Role of fibrosis and senescence in ageing mice liver

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The talk that I gave at the 2008 Annual Scientific Meeting "Ageing: Molecules to Man" in Brighton showed the results of my work during the first year of my PhD. I am very interested in the relationship between Non-Alcoholic fatty liver disease and ageing. For a long time it was considered, that the liver is almost unaffected by ageing and age-related disease. However, more recently several functional and morphological changes have been observed in the liver with age, like reduced blood flow and liver mass and a very important change: the reduced ability to regenerate.

The modern lifestyle characterised by less exercise and excess food intake is the main contributor to obesity in western countries. These factors are not only responsible for the onset of diseases such as diabetes and cardiovascular complications, but also for metabolic syndrome and Non-Alcoholic Fatty Liver Disease (NAFLD). NAFLD is the term used for the liver disorder similar to alcoholic liver disease, which is observed in patients with the metabolic syndrome and is characterized by increased accumulation of triglycerides in the hepatocytes (hepatic steatosis). Fibrosis, an advanced stage of NAFLD, is the result of chronic liver injury due to alcohol intake, drugs, infections (hepatitis C virus [HCV]) or metabolic disorders. Normally it evolves over decades, but can progress over weeks/months in some patients such as recurrent HCV infection in immunocompromised patients post liver transplantation. In all fibrotic reactions, the underlying cellular and molecular mechanisms involve leukocyte infiltration, persistence of inflammation in the tissue and proliferation of cells with a myofibroblast phenotype. Fibrosis can be considered as a wound-healing response that has become excessive due to a failure of the liver to degrade the excess of extracellular matrix and scar tissue appropriately, which eventually leads to adverse effects on liver function.

In liver an increase in SA-β-Gal activity (marker of cellular senescence) has been observed in liver cirrhosis, which is the end stage of chronic liver disease. This is consistent with the hypothesis that senescence is associated with liver disease. Also, an increase in senescent cells with age has been described in various tissues in mammals. To determine the effect of senescence on liver ageing we measured γ-H2A.X foci frequency in hepatocytes from mice at 4 different age groups (12, 22, 36 and 42 month old). Our results show a significant increase in the frequency of γ-H2A.X positive hepatocytes in liver with mouse age. Then, we decided to look in more detail at the spatial disposition of the γ-H2A.X positive cells. We found that γ-H2A.X positive cells could be found predominantly in specific areas of the liver.

4-Hydroxy-2-Nonenal (HNE) is one of the major aldehydic metabolites of lipid peroxidation and the most reliable marker of lipid peroxidation. HNE may directly activate hepatic stellate cells and lipid peroxidation may play a role in hepatic fibrogenesis. Increased HNE adducts have been reported in liver tissues from animals with experimental iron overload and in patients with different chronic liver diseases. Since oxidative stress has been shown to play a role in cellular senescence and in liver disease in both humans and mice, we decided to look at HNE prevalence in mouse liver with age. We observed an age-dependent increase in HNE adducts with age. We also found an age-dependent increase in alpha-SMA which is a marker of activated hepatic stellate cells (HSCs). The initial activation of HSCs is likely to be a result of stimuli produced by neighbouring cells namely hepatocytes, Kupffer cells, circulating leukocytes, platelets and sinusoidal endothelial cells in response to liver injury. Such stimuli include reactive oxygen species (ROS) / lipid peroxides, growth factors and inflammatory cytokines. It could be that increased lipid peroxidation with age in mouse liver, as seen using HNE, is an important stimulus for the activation of HSCs and finally increased liver fibrosis. Another possibility is that senescent hepatocytes themselves contribute to activation of HSCs. It has been well documented that senescent fibroblasts overexpress proteins that remodel the extracellular matrix or mediate local inflammation, altering the surrounding microenvironment. Moreover, our group has shown that senescent cells produce high levels of ROS. It is possible that these factors together could contribute to the activation of HSCs and the onset of liver fibrosis. However, our results only show a correlation between these factors and no causality has yet been established.