2A WILLIAMS AND BURNAND PRIZE

O51 OPTIMIZATION OF CARTILAGE DECELLULARIZATION FOR EAR RECONSTRUCTION

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Introduction: Decellularization of ear cartilage can induce chondrogenic differentiation for ear reconstruction. However no defined protocol within the literature exists. We propose a novel method for optimizing the decellularization process to produce a scaffold for ear reconstruction.

Method: Three different enzymatic/detergent protocols were applied to human ear cartilage and characterization of the matrix was conducted for histology, scanning electron microscopy, deoxyribonucleic acid, collagen and glycosaminoglycan content. Protocol A consisted of a combination of deoxyribonuclease and sodium deoxycholate. Protocol B and C were adaptations of this employing trypsin and ethylenediaminetetraacetic acid respectively as additional steps. Characterization was conducted at day 0, 7, 14, 21, 28 and day 35.

Result: Protocol B with trypsin showed that it was able to accelerate the rate of decellularization. A significant reduction in deoxyribonucleic acid content after 14 days was achieved compared to native ($P<0.05$). Absence of cells was confirmed with immunofluorescence staining(4,6-diamidino-2-phenylindole) as well as with haematoxylin and eosin. Whilst effectively depleting the cell content, scanning electron microscopy demonstrated the trypsin protocol (B) to have preserved the three dimensional structure of the extracellular matrix. There was a significantly lower reduction of glycosaminoglycans at the end of protocol B when compared to protocol A at day 35. Application of analysis of variance($P>0.05$) showed no difference in collagen content between the different protocols.

Conclusion: Protocol B with trypsin can optimize decellularization of ear cartilage within 14 days. It offers great promise for future development of a native scaffold for stem cell seeding.

Take-home message:
Employing trypsin in protocol B can optimize the decellularization of ear cartilage to produce a suitable scaffold for stem cell seeding.

O52 FUNCTIONAL EPIGENETIC ANALYSIS OF THE OSTEOARTHRITIS SUSCEPTIBILITY AT THE SUPT3H-RUNX2 LOCUS

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Introduction: Genetic association studies have identified a male-specific osteoarthritis (OA) susceptibility locus at SUPT3H-RUNX2 marked by the rs10948172 polymorphism. Cartilage methylome analysis identified that rs10948172 genotype correlates with methylation levels at four CpG sites, defining it as a cartilage meQTL. Our aim is to investigate if this meQTL is present in blood, synovium and fat pad tissues, and to examine the functional effect of methylation on the meQTL region.

Method: Methylation analysis using bisulphite pyrosequencing was performed on 72 blood, 85 synovium and 79 fat pad samples from knee OA patients for six different CpG sites. SUPT3H and RUNX2 gene expression in synovium and fat pad was assessed using RT-PCR. The functional effect of the rs10948172 meQTL region was investigated using luciferase reporter assays of in vitro methylated and non-methylated plasmids.

Result: The meQTL was present in all six CpG sites assayed in synovium and fat pad (Jonckheere Terpstra trend test $p=<0.005$) in the same direction as cartilage, with the risk allele correlating with reduced methylation. In fat pad and blood there was reduced methylated levels in males. In synovium, SUPT3H correlates to rs10948172 genotype and CpG methylation. Methylation and risk genotype enhanced luciferase activity of the region.

Conclusio : The RUNX2/SUPT3H rs10948172 meQTL found in cartilage is present in synovium and fat pad. Reduced methylation of the region in males could contribute to the male specificity of the OA signal. Methylation influences the regulatory activity of the region. meQTL: methylation quantitative trait locus RT-PCR: real time polymerase chain reaction

Take-home message:
While several genes have been identified as osteoarthritis risk loci, studying the functional effect of these is needed for translational work. This study shows how SUPT3H-RUNX2 is modifying gene expression via the intermediary of differentially methylated regions.

O53 DEEP VEIN THROMBOSIS EXHIBITS CHARACTERISTIC SERUM AND VEIN WALL METABOLIC PHENOTYPES IN THE INFERIOR VENA CAVA LIGATION MOUSE MODEL

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Introduction: Deep vein thrombosis (DVT) is a highly prevalent clinical condition with high morbidity and mortality worldwide and represents profound economic burden on healthcare systems. The diagnosis of DVT relies heavily on ultrasound imaging to confirm the diagnosis but it is not always available. In addition, there is no single reliable biomarker that accurately rule in patients with DVT.

Method: We have used the well-established inferior vena cava ligation mouse model to perform untargeted metabolic profiling of serum and vein wall extracts using liquid-chromatography coupled

mass spectrometry and NMR spectroscopy.
Result: Using multivariate and univariate statistical analysis, we have shown that DVT mice have different metabolic profiles compared to controls, and extracted statistically significant metabolites that were responsible for defining the metabolic phenotype of the DVT mice. Most notably serum of DVT mice was characterised by decreased adenosine and tricyclic acid cycle (TCA) intermediates, increased carnitine and altered expression level of various classes of lipid moieties including sphingomyelins, phosphatidylcholines and triglycerides. Interestingly, a similar phenotype was found in vein wall. Subsequent Spearman's correlation analysis showed the biochemical relationships between assigned metabolites and helped to unravel potential mechanistic deregulations in DVT, revolving around energy metabolism, sphingolipid metabolism and adenosine metabolism.
Conclusion: In summary, our findings showed a perturbed metabolism in a DVT mouse model, which could improve our understanding of underlying metabolic alterations of DVT and eventually translate into clinical application to improve diagnosis of DVT.
Take-home message:
Metabolomic profiling of DVT animal model shows a perturbed metabolism that could improve our understanding of DVT pathology.

O54 THE MESENTERY - ANTAGONIST IN CROHN'S DISEASE?

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Introduction: Inclusion of the mesentery in surgical resections for colorectal cancer is standard practice and is associated with improved outcomes. It has yet to be evaluated in Crohn's disease.
Method: Surgical recurrence rates were compared between two cohorts; cohort A (n=32) underwent conventional ileocolic resection while cohort B (n=39) underwent resection where the mesentery was included. Mesenteric disease severity was quantitated using a novel disease activity index. The relationship between mesenteric disease and mucosal disease, systemic disease, i.e. circulating fibrocytes and behaviour of mesenteric derived fibroblasts was investigated.
Result: Cumulative reoperation rates were 27% and 2.5% in cohorts A and B, respectively ($p=0.021$). Fibrocytes were increased in patients from cohort B when compared to healthy controls, both within the mesentery and systemically (8.0 ± 5.64 vs. 2.6 ± 1.68%, $p=0.003$). Mesenteric disease severity increased as mucosal disease worsened ($r=0.76$, $p<0.0001$) and as Crohn's disease activity index ($r=0.70$, $p<0.001$) and circulating fibrocytes ($r=0.81$, $p<0.05$) increased. Fibroblasts which were derived from the Crohn's mesentery showed increased adhesion and proliferation when compared to a fibroblast cell line. The mesenteric derived fibroblasts from an ileocolic Crohn's disease patient showed the greatest increase in proliferation. Patients from which the fibroblasts were harvested were given the same mesenteric disease activity index.
Conclusion : Crohn's patients displayed improved outcomes when the mesentery was included in resections. Severity of mesenteric disease increased as mucosal and systemic manifestations of disease worsened. Mesenchymal cells derived from the Crohn's mesentery can adhere and proliferate faster than fibroblast cell lines.
Take-home message:
Inclusion of the mesentery in Crohn's disease resections improves outcomes in patients.

O55 DECEASED ORGAN DONORS WITH A HISTORY OF INCREASED RISK BEHAVIOUR FOR THE TRANSMISSION OF BLOOD BORNE VIRAL INFECTION: THE UK EXPERIENCE

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Introduction: Donors with a history of increased-risk behaviour (IRB) for viral disease transmission are often not used for transplantation. This study aimed to quantify the risk of using organs from IRB donors along with the consequences of discarding them.
Method: UK Transplant Registry data on recipients of transplants from deceased donors with a history of intravenous drug use, imprisonment, or high-risk sexual behaviour in the UK between 2003 and 2016 were reviewed. Kaplan-Meier tables and Cox proportional hazards were used to compare graft and patient survival

Result: 659 consented IRB donors were identified. Only 454 proceeded to organ donation, a significantly lower proportion than that of all other consented donors (p=0.04). This resulted in 1203 transplants. IRB donors were significantly younger compared to all non-IRB donors (median 39 years IQR (30-48) vs. 50 years IQR (38-61), p<0.001) and had significantly less medical comorbidity. 3 recipients unexpectedly developed HCV post transplantation from the same seronegative IRB donor. Patient and graft survival adjusted for donor and recipient age, cold ischaemic time and HLA mismatch were similar for recipients of kidneys from IRB donors and non-IRB donors (Hazard Ratio 1.075 (95% CI 0.815-1.417), p=0.610). Graft and patient survival was similar for liver and heart transplant recipients from IRB donors compared to non-IRB donors Discussion: Patient and graft survival was similar in patients receiving organs from IRB donors compared to non-IRB donors. Unexpected disease transmission was low. This data suggest that organs from IRB donors should be considered for transplantation with informed recipient consent.

Take-home message:

Organ donors with a history of increased behaviour for viral disease can be used safely for transplantation and should not be unnecessarily discarded, as patient and graft survival was good and disease transmission was low.

O56 NEUROPROTECTION IN TEVAR: CEREBRAL EMBOLIC PROTECTION TO SAFEGUARD THE BRAIN, A PILOT STUDY

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Introduction: Stroke occurs in 2-8% and silent cerebral infarction (SCI) in 70% of patients undergoing thoracic endovascular aortic repair (TEVAR). This study investigates the feasibility of the cerebral embolic filter protection device (CEPD) to protect the brain from neurological injury during TEVAR.

Method: Patients anatomically suitable underwent TEVAR with CEPD, together with intra-operative transcranial Doppler (TCD) of the middle cerebral arteries (MCA), pre- and post-operative diffusion weighted magnetic resonance imaging (DW-MRI), and pre, post and 6- week neurocognitive testing to detect sub-clinical neurological deficit.

Result: Ten patients, mean age 68yrs underwent TEVAR with CEPD. Proximal landing zones range was 1-3; atheroma grade of the aortic arch 1-4. CEPD was successfully deployed and retrieved in all cases. Arterial wall and thrombus was captured in all filters on histopathological analysis. TCD was performed and high intensity transient signals(HITS) were detected in 8 patients. Maximum HITs were detected during stent manipulation &deployment (median76: IQR61.5-153.5), contrast runs (median69:IQR44.5-107.5). CEPD manipulation(median78:IQR44-111). There were no post-operative strokes. Two patients had no new lesions on post-operative DW-MRI, 8 patients had new low volume SCI lesions; number lesions (range 1-5), mean total surface area was 54 mm2. There has been no neurocognitive decline postoperatively or at 6-weeks. In comparison, previous unit data showed in 31 patients undergoing TEVAR without CEPD, cerebral infarction was detected in 81% and 68% SCI. Mean lesion volume was 94mm2. There was an 88% neurocognitive decline in patients with SCIs.

Conclusion: This is the first study to report use of CEPD in TEVAR. It appears safe and feasible with encouraging early results.

Take-home message:

Rate of silent cerebral infarction and cognitive decline is high in TEVAR. Cerebral embolic protection in these early cases has shown a marked reduction.

O57 EX-VIVO NORMOTHERMIC KIDNEY PERFUSION ASSESSMENT AND TRANSPLANTATION OF DECLINED HUMAN KIDNEYS FROM DONATION AFTER CIRCULATORY DEATH DONORS

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Introduction: A significant proportion of donation after circulatory death (DCD) kidneys are declined for transplantation. Ex-vivo normothermic kidney perfusion (EVKP) can be used to assess the quality of a kidney and determine suitability for transplantation.

Method: Forty two declined human DCD kidneys underwent EVKP for 60 minutes with a red-cell based plasma-free solution at 36°C. During EVKP the quality of each kidney was assessed. Kidneys were graded from 1 (best) to 5 (worst). In a second clinical study, approval was granted to transplant declined human research kidneys after EVKP assessment. Seven kidneys from 5 DCD donors were recruited.

Result: In the series of 42 kidneys, 43% were declined due to poor in-situ perfusion, 17% technical/anatomical reasons, 19% donor age, 9% history, 9% hypothermic machine perfusion parameters and 2% histology. 28 of the kidneys (67%) had an EVKP quality score of 1 to 3 and considered suitable for transplantation. Five of the kidneys from the clinical study were declined due to poor in-situ perfusion and 2 donor age. Based on the EVKP score, 3 of these kidneys (43%) were successfully transplanted. Two kidneys were rejected due to a high Remmuzi score, 1 logistical reasons, and the other a kidney that had undergone normothermic re-circulation in the donor and had abnormal perfusion parameters during EVKP. Two of the transplanted kidneys had initial graft function and the other delayed graft function.

Conclusion EVKP technology can be used to increase the number of DCD kidney transplants by assessing their quality prior to transplantation.

Take-home message:

EVKP technology can be used to increase the number of DCD kidney transplants by assessing their quality prior to transplantation.

O58 THERAPEUTIC APPROACHES TO THE METABOLIC RELATIONSHIP BETWEEN PANCREATIC DUCTAL ADENOCARCINOMA AND PANCREATIC STELLATE CELLS THROUGH TARGETING OF LACTATE DEHYDROGENASE

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Introduction: Pancreatic stellate cells (PSCs) play a key metabolic role within the tumour microenvironment (stroma) of pancreatic ductal adenocarcinoma (PDAC), which is notoriously glycolytic and associated with pro-tumorigenic acidification from excess lactate. This study investigates therapeutic approaches to the PDAC-PSC metabolic relationship through targeting of the glycolytic enzyme lactate dehydrogenase (LDH), which consists of 2 isoforms (A and B).

Method: An in vitro transwell system was adopted for coculture of PSCs and 3 PDAC cell lines (Miapaca2, Panc1 and Bxpc3). Cells were treated with the novel LDHA&B inhibitor galloflavin, and outcomes analysed regarding proliferation, apoptosis, lactate production and lactate transporter (MCT4) expression. LDHB immunohistochemistry (IHC) staining was performed on 59 resected PDAC tumours.

Result: Galloflavin reduced proliferation in Miapaca2 and Panc1, however not Bxpc3 (KRAS mutant), in solo and co-culture ($p<0.05$), whilst a significant apoptotic effect was only seen in Miapaca2 and Bxpc3 coculture. An associated significant reduction in media lactate content (mimicking extra-cellular levels) was only in observed in co-culture with PSCs ($p<0.05$). Treatment of Miapaca2 and Panc1 significantly increased MCT4 expression ($p<0.01$). Whilst LHDA expression has previously been linked to poor prognosis, IHC revealed stromal and tumoral LDH-B expression had no impact on survival.

Conclusion: Therapeutic approaches to LDH specifically disrupt a PDAC-PSC relationship, and show potential for targeting the acidic PDAC microenvironment and thereby reducing tumour invasiveness and metastasis; however compensatory upregulation of MCT4 may reverse this effect in vivo. Specific inhibitors for LDHA may be more efficacious in clinical practice.

Take-home message:

Targeting lactate dehydrogenase in vitro reduces pancreatic ductal adenocarcinoma (PDAC) proliferation, decreases pro-tumorigenic acidification and disrupts a metabolic relationship between PDAC and pancreatic stellate cells, which has the potential to reduce tumour invasiveness and metastasis in vivo.