Regeneration and carcinogenesis in a model of chronic inflamed liver monitored by MRI

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Background / Aims:

The mainstay treatment for Hepatocellular carcinoma (HCC) is liver resection, however, survival rates are not optimal due to ineffective regeneration and tumor recurrence. Thus, we aim to examine the effect of partial hepatectomy (PHx) on tumor development and progression. In addition, since in the clinic PHx is performed on inflamed and malignant tissue, we intend to investigate the effects of these conditions on the kinetics of liver regeneration.

Methods:

MRI: Experiments were preformed on a 4.7T Bruker Biospec spectrometer using a 3.5-cm birdcage coil. Hepatic volumetric assessment is acquired by serial coronal and axial 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 (Mdr2-/-) mice lack the liver-specific P-glycoprotein responsible for phosphatidylcholine transport across the canalicular membrane[1]. The absence of phospholipids from bile leads to portal inflammation at an early age (3 months) which is followed by slowly developing HCC (12 -15 month old). PHx (30%)[2] or sham surgery was performed on 3 months old (inflamed liver) and 9 months old (early HCC stages) Mdr2-/- and on equivalents heterozygote (+/-) mice (n=8mice/group). Liver MRI was acquired: at baseline and after surgery - daily for a week and once a month until the age of 12 months. 9 months old mice were sacrificed on days 0 (the resected lobe), 2 and 6 posthepatectomy and livers were taken for histology and molecular biology analysis. H&E staining were observed by a professional pathologist, and differences in the mitotic profile of the livers were confirmed by BrdU staining.

Results and Discussion:

***Tumor progression*:** In order to study the effect of PHx on tumor progression we compared the liver tumor burden in 1 year old mice operated at the age of 9 months (Table 1). Total tumor volume (sum of all tumors' volume per mouse) was bigger in the hepatectomized mice compared to sham operated mice, although there was no difference in total liver volume. Moreover, there were more tumors and the maximal tumor volume was significantly higher in the hepatectomized mice (Table 1). Since the tissue is saturated with growth factors as a result of the regenerative process, this may encourage growth acceleration of existing early dysplastic foci**.** Our results reveal that liver regeneration promotes tumor progression.

	Sham	PHx	P value
Total tumor volume per mouse (mm^3)	157±156	484±309	<.01
Liver volume (mm^3)	3318±480	3351±425	ns
Max tumor volume per mouse (mm^3)	85±103	333±244	<.01
Number of tumors per mouse	6±3	9±4	<.05

Table 1; n = 5 (sham), n = 10 (PHx)

***Liver regeneration*:** As expected, and regardless of the inflammatory state, younger mice reached full liver volume earlier than older mice (Fig.1). As was seen by MRI, histological observation revealed that the peak of hepatocytes proliferation in the older mice is detained (day 6 instead of day 2). Temporarily, regeneration was attenuated in Mdr2-/-, though eventually they reached the original (100%) liver volume and more (Fig.1). Moreover, in these mice there was less proliferation compared to Mdr2+/- mice (Fig.1). This delay may be the outcome of exhaustion of the epithelial cell compartment, resulting in activation of the progenitor cell compartment. When observing the liver status of the Mdr2-/- mice it seems that there is a reduction in inflammation on day 6 after PHx compared to pre PHx.

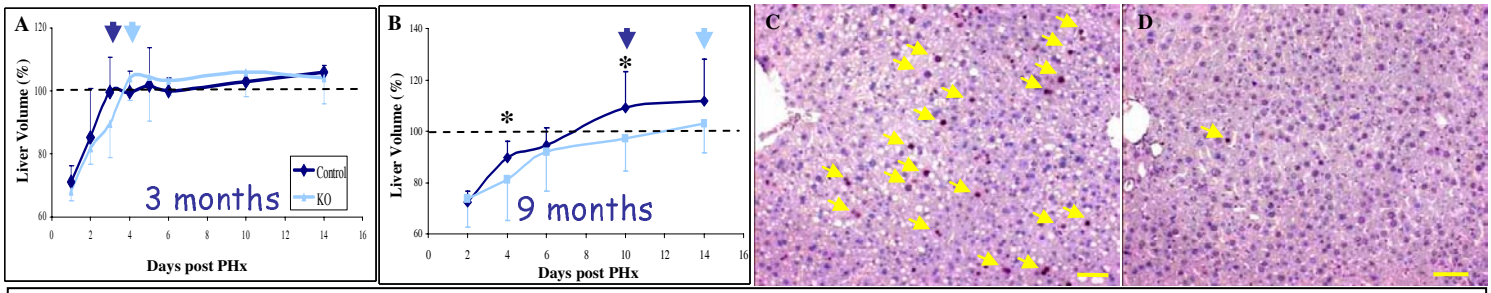

Fig 1 Average ± SD liver volume of (A) 3 months and (B) 9 months control and Mdr2-/- (KO) mice post PHx. Blue arrows indicate the time point each group reached it's original liver volume (100%) indicated by the dashed line;*p < 0.05, compared to control; n = 5 per group per time point. BrdU (C, D) immunostaining of sections obtained from 9 months control (C) and KO (D) mice on day 6 post PHx. Yellow arrows indicate BrdU positive cells; Bar = 100µm.

Utilizing MRI methods we were able to follow liver volume and tumorigenesis monitoring the same animals through the whole period. This makes comparison more accurate and easy and reduces experimental animal numbers. It enabled us to choose the critical time points for sampling histology and molecular biology. In summary, results from this study suggest that liver resection has a dramatic effect on tumor progression in the remaining parenchyma and vice versa. Further study of these changes using molecular methods and analyzing the hemodynamical changes occurring within the liver could reveal the mechanism underlying these processes. In future, this data could assist in improving long-term prognosis and survival in HCC treated patients.

Bibliography:

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