PRESENTER ABSTRACTS

A phase I/IIA trial to test safety and feasibility of an autologous iPS cell-derived retinal pigment epithelium patch in age-related macular degeneration patients

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Induced pluripotent stem cells (iPSCs) can provide autologous and allogeneic replacement tissues, potentially for all degenerative diseases. Autologous tissues have the advantage of not requiring immune-suppressive drugs that have deleterious side-effects. The safety and feasibility of autologous iPSC-based therapies hasn't been established.

Here, we developed an autologous iPSC-based therapy for age-related macular degeneration (AMD), a blinding eye disease that affects over 30 million people world-wide. AMD is caused by the progressive degeneration of retinal pigment epithelium (RPE), a monolayer tissue that maintains photoreceptor function and survival. We tissue engineered a clinical-grade iPSC-RPE-patch on a biodegradable scaffold using autologous cells from AMD patients. Preclinical investigational new drug (IND)-enabling studies performed on iRPE-patch derived from multiple AMD patients demonstrated reproducible manufacturing, validating our manufacturing process - a key requirement for an autologous phase I trial. Functional validation of clinical-grade iRPE-patches allowed determination of tissue barrier resistance, purity of RPE cells, and RPE cell shape metrics as key critical quality attributes now used as clinical product release criteria. Preclinical animal studies performed in immune-compromised rats confirmed safety of the auto-iRPE-patch and efficacy studies performed in a porcine laser-induced RPE injury model that mimics AMD-like eye conditions demonstrated integration and functionality of RPE patches. A phase I/IIa IND-application for an auto-iRPE-patch to treat AMD was recently cleared by the FDA. This Phase I/IIa clinical trial will test safety, feasibility, and integration of an auto-iRPE-patch in twelve advanced AMD patients. The trial is currently being run as a single site study at the NIH and targets patient enrollment in two cohorts: visual acuity 20/100 and above for the first cohort, and 20/80 and above. One patient was successfully transplanted in August 2022; we plan to transplant additional patients this year.

Reconciling clinical observations with retinal organoid disease phenotypes
David Gamm, M.D., Ph.D.

Developmentally, human retinal organoids (ROs) can maximally achieve a late fetal stage of maturation over the course of 200 days in vitro. While ROs can be maintained in culture for many months thereafter, most diseases that we attempt to model with ROs – even those that are the most severe and early in onset – take many years to manifest an observable phenotype in human patients. We can bypass this temporal conundrum by focusing on measurable, disease-relevant perturbations in subcellular biochemical pathways that are presumably present even before birth. However, on occasion, a true outwardly apparent RO phenotype is observed much earlier than predicted by clinical observations. We examined this phenomenon in ROs harboring mutations in interphotoreceptor matrix proteoglycan 2 (IMPG2), which cause a rare form of early onset autosomal recessive retinitis pigmentosa (RP).

IMPG2 is a chondroitin sulfate proteoglycan expressed by photoreceptors and secreted into the interphotoreceptor matrix (IPM), which envelops their light-sensing outer segments. However, the mechanism of IMPG2-associated retinal degeneration in humans remains unclear, and existing mouse models of IMPG2-RP do not recapitulate the disease severity. We characterized ROs generated from patient-specific iPSCs and gene-edited ESCs and differentiated the ROs to stage 3 (>200 days of differentiation), which is characterized by the light microscopic appearance of photoreceptor outer segments. ROs harboring IMPG2 mutations failed to display outer segments or an IPM, in stark contrast to isogenic, age-matched controls. Further investigation revealed that mutations in IMPG2 resulted in a lack of post-translational modifications. We hypothesized that IMPG2 mutations cause destabilization of the IPM which, combined with exposure to physical forces inherent to the tissue culture environment, truly accelerates outer segment loss relative to what is observed in patients. To test this hypothesis, IMPG2 mutant and control ROs were transplanted into the subretinal space of immunocompromised rats, whereupon all grafts produced elongated outer segments. Beyond providing a highly robust, developmentally accelerated system to study IMPG2-based RP, this human RO disease model can be used to address challenges common to photoreceptor-targeted gene and genome-based therapeutics regardless of the causative mutation.

Modeling neurodegeneration with iPSCs: challenges and opportunities
Li Gan, Ph.D.

A human stem-cell model of neural tube folding morphogenesis
Eyal Karzbrun, Ph.D.

Understanding human organ formation is a scientific challenge with strong medical implications. Three-dimensional stem cell cultures have provided insights into human cell differentiation. However, current approaches use scaffold-free stem-cell aggregates, which develop unreproducible tissue shapes and variable cell-fate patterns. This limits their capacity to recapitulate organ formation. Here, we present a chip-based culture system that enables the self-organization of micropatterned stem cells into precise three-dimensional cell-fate patterns and organ shapes. We use this system to recreate neural tube folding from human stem cells in a dish. Upon neural induction, the neural ectoderm folds into a millimeter-long neural tube covered with non-neural ectoderm. Folding occurs

at 90% fidelity and anatomically resembles the developing human neural tube. We find that the neural and non-neural ectoderm are necessary and sufficient for folding morphogenesis. We identify two mechanisms that drive folding: 1) apical contraction of neural ectoderm, and 2) basal adhesion mediated via extracellular matrix synthesis by non-neural ectoderm. Targeting these two mechanisms using drugs leads to neural tube defects. Finally, we develop a mechanical model which captures neural-tube morphology under all experimental conditions, further highlighting the role of tissue mechanics in organ morphogenesis. Our approach provides a new path to study human organ morphogenesis in health and disease.

Parkinson Cell Atlas: Through a Spatio-Temporal Metaverse to Precision Medicine
Clemens Scherzer, M.D.

Molecular and cellular evolution of the primate dorsolateral prefrontal cortex
Andre Sousa, Ph.D.

The granular dorsolateral prefrontal cortex (dlPFC), an evolutionary specialization found only in anthropoid primates, lies at the center of high-order cognition and complex social behaviors, which are highly derived traits in primates, especially in humans. Alterations in the molecular and cellular mechanisms underlying its intricate circuitry have been implicated in myriad neuropsychiatric diseases. However, little is known about the full repertoire of cell types in the primate dlPFC and how conserved these cell types are between human and other primate species.

We have generated single-nucleus transcriptome data profiling more than 600,000 nuclei from the dlPFC of adult humans, chimpanzees, rhesus macaques, and common marmosets, thus spanning major primate phylogenetic groups. Although most cell types were conserved across the four species, we uncovered prominent species differences both at the molecular and cellular levels.

Within homologous cell types across species, we identified prominent molecular changes, including the loss of expression of tyrosine hydroxylase (TH), the rate- limiting enzyme in catecholamine (including dopamine) biosynthesis, in the inhibitory neurons of chimpanzees that are homologous to TH-expressing inhibitory neurons in the other species studied. Among TH-expressing homologous cell subtypes in humans, macaques, and marmosets, we identified a human-specific posttranscriptional switch between the neuropeptide SST and TH, and the human-specific expression of genes involved in dopaminergic function. Using primary cultures of neural progenitors from the ganglionic eminences and neurons differentiated from induced pluripotent stem cells, we were able to delineate the developmental origins of human TH-expressing interneurons and to demonstrate that a subpopulation of these neurons can produce, transport, and release dopamine.

Our integrated analysis revealed diverse molecular and cellular features of the phylogenetic reorganization of the primate dorsolateral prefrontal cortex across multiple levels, with relevance for brain function and disease.

iPS cell-based therapy for Parkinson's disease

Jun Takahashi, M.D., Ph.D.

Human induced pluripotent stem cells (iPSCs) can provide a promising source of midbrain dopaminergic (DA) neurons for cell replacement therapy for Parkinson's disease (PD). Toward the clinical application of iPSCs, we have developed a method for 1) scalable DA neuron induction on human laminin fragments and 2) sorting DA progenitor cells using a floor plate marker, CORIN. The grafted CORIN+ cells survived well, functioned as midbrain DA neurons in the 6-OHDA-lesioned rats, and showed a minimal risk of tumor formation. In addition, we performed a preclinical study using primate PD models. Regarding efficacy, human iPSC-derived DA progenitor cells survived and functioned as midbrain DA neurons in MPTP-treated monkeys. Regarding safety, cells sorted by CORIN did not form any tumors in the brains for at least two years. Moreover, we found that MRI and PET imaging was useful in monitoring the survival, expansion, and function of the grafted cells as well as the immune response by the host brain.

Based on these results, we started a clinical trial to treat PD patients at Kyoto University Hospital in Kyoto, Japan, in 2018. The trial evaluates the safety and efficacy of transplanting human iPS cell-derived DA progenitors into PD patients' putamen. Using a stereotaxic surgical technique, we implant approximately 5 or 10 million cells into the bilateral putamen of the patients. The target is seven patients, and we will observe each of them for two years. The trial is now ongoing without any severe adverse events. I will summarize these processes and discuss future perspectives.

Human brain organoids to study neural development and disease

Momoko Watanabe, Ph.D.

The human brain has many structural and functional features that are distinct from lower species traditionally used for medical research. To identify mechanisms of human brain development and find cures for human-specific neurological disorders such as autism, we ideally need a human brain model. However, experimentation with human brain tissue, particularly at fetal stages, is inherently challenging, curtailing long-term gene manipulation studies and environmental perturbations. Cerebral organoids generated from human pluripotent stem cells (hPSC) are thus emerging as a promising alternative system for studying human neocortical development and disease. We established reproducible and efficient methods for cortical organoid differentiation that faithfully recapitulate in vivo neocortical development. Neurons within the organoids are functional and exhibit network-like activities. We further demonstrate the utility of the organoid system for modeling neurodevelopmental disorders. Finally, we started employing tissue engineering technologies to improve the established telencephalic organoids. Together, these findings provide an essential foundation for the utilization of human brain organoids to study human neural development and disease.