**A-03**

**Perfusion fixation method is critical for immunoelectron microscopy and ultrastructural evaluation on changes of caveolin-1 and caveolae relates with capillarization of liver sinusoidal endothelial cells in human cirrhotic liver**

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**Backgrounds and aims:** Most vascular endothelial cells are continuously exposed to shear stress in vivo. Caveolae, omega-shaped membrane invaginations on endothelial cell (EC), also are plasmalemmal domain enriched in cholesterol, caveolins, and signaling molecules. Previous studies have proposed a role for caveolin(CAV)-1 in the regulation of angiogenesis and sinusoidal differentiation. This study was designed to elucidate the ultrastructural localization and change in CAV-1 expression on human liver sinusoidal endothelial cells (LSECs) during the progression of cirrhosis, using sections prepared by perfusion fixation method.

**Methods:** Normal control and Child-Pugh A and C cirrhotic liver specimens by surgical procedure were studied. CAV-1 protein and gene expression was examined by immunohistochemistry. Western blotting, laser-capture microdissection (LCM)-PCR. For immunoelectron microscopy, CAV-1 expressions in sinusoid was examined by perfusion fixed liver tissue.

**Results:** In control liver tissue, CAV-1 was localized on caveolae mainly in arterial and portal endothelial cells of the portal tract, and was also found on vesicles and some fenestrae in LSECs around the central vein. In cirrhotic liver tissue, aberrant CAV-1 expression was observed on caveolae-like structures and a few vesicles in LSECs. Significant overexpressions of CAV-1 at protein and mRNA level in cirrhotic liver was demonstrated by Western blotting and LCM-PCR (p<0.01 Child-Pugh A and C vs control, p<0.01 Child-Pugh A versus C).

**Conclusion:** CAV-1 was strongly expressed on caveolae-like structures and vesicles on LSECs in the sinusoids of cirrhotic liver, suggesting an association of CAV-1 with angiogenesis and differentiation of LSECs in cirrhosis.

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**A-04**

**Brain-derived neurotrophic factor promotes angiogenesis via oxidative stress in human vascular endothelial cells: Implication for atherogenesis?**

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**Aim:** Brain-derived neurotrophic factor (BDNF), a major type of neurotrophins, promotes synaptic plasticity and neuronal cell survival, which contribute to the maintenance of structure and function of neuronal cells. Recent studies also indicate a possible involvement of BDNF in the atherogenesis. However, the detailed mechanisms for this remain to be fully clarified. We hypothesized that BDNF may at least partly play a role in the atherosclerotic plaque development through the promotion of angiogenesis. To gain mechanistic insights, we examined whether BDNF causes angiogenesis and underlying mechanisms with focusing on reactive oxygen species (ROS) and related intracellular signals in human cultured vascular endothelial cells (ECs).

**Methods and results:** In vascular ECs, BDNF increased ROS generation as measured fluorometrically using 2’-7’-dichlorofluorescein diacetate as well as NADPH oxidase (NOX) activity as determined by a chemiluminescent measurement. BDNF increased ROS generation and NOX activity were inhibited by K252a, an inhibitor of tropomyosin-related kinase B (TrkB) receptor. BDNF caused phosphorylation of p47 phox, a regulatory component of NOX, which was inhibited by K252a as determined by Western blotting. In matrigel, BDNF caused angiogenic tube formation of ECs, which were inhibited by K252a or gp91 ds-tat, a specific inhibitor of NOX. BDNF induced phosphorylation of Akt but not ERK in ECs, which was inhibited by K252a or gp91 ds-tat. It was further confirmed that small interfering RNA (siRNA) against TrkB inhibited BDNF-induced ROS generation and tube formation.

**Conclusion:** The present results for the first time showed that BDNF promotes angiogenesis through NOX-derived ROS generation via the activation of p47 phox in a TrkB receptor-dependent manner.