potential roles of these ion channels in regulating proliferation and migration.

Methods: Multiple experimental approaches were employed in this study, including whole-cell patch voltage-clamp, RT-PCR, Western blots, cell proliferation and migration assays, etc.

Results: Several ionic currents were heterogeneously expressed in human cardiac c-kit$^+$ progenitor cells, including a large conductance Ca^{2+}-activated K^+ current (BKCa) in most (93%) of cells, an inwardly-rectifying K^+ current (I_{Kir}) in 87% of cells, a transient outward K^+ current (I_{to}) in 39% of cells, a voltage-gated tetrodotoxin-sensitive Na^+ currents ($I_{Na,TTX}$) in 76% of cells. Molecular identities of these ionic currents were determined with RT-PCR and Western blot analysis. KCa.1.1 (for BKCa), Kir2.1 (for I_{Kir}), Kv4.2, Kv4.3 (for I_{to}), NaV1.2, NaV1.3, NaV1.6, NaV1.7 (for $I_{Na.TTX}$) were expressed in human cardiac progenitor cells. Inhibition of BK_{Ca} with paxilline, I_{to} with 4-aminopyridine, but not $I_{Na.TTX}$ with TTX and I_{Kir} with Ba^{2+}, decreased cell proliferation. Silencing of KCa.1.1, Kv4.2 or Kv4.3, but not Kir2.1, with siRNA targeting corresponding channels reduced proliferation. Inhibition of KCa1.1 or Kv4.2 or Kv4.3 channels accumulated cells at G0/G1 phase. Interestingly, down regulation of KCa1.1, Kv4.2 or Kv4.3 channels decreased, while of Kir2.1 channels increased migration in human c-kit$^+$ progenitor cells.

Conclusions: These results demonstrate for the first time that multiple ion channels are expressed in human cardiac c-kit$^+$ cells. KCa1.1, Kv4.2, and Kv4.3 channels, but not Na^+ channels and Kir 2.1 channels, participate in regulating proliferation. KCa1.1, Kv4.2 or Kv4.3 channels promote, while Kir2.1 channels reduce cell migration in human cardiac c-kit$^+$ progenitor cells.

P17

CHRONIC INTERMITTENT HYPOXIA INDUCES OXIDATIVE STRESS AND INFLAMMATION VIA ANGIOTENSIN II RECEPTOR 1 IN RAT LIVER

Pan Jingfei, Xiao Jia[1], George L.Tipoe[1], Man Lung Fung

Departments of Physiology and Anatomy[1], Li Ka Shing Faculty of Medicine, University of Hong Kong, Pokfulam, Hong Kong SAR, China

Chronic intermittent hypoxia (IH) associated with obstructive sleep apnea (OSA) is characterized by repetitive cycles of hypoxia and reoxygenation, leading to excessive production of reactive oxygen species and oxidative stress in tissues and organs. However the mechanistic effects of chronic IH on the liver are not clear at present. We hypothesized that renin-angiotensin system (RAS) plays a role in the IH-induced oxidative stress and tissue inflammation in the rat liver.

Adult Sprague-Dawley rats were exposed to air (normoxic (Nx) control) or IH treatment (with inspired oxygen fraction in the normobaric chamber cyclic between 5-21% ± 0.5% per min, 8 hours per day) for 14 days. Rats were fed with an angiotensin II type 1 (AT1) receptors blocker telmisartan (10mg/kg body weight), or vehicle daily before the IH treatment. Hepatic expression levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β were detected with ELISA assay; serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were examined for liver injury; also the level of oxidative stress with malondialdehyde (MDA) in the liver.

Our results showed that the protein expression of IL-6, TNF-α and IL-1β were significantly higher in the hypoxic group than that of the Nx control and telmisartan-treated hypoxic (TIH) groups, suggesting that inhibition of the binding of angiotensin II to AT1 receptors attenuates IH-induced tissue inflammation in the rat liver. In addition, the MDA level was significantly elevated in the hypoxic group but was normalized by the telmisartan treatment. Furthermore, the serum ALT to AST ratio was increased significantly in the hypoxic group when compared to the Nx and TIH groups.

In conclusion, blockade of the AT1 receptor mitigates oxidative stress, tissue inflammation and cellular injury in the liver of rats exposed to chronic IH mimicking a severe OSA condition, thus supporting a pathogenic role of RAS in the IH-induced hepatic injury.

P18

NADPH OXIDASE UPREGULATED BY AT1 RECEPTOR MEDIATES CHRONIC INTERMITTENT HYPOXIA-INDUCED OXIDATIVE STRESS AND INFLAMMATION IN RAT ADRENAL MEDULLA

Y. Liu[1], G.L. Tipoe[2], J. Xiao[2], Y. Huang[3], M.L. Fung[1]

[1]Departments of Physiology and [2]Anatomy, The University of Hong Kong; [3]School of Biomedical

Sciences, The Chinese University of Hong Kong

Our previous study found that chronic intermittent hypoxia (CIH) associated with recurrent apnea induced oxidative stress and inflammation in rat adrenal medulla. However, the underline mechanism was not clear. We hypothesized that, under CIH, the up-regulation of NADPH oxidase mediated by renin-angiotensin system (RAS) via an activation of angiotensin II receptor 1 (AT1) might take part in the oxidative stress and local inflammation in the adrenal medulla. Adult male SD rats were exposed to air (normoxic) control or CIH treatment (8 hours/day) which mimicked a severe recurrent sleep apneic condition for 14 days. Oral feeding of Telmisartan (10 mg/kg), a specific AT1 receptor blocker, or an intraperitoneal injection of apocynin (25 mg/kg i.p.), an inhibitor of NADPH oxidase, or vehicle was performed before the daily hypoxic treatment. The adrenal medulla was harvested for the measurement of markers for oxidative stress (MDA and NTR), macrophages infiltration (ED1), apoptosis, and inflammation (pro-inflammatory mediators) using TUNEL assay, real-time PCR, ELISA and Western blot. Levels of MDA and NTR were significantly increased in the hypoxic (CIH) group when compared with the normoxic control, but were normalized in the hypoxic groups treated with apocynin (AIH) or telmisartan (TIH). The expression levels of macrophage marker ED1-immunoreactivity and the pro-inflammatory mediators (TNFa, IL6) were also elevated in the CIH group, but were significantly ameliorated by the apocynin or telmisartan treatment. In addition, the amount of apoptotic cells in the CIH group was significantly higher than that of the AIH and TIH groups. Moreover, the mRNA levels of NADPH oxidase subunits (Nox2, Nox4) were increased significantly in the CIH group when compared with that of the AIH and TIH groups. Also, the protein expression of RAS components (AGT, AT1) was also increased in the CIH group. In conclusion, we showed that an up-regulation of NADPH oxidase via AT1 receptor activation mediates CIH-induced oxidative stress and inflammation in rat adrenal medulla.

P19

REDUCTION IN HEPATIC APOPTOSIS MODULATED BY GARLIC DERIVED S-ALLYLMERCAPTOCYSTEINE (SAMC) IN NON-ALCOHOLIC FATTY LIVER DISEASE RAT MODEL THROUGH P53-DEPENDENT PATHWAYS

Jia Xiao[1], Man-Lung Fung[2], Emily C. Liong[1], Raymond Chuen Chung Chang[1], Yick-Pang Ching[1], George L. Tipoe[1]

[1] Department of Anatomy, [2] Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China.

Purpose Previous study demonstrated that administration of garlic-derived antioxidant S-allylmercaptocysteine (SAMC) ameliorated hepatic injury in a non-alcoholic fatty liver disease (NAFLD) rat model. In the present study, we investigated the effect and mechanism of SAMC on NAFLD-induced cellular apoptosis in the liver.

Methods Adult Sprague-Dawley female rats were fed with a diet comprising of highly unsaturated fat diet (30% fish oil) for 8 weeks to develop NAFLD with or without intraperitoneal injection of 200 mg/kg SAMC three times per week. After chemical euthanasia, liver samples were collected for histological, biochemical and molecular analyses.

Results During NAFLD development, increased apoptotic cells were observed in the liver. Hepatic apoptosis was accompanied by activated intrinsic apoptotic pathway as shown by expressional changes of cytochrome c and Bcl-2 family genes. Extrinsic apoptotic pathway was also activated as shown by expressional changes of Fas, TRAIL, FADD and cleaved caspase-8. Increased activity of caspase-3 further confirmed the activation of apoptosis. In addition, reduced activity of LKB1/AMPK and PI3K/Akt pathways could be observed with increased expression of pro-apoptotic regulator p53 in NAFLD rats. Administration of SAMC reduced the number of apoptotic cells through down-regulation of both intrinsic and extrinsic apoptotic mechanisms. Phosphorylation status of LKB1, AMPK, PI3K, and Akt were also restored by SAMC co-treatment, leading to the reduction of p53 expression.

Conclusion Administration of SAMC during NAFLD development in rats protects liver from apoptosis through p53-dependent intrinsic and extrinsic apoptotic pathways.