Results: A total of 116 patients were included: 45 LT-HCC and 71 LT-non-HCC. LT-HCC patients were older than LT-non-HCC (median 60 vs 53 years, p = 0.011), but comparable for sex, liver disease etiology, immunosuppressive schedule. At baseline, levels of activated CD8, memory B-cells and senescent CD4, CD8 and B-cells were significantly higher in LT-HCC patients than LT-non-HCC ones. During 27.4 (7.7–41.7) months of follow-up, 6 PTM occurred: 4 in LT-HCC (8.9%) and 2 in LT-non-HCC (2.8%). Patients developing PTM showed significantly higher baseline levels of immune activation than patients without malignancies. Within LT-HCC group, levels of senescent cells were significantly higher in patients with PTM compared to the others [%CD8+CD28-CD57+: 22.45 (17.72–25.86) vs 10.82 (5.21–25.16), p = 0.098; %CD4+CD28-CD57+: 14.33 (10.23–21.12) vs 2.65 (1.10–13.11), p < 0.001].

Table: Differences of immunological parameters between LT-HCC and LT-non-HCC at baseline

Median (IQR)	LT-HCC (N = 45)	LT-non-HCC (N = 71)	p value*
%CD8 activation (CD8+CD38+HLA-DR+)	10.89 (5.61–18.52)	6.59 (4.26–9.25)	**0.003**
%CD4 activation (CD4+CD38+HLA-DR+)	7.23 (4.11–14.12)	6.21 (3.49–9.23)	0.092
%B activated memory (CD19+CD10-CD21-D27+)	10.97 (5.59–20.68)	7.60 (3.00–13.72)	**0.040**
%CD8 senescence (CD8+CD28-CD57+)	11.06 (6.24–25.16)	5.92 (3.54–10.97)	**0.006**
%CD4 senescence (CD4+CD28-CD57+)	3.80 (1.36–14.03)	1.80 (0.48–3.41)	**0.002**
%B senescence (CD19+CD27-IgD-)	12.20 (6.28–17.67)	6.59 (4.24–12.50)	**0.019**

*Adjusted by age.

Conclusion: Our findings suggest that patients undergoing LT for HCC have a higher immune activation and senescence profile compared to other recipients, possibly representing an additional risk factor for PTM. Moreover, immune activation and senescence may be prognostic factors for PTM occurrence regardless of the cause of transplantation.

SAT319

The crucial role of PARP [poly (ADP-ribose polymerase] on the post-ischemic liver injury and inflammation

Michitaka Ozaki[1], Sanae Haga[1], Naoki Morita[2]. [1]*Hokkaido University, Department of Biological Response and Regulation, Sapporo, Japan;* [2]*National Institute of Advanced Industrial Science and Technology (AIST)*
Email: ozaki-m@med.hokudai.ac.jp

Background and aims: Ischemia/reperfusion (I/R) of liver is an unavoidable event in liver surgery and transplantation, which sometimes causes serious post-operative liver failure/complications. The mechanism of I/R-induced liver injury, however, is complicated so that we have not elucidated yet. I/R-induced ROS, various types of programmed cell death and the following sterile inflammatory reaction seem to be crucial. In the present study, we investigated the roles of PARP [Poly (ADP-ribose) polymerase] on hypoxia/reoxygenation (H/R)-induced, ROS-mediated programmed cell death and inflammation in mouse liver cells and macrophages.
Method: AML12 cells and Raw264.7 cells were used for the experiments as hepatocytes and macrophages, respectively. Cells were cultured in high-glucose DMEM supplemented with 10% FBS. H2O2 were administered directly to the culture medium for oxidative stress. Cellular hypoxic conditions were created and maintained in a chamber by flushing with a 95% N2/5% CO2 gas mixture for 10 min and then sealing the chamber. Following 6 h of hypoxia, cells were reoxygenated by opening the chamber and replacing the hypoxic medium with oxygenated medium. Protein and gene expressions were measured by western blot analysis/capillary-based immunoassay and RT-PCR, respectively. Cell survival and death were determined by plating the cells in the xCELLigence System (Roche) and the LDH release.
Results: H/R-induced cell death was inhibited significantly by PJ34, a specific inhibitor of PARP. Because an oxidative stress is one of the major causes of H/R-induced cell death, we challenged 1 mM H2O2 to AML12 liver cells. H2O2 induced cell death, PAR [Poly (ADP-ribose)] production and nuclear translocation of AIF (apoptosis inducing factor), which were suppressed significantly by the pre-treatment of PJ34. H2O2 also induced Receptor-Interacting Protein (RIP)1/RIP3 binding, which indicates the involvement of necroptosis in H2O2-induced cell death. H2O2-induced RIP1/RIP3 binding was inhibited by PJ34. These facts indicate that H/R and ROS potentially induce parthanatos and necroptosis in hepatocytes. Furthermore, H/R-induced inflammatory reaction (proinflammatory gene expression) was inhibited by PJ34 both in AML12 cells and Raw264.7 cells.
Conclusion: PARP is potentially involved in H/R- and oxidative stress-induced cell death in mouse liver cells, by inducing programmed cell death (parthanatos and necroptosis) and inflammatory reaction.

SAT320

Mitochondrial transplantation attenuates murine in vivo hepatic ischemia/reperfusion injury

Avinash Mukkala[1,2], Raluca Petrut[2,3], Zahra Khan[1,2], Mirjana Jerkic[2], Menachem Ailenberg[2], Shawn Rhind[4], Marc Jeschke[5,6,7], Ana Andreazza[8], Andras Kapus[2,7,9], Ori Rotstein[1,2,7,10]. [1]*University of Toronto, Insitute of Medical Science, Toronto, Canada;* [2]*St. Michael's Hospital, Keenan Research Centre for Biomedical Science, Toronto, Canada;* [3]*University of Toronto, Temerty Faculty of Medicine, Toronto, Canada;* [4]*Defence Research and Development Canada/Recherche et développement pour la défense Canada, Ottawa, Canada;* [5]*University of Toronto, Department of Immunology, Toronto, Canada;* [6]*Sunnybrook Health Sciences Centre, Ross Tilley Burn Centre, Toronto, Canada;* [7]*University of Toronto, Department of Surgery, Toronto, Canada;* [8]*University of Toronto, Department of Pharmacology and Toxicology, Toronto, Canada;* [9]*University of Toronto, Department of Biochemistry, Toronto, Canada;* [10]*St. Michael's Hospital, Department of Surgery, Toronto, Canada*
Email: avinash.mukkala@mail.utoronto.ca

Background and aims: Liver ischemia/reperfusion injury (LIRI) is a disease process implicated in organ dysfunction following hepatic surgery, traumatic injuries, and liver transplantation. LIRI causes mitochondrial perturbations which lead to organ death. Stringent mitochondrial quality control is vital in reinstating organ integrity in LIRI. Mitochondrial transplantation (MT) has recently surfaced as a biotherapeutic option in IRI. The effect and mechanism of MT in IRI are poorly characterized. We hypothesized that MT, using mitochondria derived from mouse skeletal muscle tissue, is hepatoprotective following murine LIRI.
Method: We utilized an *in vivo* murine model of LIRI by occlusion of the hepatic artery leading to the left lobe for 1 h to achieve lobe-specific ischemia. Mitochondria were isolated from hindlimb mouse skeletal muscle tissue by differential filtration followed by characterization for particle size and number, ATP, membrane potential, ultrastructural morphology, and protein purity. Inactivation of mitochondria was conducted using liquid nitrogen (LN_2) immersion or incubation at 95°C. Active or inactive mitochondria were intrasplenically injected, to access the portal system, at the start of reperfusion. Mouse livers were reperfused for 2 h followed by sacrifice for blood and liver tissue collection for various molecular/histological analyses.
Results: Isolated active mitochondria (1–3um) retained membrane potential, cristae architecture and morphology, protein purity, and ATP content. LIRI animals had significantly greater circulating AST and ALT compared to sham animals. In addition, LIRI animals had extensive, left lobe-specific liver necrosis, congestion, and vacuolization, when compared to sham animals. Intrasplenic injection of 80 million active mitochondria into LIRI animals, significantly reduced plasma AST and ALT concentrations and normalized histology. Blinded Suzuki scoring of liver histology showed a significant attenuation of congestion, necrosis, and vacuolization as a result of MT in LIRI animals. Interestingly, inactivated mitochondrial particles (LN_2 or 95°C) also significantly reduced plasma AST and ALT, albeit to