and we found that expression of the prostaglandin cyclooxygenase enzyme (Cox1) and prostaglandin regulator Pge2 was reduced in Emyr deficient embryos. Treatment with dnPGE2 or Cox1 overexpression was sufficient to rescue renal and cilia defects.

Conclusions: These data position Emyr as a novel link between ciliogenesis and nephropathies through regulation of prostaglandin signaling, and highlight Emyr as a potential new target for future ciliopathic treatments.

PO0643

Single-Cell Analysis of Senescent Epithelia Reveals Targetable Mechanisms Promoting Fibrosis

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Background: Progressive fibrosis and maladaptive organ repair result in significant morbidity and millions of premature deaths annually. Senescent cell accumulates with ageing and after injury and are implicated in organ fibrosis, but the mechanisms by which senescence influences repair are poorly understood. Here, we address the role of senescence in maladaptive repair and identify new anti-fibrotic targets.

Methods: We analyse human kidney tissue samples post deobstruction and corresponding murine models to test involvement of senescent cells in maladaptive repair via pharmacological depletion. We use single cell RNA-Seq to examine these cells in more detail. We validate our findings using in-vitro models of senescence and fibroblast activation. Finally we use murine models of injury to test inhibition of in silico targets as anti-fibrotic.

Results: We demonstrate for the first time in man that senescence and fibrosis persist in kidneys in the aftermath of a resolved obstructive injury. Using a relevant murine model of injury and repair we show senescent epithelia persist after relief of ureteric obstruction and that depletion of senescent epithelia reduces fibrosis and promotes repair. We next characterise senescent epithelia in murine renal repair using single cell RNA-Seq to examine these cells in more detail. We identify conserved pro-fibrotic molecules that we validate in vitro and in human disease. Inhibition of one of these molecules is essential for TGFβ mediated fibroblast activation. Importantly for translation, inhibition of this molecule in vivo significantly reduces kidney fibrosis after injury.

Conclusions: Our data shed light on the role of senescent epithelia in renal disease and identify a new anti-fibrotic molecule. Analysis of signaling pathways of senescent epithelia connects the important pathways such as the cell stress response to organ fibrosis, permitting rational design of anti-fibrotic therapies.

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PO0644

Treatment of Diabetic NOD/SCID Mice with Human “Neo-Islets,” 3D Organoids of Mesenchymal Stromal and Pancreatic Islet Cells, Normalizes Blood Glucose Levels: Significance for Clinical Trials

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Background: We reported that allogeneic “Neo-Islets” (NI) are immune protected and permanently correct autoimmune diabetes in NOD mice by omental engraftment and endocrine cell redifferentiation. This new “endocrine pancreas” delivers islet hormones physiologically into the hepatic portal vein. Further, treatment of insulin-dependent dogs with allogeneic canine NIs (ongoing FDA-approved Pilot Study) consistently improved glycemic control without the need for anti-rejection drugs. The current preclinical study was undertaken in anticipation of a Phase 1 Clinical Trial with two objectives: to determine (a) whether human NIs (hNIs) can also restore euglycemia, and (b) whether redosing of suboptimally controlled diabetic animals could restore euglycemia in streptozotocin (STZ)-diabetic NOD/SCID mice, as has been previously shown for mouse and dog cell-derived NIs.

Methods: Passaged cells that were to be used to treat diabetic NOD/SCID mice were characterized for gene expression profiles by rPCR. For in vivo testing, NOD/SCID mice were made diabetic with STZ, then randomized based on blood glucose levels into groups of 6 each, treated with insulin pellets, and once blood glucose levels were stabilized near normal animals were treated i.p. either with -2x10e5 human cell-derived NIs/kg bw (n=6) or vehicle (n=6), then followed for 8 weeks. Once blood glucose levels were determined to be no longer significantly improved compared to controls without administration of exogenous insulin, mice in each group were again treated with either 2x10e5 NIs/kg bw or vehicle, and followed for an additional 6 weeks. Therapeutic efficacy was assessed by survival, 2x weekly blood glucose monitoring, and glucose tolerance tests administered 57 and 41 days post the 1st and 2nd doses, respectively.

Results: Human NI therapy significantly improved glycemic control and survival vs. vehicle. A 2d dose given to the initial group normalized blood glucose levels long-term. Conclusion: Despite the limitations of the diabetic NOD/SCID model, these data show that human NIs are curative, and in conjunction with data from the dog study, where allogeneic NI therapy reduces the need for insulin without need for antirejection drugs, have high translational relevance and support the planned conduct of human NI clinical trials.

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PO0645

IL-33 as a Novel Target for the Treatment of Diabetic Kidney Disease

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Background: Diabetic kidney disease (DKD) has classically been thought as a microvascular disorder, although inflammation has emerged as a key pathophysiologic mechanism involved in the development of diabetic kidney injury. Consequently, inflammatory mediators have aroused as promising therapeutic targets.

Methods: IL-33 is a broad-acting cytokine, expressed in endothelial and epithelial barriers, that mediates local tissue inflammation. It exerts its function by binding to a heterodimer formed by its specific receptor ST2 and co-receptor IL-1RAcP. Due to the evidence of the role of IL-33 in kidney injury, we generated MEDI3506, a potent IL-33 blocking mAb for the treatment of DKD.

Results: Transcriptomic analysis showed that expression of IL-33 RNA is upregulated in both the glomeruli and tubulointerstitium of DKD patients in two independent cohorts. Assessment of expression in both human and experimental DKD demonstrated that IL-33 is among the most regulated inflammatory genes. Preliminary data on IL-33 protein levels in human kidney biopsies indicates that IL-33 is increased in DKD versus controls. Preclinically, the db/db uninephrectomy model of DKD showed IL-33 protein levels in kidney lysates positively correlated with histological glomerular damage from week 7 to 21. More importantly, blockade of ST2 signalling by using a mAb, prevented the progression of albuminuria. In vivo mechanistic studies using primary human glomerular endothelial cells (GECs) and mesangial cells (MCs) showed that both cell types expressed ST2 and upregulated IL-33 in response to TNFα and INFγ, commonly upregulated in diabetic kidney microenvironment. Moreover, GECs and to a lesser extent MCs, displayed a significant IL-33 induced proinflammatory cytokine release (e.g. IL-8, IL-6...) mediated by MAP kinase activation and NF-kB translocation. All these effects were inhibited by MEDI3506.

Conclusions: Upregulation of IL-33 in diabetic kidney, generates localised chronic kidney inflammation through autocrine signalling in GECs and MCs. This data suggest that targeting IL-33 with MEDI3506 arises as a promising therapeutic intervention for DKD, currently in Phase 2b trial.

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