**Angiogenic Changes in a New Mouse Model for Hepatocellular Carcinoma**

**BACKGROUND**

The increasing incidence of hepatocellular carcinomas in Western countries has led to an expanding interest in this field. A vast need of experimental models that mimic the natural pathogenesis of hepatocellular carcinoma is in short time present. The goal of our study was (1) to develop an efficient mouse model for hepatocellular carcinomas research, (2) to assess time-dependent changes angiogenic pathways and (3) to investigate tumour growth and neo-vascularization using state-of-the-art imaging techniques.

**METHODS**

5-week-old mice received weekly intraperitoneal injections with N-nitrosodiethylamine (Den) (35 mg/kg bodyweight) and samples were taken at several time points. Histology, IHC, ELISA and immunohistochemical staining were used to document the HCC lesions and to quantify angiogenic factors VegF and PlgF; and their receptors. HCC-livers (Den) were perfused with Batson’s n°17 solution (25W) were perfused with Batson’s n°17 solution to produce vascular casts (arterial and venous). A state-of-the-art microPET/CT was used for in vivo detection for 3D-reconstruction of the vascular casts.

**RESULTS**

After 16W of Den-injections a mild fibrose (FI-F2) and doppleric lower grade activity appears as a pre-malignant environment. An increase of angiogenic factors VegF and PlgF takes place, but not enough to induce an increase in endothelial cells, which were upregulated after 25W. After 25W Den-injections, the doppleric lesions have progressed to vascularized neoplastic tumours which are macroscopically visible and give rise to a further increase in angiogenic factors, activating the angiogenic pathway and leading to the formation of new blood vessels.

The vascular casts of HCC-livers clearly revealed the chaotic pattern and hennarchy disorganization of tumour induced blood vessels. Arteries formed a circumferential mantle around the hepatic tumours, while the central tumour regions showed a lower arterial density. Electron microscopy revealed several angiogenic sprouts, mostly sprouting angiogenesis, furthermore intussusceptive angiogenesis was also seen.

**CONCLUSION**

While most Den induced models take about one year to develop tumours, weekly injections with Den give rise to tumour occurrence after 25W. The well vascularized orthotopic tumours are a representative model for HCC and can serve as an excellent platform for the development of new therapeutic targets. The histological and morphological increase of angiogenic factors VegF and PlgF clearly gave rise to new blood vessel formation, confirmed by endothelial cell quantification and CT-reconstructions of vascular casts.

High levels of VegF can predict vascular invasion of HCC and correlated with poor prognosis [1-2]. Elevated VegF is also a marker for poor response to locoregional treatment and is correlated with early recurrence [3-4]. PlgF levels increase to be elevated in a variety of cancers and is associated with poor prognosis in HCC [5-6]. The up-regulation of PI GF in the Den model supports the theory that PlgF plays an essential role in the angiogenesis of HCC.

**REFERENCES**


Angiogenic changes in a new mouse model for hepatocellular carcinoma

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BACKGROUND

The increasing incidence of hepatocellular carcinoma in Western countries has led to an expanding interest in this field. A vast need of experimental models that mimic the natural pathogenesis of hepatocellular carcinoma in a short time period is present. The goal of our study was (1) to develop an efficient mouse model for hepatocellular carcinoma research, (2) to assess time-dependent changes angiogenic pathways and (3) to investigate tumour growth and neo-vascularisation using state-of-the-art imaging techniques.

METHODS

5-week-old male mice received weekly intraperitoneal injections with N-nitrosodiethylamine (DEN, 55 mg/kg bodyweight) and samples were taken at several time points. Histology, ELISA and immunohistochemical stainings were used to identify the HCC-livers and to quantify angiogenic factors: VEGF and PlGF, and their receptors. HCC-livers (25W) were perfused with Batson’s n°17 solution to produce vascular casts (arterial and venous). A state-of-the-art multimodal PET/CT was used for in vivo detection for 3D-reconstruction of the vascular casts.

RESULTS

After 16W of DEN-injections a mild fibrosis (F1-F2) and dysplastic lesions appear, resulting in a pre-malignant environment. An increase of angiogenic factors VEGF and PlGF takes place, but not explicit enough to induce an increase in endothelial cells, which were upregulated after 25W. After 25W of DEN-injections, the dysplastic lesions have progressed to vascularised exophytic tumours which are macroscopically visible and give rise to a further increase in angiogenic factors, activating the angiogenic pathway and leading to the formation of new blood vessels.

The vascular casts of HCC-livers clearly revealed the chaotic pattern and hierarchically organisation of tumour induced blood vessels. Arteries formed a circumferential mantle around the hepatic tumours, while the central tumour regions showed a lower arterial density. Electron microscopy revealed several angiogenic spots, with mostly sprouting angiogenesis, furthermore intussusceptive angiogenesis was also seen.

CONCLUSION

While most DEN-induced models take at least one year to develop tumours, weekly injections with DEN give rise to tumour occurrence after 25W. The well vascularised orthotopic tumours are a representative model for HCC and can serve as an excellent platform for the development of new therapeutic targets. The histological and atherosclerotic increase of angiogenic factors (VEGF & PlGF), clearly give rise to new blood vessel formation, confirmed by endothelial cell quantification and CT-reconstructions of vascular casts.

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