# Poster Presentations

## 8 GENERATING A GENOMIC-WIDE TRANSCRIPTOMIC ATLAS OF THE MAMMALIAN CARDIOVASCULAR SYSTEM

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The various highly specialised tissues and structures that form the cardiovascular system enable the transport of blood, oxygen and other important molecules throughout the body. Perturbations in this system increase the risk of developing cardiovascular-related disease.

A sheep cardiovascular transcriptomic atlas was generated using RNA-seq to explore gene expression patterns in the mammalian cardiovascular system. Tissues included the cardiac valves, as well as left and right auricles and ventricles. Detailed functional clustering of the sheep transcriptome was performed, where transcripts were grouped according to their expression pattern. This analysis, using the innovative Miru (Kajeka) bioinformatics tool, was based on a gene-to-gene comparison of the expression patterns across analysed samples, using a Pearson correlation matrix (correlation value $R \geq 0.99$). Expressed genes in clusters were grouped together according to region-specific roles and specialised cellular functions. Notably, one cluster contained genes with high expression in the auricles in this dataset. The cluster genes were involved in cation channel activity (GO term enrichment analysis returned a Benjamini corrected p-value of $3.4 \times 10–2$). Genes in this cluster included potassium channel subfamily K member 3 (KCNK3; also known as TWIK-related acid-sensitive K+ channel, TASK1), potassium voltage-gated channel subfamily J member 3 (KCNJ3), and myosin light chain 4 (MYL4). Additionally, a number of genes within this cluster have been implicated in atrial fibrillation, and further genes in this cluster may also be important in atrial function.

This dataset provides a highly valuable resource for understanding gene expression in the mammalian cardiovascular system.

## 9 $COCL_2$ INDUCED CARDIOTOXICITY ASSOCIATED WITH COCR ALLOY ORTHOPAEDIC IMPLANTS- AN *IN VIVO* AND AN *IN VITRO* STUDY

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Cobalt/chromium (Co/Cr) alloy metal-on-metal bearings used in prosthetic hip replacements can cause adverse effects in some patients because they release metal ions into the bloodstream during wear. Co toxicity may be a cause of many severe systemic manifestations including neurologic and cardiac symptomatology.

This study examines the effects of chronic Co exposure in rats treated for 28 days with $CoCl_2$ (single i.p. injection of 1 mg/kg, daily) and examines Co uptake *in vitro* into primary adult cardiac fibroblasts (CFs). Co treatment was associated with accumulation into various organs with significant increases detected in liver, kidney and heart ($245.31 \pm 23.64$, $204.80 \pm 11.19$ and $41.04 \pm 4.77$ µg/L respectively). Echocardiography performed on the same animals showed functional changes correlating with compromised cardiac contractility. Fractional shortening was significantly reduced in $CoCl_2$-treated rats following 28 days treatment when compared with control animals ($54.01\% \pm 0.90\%\%$ vs $60.29 \pm 0.53\%\%$, $n=6$, $p \leq 0.01$) and there was evidence of diastolic dysfunction. In order to investigate how Co may accumulate in the heart, primary adult CFs were isolated and uptake of $CoCl_2$ into CFs was compared with uptake into a standard fibroblast 3T3 cell line (3T3s). Uptake of metal ions was measured using inductively coupled plasma mass spectrometry. Co uptake into both 3T3s and CFs increased to between 0–50 and 0–120 µg/L, respectively as the medium concentration of Co (0–300 µM) increased. Interestingly, uptake of Co into CFs was significantly greater than into 3T3 cells. The greater accumulation of $CoCl_2$ into CFs suggests that Co ions *in vivo* could accumulate in these cells and have functional consequences on cardiac performance. Overall, our data provides strong evidence that Co accumulates in the heart resulting in cardiac dysfunction. Importantly, we have shown for the first time that Co could accumulate in the heart via efficient uptake into CFs. Future work will focus on determining the underlying mechanism for uptake which could have important therapeutic implications.

## 10 MACROPHAGE-DERIVED WNTS ARE REQUIRED FOR SCAR-FREE REGENERATION OF THE NEONATAL MOUSE MYOCARDIUM

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**Objective** In contrast to the adult, neonatal mice regenerate their myocardium following injury, at least during the first week after birth.[1] Macrophages (Mϕ) contribute to vessel formation and scar removal following neonatal myocardial infarction (MI)[2]. In the kidney[3] liver[4,7] and gut[5] Mϕ-derived WNTs are required for scar free regeneration following injury. Secretion of WNTs is dependent on acylation by Porcupine (PORCN). In the present study it was hypothesised that neonatal cardiac regeneration would be impaired in mice with Csf1r-Cre driven Mϕ specific *Porcn* deletion.[5]

**Methods** Csf1r-EGFP(MacGreen), $Porcn^{fl}/Csf1r^{Cre-ve}$ and $Porcn^{fl}/Csf1r^{Cre+ve}$ mice underwent coronary artery ligation at post-natal day 1 (P1). Functional loss 1 day after MI, and recovery by P21 were assessed by high-resolution ultrasound. Heart sections were stained with isolectin B4 (vessel density) and picrosirius res (fibrosis). Myocardial gene expression was determined by PCR array in wild-type (WT) mice after injury.

**Results** At day 1 and day 7 post-MI, Csf1r-expressing cells accumulated within the injured myocardium, consistent with a role in regeneration.[2] At day 1 post-MI, fractional area change (FAC) decreased from 40.9% ± 1.6 to 18.0% ± 2.4% ($p<0.0001$) and from 41.0% ± 1.3 to 16.5% ± 2.7% ($p<0.0001$) in *Porcn*$^{fl/}$Csf1r$^{Cre-ve}$ and Porcnfl/Csf1rCre-ve mice respectively. By 21 days after MI, FAC had recovered to 47.4% ± 2.5% ($p<0.0001$) in *Porcn*$^{fl/}$Csf1r$^{Cre-ve}$ and 45.8% ± 1.9% ($p<0.0001$) in *Porcn*$^{fl/}$Csf1r$^{Cre-ve}$ littermates. Coronary vascularisation was restored in the infarct area by 21 days in both lines, but interstitial fibrosis was significantly higher in *Porcn*$^{fl/}$Csf1r$^{Cre-ve}$ (6.0 ± 0.9% LV) compared to *Porcn*$^{fl/}$Csf1r$^{Cre-ve}$ (3.8 ± 0.5% LV, $p<0.05$). In WT neonatal hearts, MI increased the expression of *Wnt5b* and *Fzd2*, genes associated with regulation of fibrosis.[8]

**Conclusion** Mϕ-derived WNTs are not required for re-vascularisation or restoration of myocardial function after neonatal myocardial injury, but are necessary for scar removal during regeneration.

## REFERENCES


1 Porrello *et al*. *PNAS* 2013;**110**:p187–92.
2 Aurora *et al*. *JCI* 2014;**124**:p1382–92.
3 Lin *et al*. *PNAS* 2010;**107**:p4194–99.
4 Boulter *et al*. *Nat Med* 2012;**18**:572–9.
5 Saha *et al*. *Nat Com* 2016;**7**:1–16.
6 Derlindati *et al*. *PLoS ONE* 2015;**10**:1–17.
7 Irvine *et al*. *Fibrogenesis Tissue Repair* 2015;**8**:19–32.
8 Laeremans *et al*. *Cardiovascular Research* 2010;**87**:514–523.


## 11 TARGETING 11β-HSD1 TO PROMOTE ANGIOGENESIS – CONSEQUENCES FOR SOLID TUMOUR GROWTH


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11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), which generates active glucocorticoids from inactive precursors, is expressed in glucocorticoid target tissues, including the arterial wall. Since active glucocorticoids are anti-angiogenic 11β-HSD1 inhibitors enhance angiogenesis and may have therapeutic potential in ischaemia. However increased angiogenesis may be detrimental in tumours. This investigation tested the hypothesis that 11β-HSD1 inhibition promotes angiogenesis and tumour growth in mouse models of squamous cell carcinoma (SCC) and pancreatic ductal adenocarcinoma (PDA).

Murine tumour cells ($1 \times 10^6$) were injected subcutaneously into mice (female, 10–12 weeks, FVB/C57Bl6/J) receiving RM-1 diet with, or without (Control), the 11β-HSD1 inhibitor UE2316 (175 mg/kg). Tumour size was measured every 2–3 days for 2–3 weeks. Sections of tumours were stained for vascular markers (CD31, alpha-smooth muscle actin) for quantification of vessel density. Steroid/drug levels were measured in plasma/tissues using liquid chromatography tandem mass spectrometry whilst 11β-HSD1 activity was assayed in tissue homogenates by high performance liquid chromatography. The effects of 11β-HSD1 inhibition on angiogenesis were examined *ex vivo* using an aortic ring assay. Data are mean±SEM.

11β-HSD1 inhibition increased SCC tumour growth in FVB mice ($p<0.01$) but did not affect the growth of PDA tumours in C57Bl6/J mice. Vessel density was unaffected in both tumour types. SCC tumours expressed more 11β-HSD1 mRNA and had higher ($p<0.001$) enzyme activity (0.291 ±0.054 nmoles product/mg/min) than PDA tumours (0.038 ±0.006). FVB mice had higher plasma UE2316 levels than C57Bl6/J mice (164.6±78.28 nM vs 14.4±6.23 nM), and reduced circulating corticosterone after UE2316 treatment. UE2316 reduced type 1 collagen in SCC tumours (9.8±0.8 vs 3.8%±0.6% area, $p<0.001$). Pharmacological inhibition of 11β-HSD1 did not alter steroid-mediated angiostasis in aortic rings.

11β-HSD1 inhibition does not promote angiogenesis in SCC or PDA tumours, but may increase SCC growth through a mechanism involving reduced fibrosis. Whether 11β-HSD1 inhibition increases angiogenesis in glucocorticoid-sensitive tumours remains to be established.

## 12 PREVENTION OF CONTRAST-MEDIA INDUCED RENOVASCULAR TOXICITY USING LIPID NANOPARTICLE ENCAPSULATION


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**Introduction** The primary goal of conventional lipid nanoparticles (LiNPs) is to target and release drugs at the site of disease. In comparison, this research aims to minimise drug interactions by entrapping the nephrotoxic contrast agent, iohexol (IOX), within protective particles. Susceptible patients, such as those with diabetes, suffer from pre-existing vascular dysfunction which is exacerbated by IOX.[1] Mechanisms of IOX-associated toxicity are unknown, therefore, cell-based assays will be used to examine toxicity and whether it may be prevented through LiNP encapsulation.

**Method** Human umbilical vein endothelial cells (HUVEC), vascular (VSMC) and kidney cells (HEK-293) and were treated with IOX or IOX-encapsulated LiNPs (n=3). A metabolic indicator assay were used to determine metabolic activity after treatment proliferation and toxicity. Cell counting and a dye exclusion assay were used to confirm toxicity.

**Results** Resazurin experiments showed a significant reduction in metabolic activity after 2 hour IOX incubation resulting in a 54%, 15% and 52% reduction in HUVEC, VSMC and HEK-293 metabolic activity respectively. These results were confirmed through cell counting analysis. Encapsulation of IOX within LiNPs was found to prevent IOX-associated renal toxicity as metabolic activity was comparable to non-treated cells (129%) ($p<0.05$).

**Conclusions** Treatment of HEK-293 and VSMCs with IOX led to a reduction in viable cells and an increase in toxicity which was mainly influenced by concentration rather than time. Encapsulation of IOX within LiNPs was found to prevent toxicity *in vitro*. Future work will be carried out to determine mechanisms behind contrast agent-associated toxicity with a focus on *ex vivo* kidney perfusion and vascular contractile experiments.

## REFERENCE


1. Weisberg, *et al*. *Kidney Int* 1994;**45**:259–265.