



**Karolinska
Institutet**

Institutionen för Medicin, Huddinge

**Experimental and clinical studies on liver
regeneration and hepatocellular carcinoma.
Roles of redox proteins, iron homeostasis
and multikinase inhibition**

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska
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av

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ABSTRACT

Compensatory liver regeneration is triggered by chronic liver injury or surgery and is crucial to maintain tissue homeostasis. The underlying mechanisms which include a whole battery of complex signaling events have been thoroughly studied for decades. The majority of hepatocellular carcinomas develop in a highly proliferative environment caused by underlying chronic liver disease in which lost liver tissue must be restored to meet the needs of the organism. The chronic inflammatory condition with chronic liver repair enhances the presence of free radicals leading to an increased risk of cell alterations.

This thesis includes four papers; the first three of which comprise studies regarding the importance of the regulation and endurance of cell proliferation, and also the sensitivity of the proliferating cells to compounds used in cancer prevention and treatment. In these studies we used a 2/3 partial hepatectomy (PH) rat model and also a chemically induced rat liver cancer model (The Solt and Farber Resistant hepatocyte model). The fourth paper is a human study in which we quantified immunohistochemical stainings for 6 different redox proteins in livers from patients resected for hepatocellular carcinoma (HCC) and colorectal carcinoma (CRC) liver metastases. The specific aims were: (I) to characterize gene expression of the different pathways involved in hepcidin regulation after PH, until liver regeneration is complete; (II) to study the effects of sodium selenite on regenerative versus neoplastic liver cell proliferation in rat, and to investigate if TrxR1 is a constitutive tumour marker or an unspecific marker for cell growth in rat liver; (III) to study the effect of the anticancer agent sorafenib, a multikinase inhibitor, on normal liver regeneration after PH in rat; and (IV) to evaluate if redox protein (thioredoxins and glutaredoxins) expressions correlate to clinical features in human hepatocellular carcinoma and if they can be used as prognostic markers after liver surgery.

Our results showed that high serum levels of IL6 induced the levels of STAT3 and the expression of hepcidin mRNA during the acute phase after PH. The gene expressions of the iron sensing proteins HFE, hemojuvelin (HJV) and transferrin receptor 2 (TfR2) were decreased during the whole regeneration, gradually decreasing hepcidin gene expression and thereby mobilizing iron to the growing liver. The expression of genes involved in iron uptake; transferrin receptor 1 (TfR1) and divalent metal transporter 1 (DMT1) were increased thereby facilitating iron uptake (paper I).

After administration of sodium selenite in a tumour preventive, supranutritional dose followed by PH no effect on body weights or gain of liver mass was seen. In the hepatocarcinogenesis model the tumour volume was significantly decreased in animals supplied with selenium during the progression phase compared tumours in rats not treated with selenium. The expression of TrxR1 was exclusively seen in the neoplastic liver lesions but not in the remodelling preneoplastic lesions (paper II). Treatment with sorafenib transiently suppressed liver regeneration and the gain of relative liver mass, but was followed by a delayed compensatory increase of liver cell proliferation one week after resection with the result that after 14 days the treated animals reached the same relative liver weights as the controls did in five days (paper III).

In the human study we saw an up-regulation of Trx1, Trx2 and Grx5 in HCC compared to its respective surrounding non-tumorous tissue. The same was observed in the CRC metastases where also the staining of Grx1 and Grx3 was significantly higher compared to non-tumorous tissue. Trx1 expression correlated well to cell proliferation but not to tumour differentiation, micro-vascular invasion or tumour recurrence. A relative down regulation of Trx1 was seen in tumours compared to the surrounding liver in males, smokers and in patients with high alcohol consumption.

We concluded that the peak of hepcidin expression during the acute phase was eventually overruled by the downregulation of the iron sensing pathway in order to promote iron mobilization to the regenerating liver. We also concluded that selenium in a supranutritional dose impaired tumour growth without impairing the normal liver cell proliferation, and that the selenoprotein TrxR1 is a constituent of the neoplastic phenotype. Sorafenib prolonged liver regeneration in proportion to the length of treatment but the liver adapted to the early inhibitory effects of the drug. Thioredoxins and glutaredoxins were ubiquitously expressed in livers exposed to oxidative stress and various malignancies and can therefore not be used as diagnostic markers for HCC. Smoking and high alcohol consumption increased the Trx1 expression in tissue surrounding the HCCs, whereas expression of Trx1 in the HCCs correlated to cell proliferation. Redox protein expression in HCCs cannot be used as predictive markers for tumour recurrence after liver resection.