Expression of STIM and Orai in liver disease

C.H. Wilson,[1] G.Y. Rychkov[2] and G.J. Barritt,[1] [1]Department of Medical Biochemistry, School of Medicine, Flinders University, Adelaide, SA 5001, Australia and [2]Department of Physiology, School of Medicine, The University of Adelaide, Adelaide, SA 5001, Australia.

Liver disease is one of the leading causes of death in people with type II diabetes. Disruption in Ca^{2+} homeostasis has been detected in numerous liver diseases. In hepatocytes, Ca^{2+} regulates glucose homeostasis, protein synthesis and lipid metabolism amongst other functions. Store-operated calcium entry (SOCE) plays a key role in maintaining intracellular Ca^{2+} and SOCE plays an important role in growth and differentiation of hepatocytes. Defects in this process may lead to the development of liver disease ranging from non-alchoholic steatosis, cirrhosis and cancer. STIM and Orai1 proteins are key components of SOCE and STIM1 and Orai are required for SOCE in primary hepatocytes (Jones *et al.*, 2008). STIM1 and Orai1 isoforms, STIM2, Orai2 and Orai3 may also be involved in disease altered SOCE. We hypothesize that defects in Ca^{2+} homeostasis associated with liver disease are due to abnormal expression, localization, and interaction of Orai1 with STIM1. In hepatocytes isolated from genetically obese (*fa/fa*) Zucker rats, a model of obesity and insulin resistance that develop steatosis, we previously detected a significant decrease in the amplitude of ISOC activated by 25 μM ATP compared to the ISOC of lean Hooded Wistar rats. To assess whether this observation was due to altered gene expression, the mRNA levels of STIM1, Orai1 and their isoforms STIM2, Orai2 and Orai3 were compared between primary hepatocytes of Zucker obese and lean control rats. In addition, gene expression was determined in cultured H4IIE rat liver cancer cells treated with or without 100 nM insulin/dexamethasone to influence differentiation. Hepatocytes were isolated from Zucker rats anaesthetized with xylazine/ketamine followed by collagenase perfusion of the liver. Gene expression was measured by relative quantitative polymerase chain reaction (qPCR) and expressed relative to B-actin. Results revealed no difference in expression of STIM1, STIM2, Orai1 and Orai3 in hepatocytes from obese and lean Zucker rats. This suggests that the observed difference in SOCE is due to changes in the distribution of STIM and Orai proteins, not altered expression. Orai2 is only expressed at low levels in liver tissue and was difficult to detect in all samples. From measurements obtained it appears that there is a small increase in Orai2 expression in hepatocytes from obese Zucker compared with lean Zucker rats, suggesting that this might contribute to the observed altered SOCE current. Insulin and dexamethasone treatment of H4IIE cells resulted in a significant ($p<0.05$) decrease in the levels of STIM2 and Orai3 accompanied by a decrease in STIM1 level, and increase in Orai1 levels. Orai2 was not detected. These initial findings suggest that STIM and Orai expression is altered in liver cancer and indicates their possible involvement in cellular differentiation. The complete absence of Orai2 indicates its possible loss in cancer tissue. Future assessment of expression levels in diseased human liver tissue samples compared to normal tissue will test whether this finding is physiologically relevant. Moreover, imunofluoresence microscopy will help determine if altered distribution of STIM1 is responsible for changes in ISOC of obese zucker rats hepatocytes. Further work will be needed to determine whether a change in distribution occurs in livers of Type II diabetes patients that might lead to disruption of liver Ca^{2+} homeostasis and development of liver disease.

Jones BF, Boyles RR, Hwang SY, Bird GS, Putney JW. (2008). Calcium influx mechanisms underlying calcium oscillations in rat hepatocytes. *Hepatology* **48(4):** 1273-81.