Session 16: Induced pluripotent stem cells and organoids for disease modelling

Lectures

L.16.1

Towards better understanding of atypical diabetes using human pluripotent stem cell technology

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Genetic analysis of an adult patient with an unusual course of ketosis-prone diabetes (KPD) and lacking islet autoantibodies demonstrated a nucleotide variant in the 5'-untranslated region (UTR) of *PDX1*, a β-cell development gene. When differentiated to the pancreatic lineage, his induced pluripotent stem cells stalled at the definitive endoderm (DE) stage. Metabolomics analysis of the cells revealed that this was associated with leucine hypersensitivity during transition from the DE to the pancreatic progenitor (PP) stage, and RNA sequencing showed that defects in leucine-sensitive mTOR pathways contribute to the differentiation deficiency. CRISPR/Cas9 manipulation of the *PDX1* variant demonstrated that it is necessary and sufficient to confer leucine sensitivity and the differentiation block, likely due to disruption of binding of the transcriptional regulator NFY to the *PDX1* 5'-UTR, leading to decreased PDX1 expression at the early PP stage. Thus, the combination of an underlying defect in leucine catabolism characteristic of KPD with a functionally relevant heterozygous variant in a critical β-cell gene that confers increased leucine sensitivity and inhibits endocrine cell differentiation resulted in the phenotype of late-onset β-cell failure in this patient. We define the molecular pathogenesis of a diabetes syndrome and demonstrate the power of multiomics analysis of patient-specific stem cells for clinical discovery.

L.16.2

Human iPS cell-derived engineered skeletal muscles for advanced modelling of neuromuscular diseases and therapeutics

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Skeletal muscle is a complex tissue composed of multinucleated myofibres supported by multiple cell types, compromised in severe incurable neuromuscular diseases such as muscular dystrophies. Lack of robust, human(ised) models is a major barrier towards development of novel neuromuscular therapies. To tackle this challenge, we developed 3D human models of skeletal muscle tissue, enabling high-fidelity modelling of different morphological and functional defects in tissue compartments impaired in muscular dystrophies. 3D muscles were developed from induced pluripotent stem cells (iPSCs) differentiated into myogenic, neural and vascular progenitor cells, and then combined within 3D biomaterials to generate aligned myofibre scaffolds containing vascular networks and motoneurons. Engineered muscles recapitulated morphological and functional features of human skeletal muscle, providing a high-fidelity platform to study muscle pathology, such as emergence of dysmorphic nuclei in muscular dystrophies caused by mutant lamins. To further validate this approach in another muscle disease with abnormal nuclear dynamics, we developed a 3D model of X-linked centronuclear myopathy, identifying promising morphological and functional readouts for therapeutic development. Finally, I will discuss current extensions of this technology to test next generation genetic therapies, laying the foundations for a multi-functional platform for precision medicine in neuromuscular diseases.

L.16.3

Induced pluripotent stem cells and gene editing for modelling and experimental therapies of Duchenne muscular dystrophy cardiomyopathy

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Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene, encoding dystrophin. DMD patients suffer from progressive muscle damage and concomitant development of dilated cardiomyopathy, which is currently the major cause of patients' mortality. In our studies we use patient-derived induced pluripotent stem cells (iPSC) and CRISPR/Cas9 gene editing, to model the cardiac DMD pathology and test experimental therapies (*Martyniak et al. J Mol Cell Cardiol. 2021 28;160:128-141.*)
Our recent studies demonstrated impaired iron metabolism in iPSC-derived DMD cardiomyocytes, and the reversal of this effect by CRISPR/Ca9 correction of the *DMD* mutation or pharmacologic treatment (*Andrysiak et al., in revision*). Moreover, in another approach we investigated the significance of utrophin, the dystrophin paralogue in functioning of DMD cardiomyocytes. Obtained results indicated that disturbance of calcium handling in DMD hiPSC-CM was more pronounced in cells additionally lacking utrophin. Reversely, utrophin upregulation by CRISPR/deadCas9-VP64 approach improved calcium oscillations in DMD cardiomyocytes (*Andrysiak et al, in preparation*).
In sum, abnormalities in iron homeostasis may explain some features of DMD-associated cardiomyopathy and could be amenable for therapeutic interventions. Moreover, utrophin appears important in the maintenance of the electrophysiological properties of DMD hiPSC-CM.

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L.16.4

Human iPSC for modelling endothelial cell dysfunction in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD), an X-linked recessive disorder caused by mutations in the dystrophin-encoding gene, *DMD,* is manifested by progressive degeneration of the skeletal and cardiac muscles. Dystrophin was suggested to be present in endothelial cells (ECs), and alterations in the angiogenesis process may have a significant impact on the severity of DMD.
We found reduced expression of angiogenic genes in ECs isolated from lungs of dystrophic *mdx* mice compared to wild-type animals. Then we used human induced pluripotent stem cells (hiPSCs) and CRISPR/Cas9 technology to establish the control and the isogenic cell line with deleted exon 50 of the *DMD* gene (ΔEx.50 *DMD*). Functional endothelial cells (hiPSC-ECs) were differentiated from control cells, as indicated by the expression of angiogenic factors and properties. In contrast, cells with excised exon 50 of the *DMD* gene exhibited decreased expression of endothelial markers and impaired angiogenic potential, lower motility, and differences in cytoskeleton architecture. The level of profibrotic factors and ROS generation was increased, while antioxidant enzymes were negatively regulated in ΔEx.50 *DMD* hiPSC-ECs.
In conclusion, we have shown altered properties of ECs lacking dystrophin. Our results indicate that hiPSC-ECs comprise a valuable tool to understand the angiogenesis-related changes in the pathology of DMD.

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Posters

P.16.1

Efficient CRISPR/Cas9-mediated correction of *DMD* exon deletion in Becker muscular dystrophy patient-derived induced pluripotent stem cells

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Becker muscular dystrophy (BMD) is an X-linked recessive disorder caused by in-frame mutations in the *DMD* gene encoding dystrophin. Induced pluripotent stem cells (iPSCs) are a novel tool for BMD modeling. We aimed to generate patient-specific BMD iPSCs expressing shortened dystrophin and relevant isogenic lines with repaired *DMD* exon deletions by CRISPR/Cas9 gene editing. BMD iPSCs with deletion of exons 3 to 9, and 45 to 47 were generated from peripheral blood mononuclear cells of two patients using Sendai vectors delivering reprogramming Oct4, Sox2, Klf4, and c-Myc genes. Following the introduction of a plasmid encoding repair template and differentiation of the cells into cardiomyocytes (iPSC-CM), RT-PCR and Western Blot analysis were performed to confirm the knock-in of missing exons. Successful generation of BMD iPSC lines was first confirmed by demonstrating the expression of pluripotency markers, the ability to differentiate into the cells of three germ layers as well as the lack of *DMD* exons. The Surveyor nuclease assay selected the most efficient of the two designed sgRNAs and the corresponding plasmid together with the repair template were introduced into the iPSCs. Obtained cells were differentiated into iPSC-CM after clonal selection and analysis. The mutation repair was finally confirmed, demonstrating the presence of full-length dystrophin protein in the cells.

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