Effect of PlGF-inhibition on survival in mice with hepatocellular carcinoma

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BACKGROUND

Inhibition of angiogenesis is currently hot topic in the search for an effective treatment against hepatocellular carcinoma (HCC). Placental growth factor (PlGF) is a VEGF analogue only involved in pathologic angiogenesis and its inhibition has the potential to restrain tumour growth, without affecting healthy organs. Therefore, we assessed whether administration of PlGF-antibodies could serve as a potential therapy for HCC in mice.

METHODS

5-week-old male mice received intraperitoneal injections with diethylnitrosamine (35 mg/kg bodyweight, 1x/week) leading to HCC after 25W. At 26W mice were treated with murine PlGF-antibodies (20 mg/kg aPlGF, 2x/week) or IgG for 5W. PlGF-knock-out mice (PlGF-/-) received weekly ip DEN-injections and were compared with their WT counterparts. Tumour vasculature was assessed with endoglin staining.

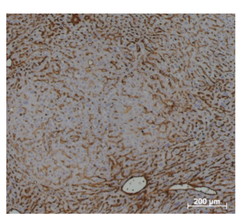
As a marker for vessel abnormality the number of cords without lumen, tortuous vessels and intercapillary distances were quantified. Arterialisation was assessed (aSMA staining) and the number and size of unpaired arteries (i.e. large SMA+ vessels not accompanied by a bile duct) determined. Stainings for HIF2alpha and PCNA were used to identify hypoxia and proliferation.

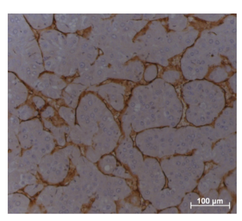
RESULTS

▲ HCC survival after therapy with aPLGF
After 5 weeks of mAb treatment, 45% of the mice receiving control IgG were dead, while only 23% mortality was observed in the aPLGF group (N=48; P<0.05)

▲ HCC survival in PIKO mice
After 30 weeks of DEN treatment, 58% of WT mice (N=24) but only 5% of PlGF-/- mice (N=21) succumbed to the disease (P = 0,002)

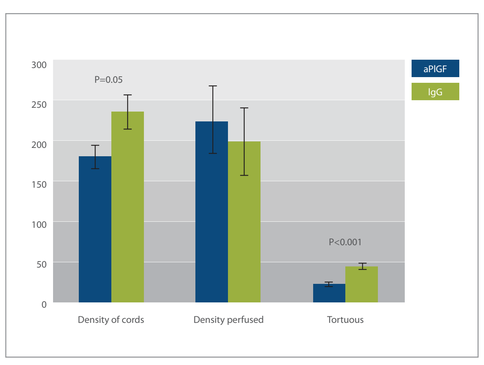
▲ Treatment
Macroscopic evaluation of livers revealed a significantly lower tumour burden in anti-PlGF treated mice (p < 0,05).

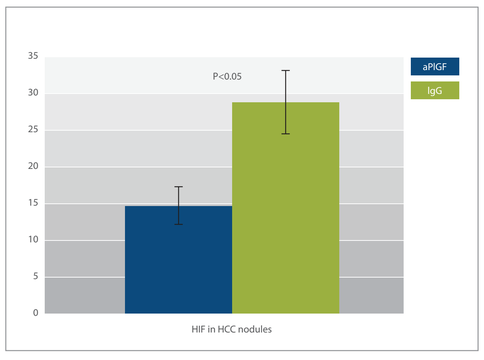


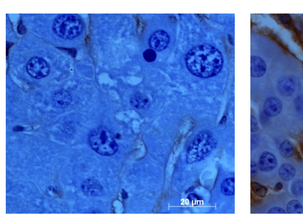


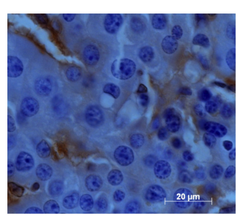
▲25W DEN + 5W aPLGF **▲25W DEN + 5W IgG**

Endoglin staining: PlGF-blockage partially normalised the abnormal blood vessel structure. In PlGF-blocked tumours, fewer capillaries were tortuous or cords, and had a more normal appearance.The structural changes in vasculature were functionally relevant, as PlGF-blocked HCC nodules expressed lower levels of HIF-2alpha. In line with reports that hypoxia promotes HCC growth, PCNA staining showed proliferation of HCC cells was higher in avascular clusters (IgG) than in vascular regions with short intercapillary distances (p=0,02).



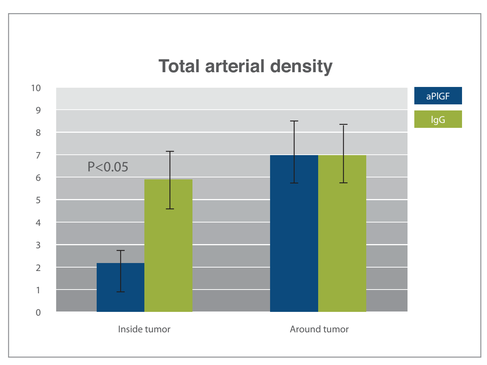


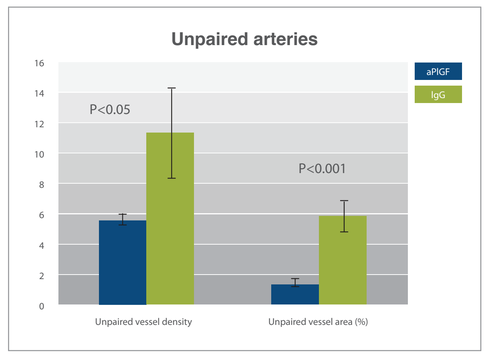




▲25W DEN + 5W aPLGF **▲25W DEN + 5W IgG**

aSMA staining: in healthy liver tissue, arteries are always accompanied by a bile duct. The number and lumen size of aSMA-positive vessels not adjacent to a bile duct was quantified as a marker for de novo arterialisation. aPlGF treatment inhibits arterialisation of the liver.





CONCLUSION

Inhibition of PlGF has a positive effect on survival in mice with HCC. aPlGF treatment decreased the tumour induced vascularisation and arterialisation, without effecting healthy tissue. Furthermore, the normalised vasculature and reduced hypoxia, decreases the proliferation of malignant cells.

Given that VEGF-inhibitors enhance metastasis by evoking hypoxia, the normalised activity of aPlGF may offer a promising target to combat HCC.

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