Chronic liver inflammation-induced double-strand DNA breaks enhance hepatocarcinogenesis

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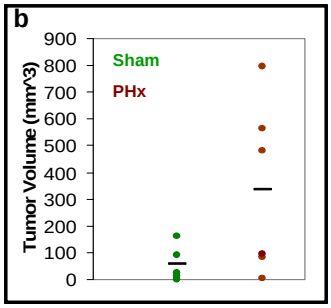
Background / Aims: Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality and considered to be the outcome of chronic liver inflammation. Surgical resection is the preferred treatment for HCC; however, survival rates are suboptimal partially due to tumor recurrence. Our objective was to understand the molecular mechanisms linking liver regeneration under chronic-inflammation to tumorigenesis. We also aimed to examine the effect of partial hepatectomy (PHx) on tumor development and progression. Moreover, since in the clinic PHx is performed on inflamed and malignant tissues, we investigated the effects of these conditions on liver regeneration.

Methods: ***MRI:*** Experiments were performed on a 4.7T Bruker Biospec spectrometer using a 3.5-cm birdcage coil. Hepatic volumetric assessment was acquired by T_1W SE images (TR/TE=400/18ms). Tumor assessment was done from T_2W fast SE images (TR/TE=2000/40ms). ***Animal:*** Mdr2-knockout (KO) mice, a model of naturally occurring HCC resulting from chronic liver inflammation, were used. PHx or sham surgery was performed on 3-month-old (inflamed liver) and 9-month-old (early HCC stages) Mdr2-KO and on equivalents heterozygote (control) mice (n=8 mice/group). Liver MRI was acquired at baseline and after surgery - daily for a week and once a month until the age of 12 months. Nine month-old mice were sacrificed on days 0 (the resected lobe), 2 and 6 posthepatectomy and the livers were harvested for histology, immunostaining and molecular biology analyses (Affimatrix array).

Results: Tumor development was enhanced in the PHx Mdr2-KO mice compared to sham-operated Mdr2-KO mice, regardless of the operational age (Fig. 1a,b). In mice operated at the age of 9-months, both the size and the number of tumors were significantly increased in the PHx group compared to sham (Fig. 1a). These results might suggest a direct effect of the growth factors and cytokines, which are involved in the regeneration process, on the preneoplastic liver parenchyma. However, PHx had a prolonged effect in view of the fact that tumor size was also enhanced in the 3-month-old operated Mdr2-KO mice (Fig. 1b). Surprisingly, liver regeneration was significantly delayed in the Mdr2-KO mice compared to controls (Fig. 1c).

a

	Sham	PHx	P value
Total tumor volume per mouse (mm^3)	157±156	484±309	<.01
Max tumor volume per mouse (mm^3)	85±103	333±244	<.01
Number of tumors >100mm³ per mouse	0.4±0.9	1.2±0.8	<.05
Number of tumors per mouse	5.6±2.7	8.8±3.7	<.05
Liver volume (mm^3)	3318±480	3352±426	ns



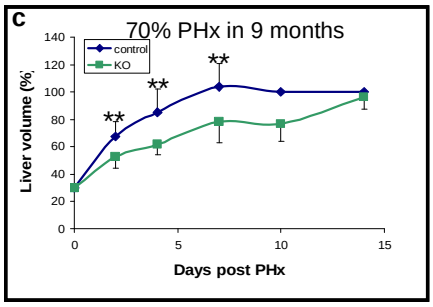
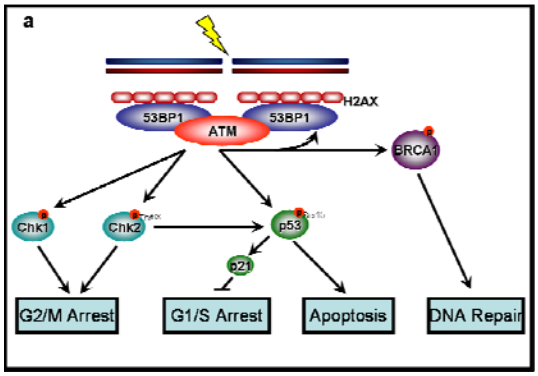


Fig 1: ***Tumorigenesis acceleration and regeneration delay.*** **(a)** The tumor status at the age of 12 months of KO mice that underwent sham (n=5) or PHx (n=10) at the age of 9 months. **(b)** Tumor volume at the age of 12 months of sham mice (green, n=5) or PHx (brown, n=6) operated at at the age of 3 months; p<0.05. **(c)** Liver regeneration kinetics in 9-month-old **(**n≥10**)** control (blue) and KO (green) mice following 70% PHx. **p<0.01.

In order to reveal the molecular basis for the enhanced tumorigenesis and the delayed proliferation rate in the PHx Mdr2-KO mice, we performed a gene expression array. In the 9-month-old Mdr2-KO mice, we found abnormal expression levels of genes involved in the DNA repair mechanism and, specifically, members of the ATM/ATR pathway that are involved with double-stranded DNA breaks (DSBs). The higher levels of γH2AX and 53BP1, known markers of DSBs, were confirmed by immunohistochemistry, indicating DSB accumulation (Fig. 2b,c). P21, a known cell cycle inhibitor downstream to the DSB repair pathway, was elevated in these mice, as revealed by immunohistochemistry (Fig. 2d,e), and probably contributed also to the proliferative delay. According to Tunel assay, there was increased apoptosis, another downstream effect of DSBs, pre- and post liver resection in the Mdr2-KO mice (Fig. 2f,g).



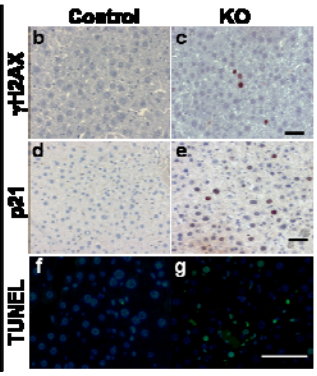


Fig 2: ***Activation of the DNA damage response.*** **(a)** The DNA damage response scheme. The cellular response to damage may involve activation of a cell cycle checkpoint, execution of DNA repair, or when the damage is severe, initiation of apoptosis. **(b-g).** Representative liver sections of control **(b,d,f)** or Mdr2-KO (**c,e,g**) mice before PHx immunostained for γH2AX **(b,c)**, P21 **(d,e,)** and for apoptosis by TUNEL assay **(f,g;** Dapi – blue; apoptosis -

Discussion: We suggest that due to a malfunction in the DSB repair mechanism, hepatocytes undergo increased cell cycle arrest or apoptosis. However, when exposed to proliferative stress resulting from liver resection, these mutated hepatocytes may reenter the cell cycle, which may explain the enhanced tumorigenesis due to resection.