactivates G1 (CKD4 and 6). p21 and p27 inactivate CDK-cycling. p21 also inhibits DNA synthesis by binding to and inhibiting proliferating cell nuclear antigen (PCNA). p21 is under transcriptional control of the p53 tumor suppressor gene. p15 and p27 increase in response to transforming growth factor β (TGFβ). TGFβ was up regulated in AH when measured by RNA Seq (Liu et al., 2015b) contributing to growth arrest (Vermeulen et al., 2003). ATM phosphorylates p53 in response to DNA damage, resulting in p21 blocking the cell cycle at the G1/S checkpoint (Vermeulen et al., 2003). ATM also induces S phase arrest by phosphorylation of NBS1. In the AH biopsy study p53 expression was significantly decreased when measured by RNA seq (Liu et al., 2015b).

p27 expression was up regulated in the AH liver biopsy study (Liu et al., 2015b) in response to miR-34a expression up regulation (Figs. 2 and 3). The miR-34 promoter contains p53 binding sites. p53 is one of the strongest inhibitors of miR34a. The mRNA level of p53 was down regulated. This suggests that miR34a was up regulated because of the down regulation of p53 and the up regulation of the expression of p27 by miR34a (Liu et al., 2015b).

3. Role of p21 up regulation inhibition of regeneration in alcoholic liver disease (ALD) and its clinical importance

Clinically p21 up regulation and inhibition of liver regeneration develops in ALD. This relationship has been clearly documented by Aravinthan et al. (2013). They studied two cohorts. The first was composed of 42 patients across the full spectrum of ALD. The second was 77 patients with ALD cirrhosis. In cohort 1, Mcm-2 expression was mildly increased in ALD measured by immunohistochemistry quantitation (IHC) indicating cell cycle entrance was increased in cohort 1. p21 was much higher in cohort 1. Cyclin A (S phase marker) was mildly increased in cohort 1. PH3 (M phase) was also mildly increased in cohort 1. When cohort 1 was compared to cohort 2 there was a positive correlation between cohort 1and p21 expression and the degree of fibrosis. Areas of liver with increased fibrosis showed an increase in stellate cell activity, which correlated with an increased p21 expression even within the same liver where fibrosis varied from area to area. p21 increase in expression did not correlate with the steatosis grade

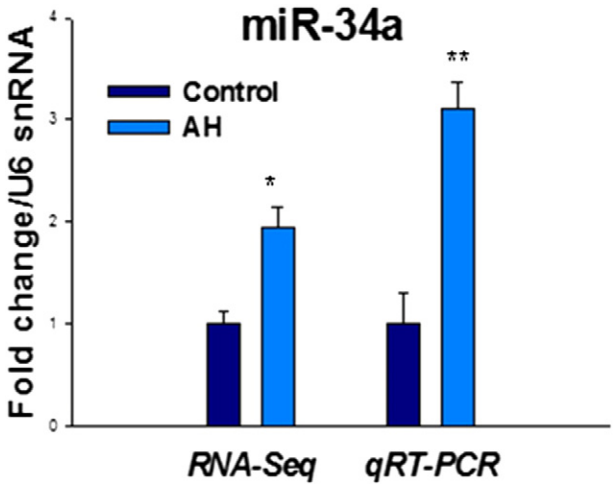

Fig. 2. Increased expression of miR-34a in the livers of alcoholic hepatitis patients was measured by RNA-Seq and qRT-PCR, respectively.
The figure was previously published in Exp. Mol. Pathol. 99: 552–557 (2015b).