

Confocal Laser Scanning Microscopic Studies for Liver Diseases

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A confocal laser scanning microscope (CLSM) is an advanced microscope that provides information of subcellular protein localization in detail with double or triple-labeling immunohistochemistry without time-consuming and complicated preparations as required in electron microscopy.

In several liver diseases such as damage of bile ducts in primary biliary cirrhosis (PBC), narrowing of bile ducts in primary sclerosing cholangitis (PSC), or obstruction of bile ducts in obstructive jaundice, serum bilirubin levels elevate. In these conditions, bile including bilirubin regurgitates to blood. Although previous electron microscopic studies have demonstrated increased permeability in the tight junction of hepatocytes and bile enter the blood of the liver sinusoid in obstructive jaundice, permeability of bile ducts has never been studied in liver diseases because of the limitation of the methodology. When we have applied CLSM and two antibodies to tight junction proteins, ZO-1 and 7H6, tight junction permeability has been demonstrated to increase at the hepatocyte level in PSC and obstructive jaundice, and at the bile duct level in PBC.

An increased serum level of gamma glutamyl peptidase (GGT) is the most reliable clinical marker for alcoholic liver diseases. However, the expression of GGT in the liver has not been well documented in alcoholic liver diseases. We have demonstrated with CLSM and an anti-human GGT antibody that GGT colocalized with DDP VI, a canalicular membrane marker, but GGT expression level was very limited in normal livers. In alcoholic liver disease, GGT was strongly expressed on the canalicular membrane as well as whole cytoplasm of the biliary epithelial cells. In situ hybridization demonstrated mRNA expression GGT in hepatocytes as well as bile ducts. Western blot analysis has shown a significant increase in GGT protein expression in alcoholic liver disease.

Conclusions: CLSM is a very powerful tool to study the subcellular protein localization, with various specific antibodies, that electron microscopy is not able to reveal.