In Focus

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1. Understanding Fragile X Syndrome Using Patient-derived Stem Cell Models

Fragile X Syndrome (FXS) is caused by an expansion of CGG trinucleotide repeats in the 5' untranslated region of the Fragile X mental retardation 1 (FMR1) gene on the X chromosome. Affected individuals possess over 200 copies of the CGG repeat, resulting in hypermethylation of the FMR1 promoter, which leads to epigenetic silencing of the gene and FMRP protein deficiency. Children with FXS display intellectual disabilities ranging from mild to severe, and are often diagnosed with autism. Intriguingly, the number of trinucleotide repeats can vary between and within tissues of the same individual, a phenomenon known as somatic repeat instability (SRI). To elucidate the molecular mechanisms involved in hypermethylation of CGG repeats, and to explore the consequences of SRI, Hagar Mor-Shaked (Jerusalem, Israel) and colleagues have developed human FXS stem cell models. Analysis of FXS-affected human embryonic stem cell (HESC) lines showed that hypermethylation is tightly linked to FMR1 transcriptional inactivation, suggesting that this epigenetic event occurs during early embryo development. Using a separate in vitro model, data from induced pluripotent stem cells (iPSCs) derived from FXS patient fibroblasts (FXS-iPSCs) was presented. These can be differentiated into clinically-relevant cell types, such as neurons, while harboring the mutations that cause the disease. Previously, exploring the neurological features of FXS in humans relied on using difficult-to-obtain postmortem brain tissue. Neurons derived from FXS-iPSCs open up the possibility of understanding the pathogenic mechanisms in more detail and potentially screening drugs to reset epigenetic modifications and correct the neurological phenotype.

2. Genetic Dissection of MYO15A in Human Hereditary Deafness

Hereditary deafness is the most common inherited sensory disorder, affecting 1 in every 1000 births. Approximately two-thirds of congenital deafness is non-syndromic (without associated disorders) and can involve mutations in an array of genes that encode proteins involved in transducing sound into nerve signals perceived by the brain. In humans, MYO15A encodes the unconventional myosin 15A, a myosin with a C-terminal tail that allows it to move in a viscous environment. MYO15A contains an N-terminal domain that is conserved in all vertebrates, and a C-terminal domain that is unique to MYO15A. The exact function of MYO15A is not yet known, but it is thought to be involved in the formation of stereocilia, which are hair-like structures on the sensory hair cells of the inner ear.

Using a mouse model of congenital deafness, researchers have identified two different isoforms of MYO15A: isoform I and isoform II. Isoform I is expressed in the tips of shorter stereocilia, whereas isoform II is expressed in the taller rows of stereocilia. This differential expression pattern is thought to contribute to the development of normal stereocilia architecture. However, as in the human situation, these mice are profoundly deaf suggesting there is a subsequent pathology. Detailed analysis demonstrated that the two isoforms show differential patterns of expression within the stereocilia, with isoform I localizing to the tips of shorter stereocilia, whereas isoform II localizes to the middle of the stereocilia. This suggests that isoform I is required for the proper development of stereocilia, while isoform II is required for maintenance of the stereocilia.

3. Genetics of Pain Sensitivity

Pain is a distressing feeling often caused by unpleasant stimulation resulting in tissue damage, and is the most common reason for general practitioner consultations in the developed world. Geoff Woods (Cambridge, UK) presented the latest developments in the study of Mendelian disorders of pain sensitivity. These disorders result from a mutation at a single genetic locus that is inherited in families in a particular pattern. An individual might display insensitivity to pain, or conversely increased pain perception, depending on the consequence of the causative mutation. The aim is to attribute mutations to specific disorders by sequencing the DNA of affected families, enabling insight into the molecular mechanisms that lead to the disease phenotype. Many acquired pain syndromes occur through mutations in ion channels that are expressed on nociceptors. Nociceptors are sensory neurons that detect stimuli that have the potential to cause tissue injury such as extremes of temperature, chemical irritants and mechanical damage. The exact function of a given nociceptor will depend on the type and distribution of ion channels that are expressed, and this heterogeneity enables an appropriate response to a range of sensory inputs. For example, voltage-gated sodium channels (Nav) allow extracellular Na⁺ to pass into the interior of the neuron, which can result in an action potential. In humans, mutations in the Na⁺ channel encoded by SCN9A, can cause congenital insensitivity to pain if a null mutation prevents the passage of Na⁺ into the cell. Conversely, if an activating mutation is present, this...
results in Na1.7 opening more easily allowing too many Na+ to pass into the cell. This can result in disorders such as inherited erythromelalgia (IEM), causing chronic pain in the hands and feet. Recent developments in drugs that selectively block Na1.7 can elicit a reversal of the pain phenotype in humans with IEM. It is hoped that subtype-specific blockade of other sodium channels might prove an effective approach to treat a range of pain perception disorders.

4. Consequences of PPA2 Mutations

In a session dedicated to metabolic and mitochondrial disorders, two talks focused on disorders associated with mutations in the mitochondrial inorganic pyrophosphatase (PPA2). PPA2 is an enzyme that catalyzes the hydrolysis of inorganic pyrophosphate (PPI) to two phosphate ions, resulting in the liberation of 19 kJ. This reaction is coupled to many energetically unfavourable reactions to drive them to completion, for example in the metabolism of lipids. Mitochondria produce the majority of the energy required by a cell, and consequently contain ~90% of cellular PPI. Therefore mutations that affect the function of this enzyme can cause huge problems, especially in tissues that have high energy requirements such as the heart. Kit Doudney (Christchurch, New Zealand) presented data on four families with a spectrum of cardiomyopathy disorders. Next-generation sequencing technologies identified mutations in the PPA2 gene that were responsible for a number of neonatal cardiac-related medical conditions including lactic acidosis and heart failure. In one family with acute sensitivity to alcohol, two brothers died at 15 and 20 years of age. Sequencing results revealed that both siblings had inherited compound mutations, one in the catalytic site and the other affecting a residue potentially involved in the dimerization of PPA2. Functional studies in E. coli demonstrated that this combination of mutations caused PPA2 to work inefficiently, with catalytic activity reduced by ~70%. However, this reduction is less than the 95% activity loss observed with purely catalytic mutations present in the majority of affected families. The small increase in PPA2 activity associated with the dimerizing proline228 mutation may explain the later age of onset associated with cardiac failure in this family. The surviving siblings also inherited these homoplasmic sequence variants in PPA2, and were subsequently fitted with artificial pacemakers to counter cardiac arrhythmias. Anne Guimier (Paris, France), then presented data on two families with a recurrent sudden unexpected death in infancy (SUDI). SUDI is the most common case of post-natal infant mortality in developed countries, although the underlying cause is largely unexplained. All babies from these families died of cardiac arrest at 4–20 months. When post-mortem tissue was analyzed by whole exome sequencing, compound heterozygous missense variations in the PPA2 gene were identified. Both studies reveal new links with PPA2 and human disorders, which may have implications for undiagnosed individuals with mutations in this gene.

5. CRISPR Cas9 Tips and Tricks

Dario Lupianez and Malte Spielmann (Berlin, Germany) held an engaging workshop titled CRISPR Cas9 tips and tricks. The aim was to demonstrate how this genome editing technology can be utilized in embryonic stem cells (ESCs) to produce genomic structural variations (SVs) in mice within ten weeks. This process would typically take a year using conventional targeting technologies, thus offering a fast alternative. SVs include deletions, inversions and duplications of genomic regions, allowing one to functionally assess gene and enhancer regions and create mouse models of human disease efficiently and with relative ease. This was exemplified by creating mouse mutants in which the EphA4 locus was manipulated to produce multiple pathogenic variations in the limb. Genomic deletions in regulatory regions resulted in brachydactyly, inversions resulted in F-syndrome (syndactly) and duplications produced polydactyly in mice. In all cases, the mouse phenotypes recapitulated those of rare limb malformations in humans, enabling the in vivo and in vitro dissection of genetic variants seen in a clinical setting. This workshop comprised an interactive demonstration of single guide RNA (sgRNA) design, with tips on how best to maximize on-target activity with the targeting sequence while minimizing off-target activity. The pitfalls of this technology were also highlighted, including the extensive variability in targeting efficiency from one locus to another and increasing the proportion of homology-directed repair (HDR), over error-prone nonhomologous end joining (NHEJ), during precise genome-editing. Strategies to increase HDR relative to NHEJ were discussed and included the use of chemical inhibitors to target the NHEJ pathway to favor HDR. As CRISPR Cas9 technology is refined further, modeling genomic rearrangements to understand human disease will become increasingly powerful.

6. Antisense Therapy to Treat Spinal Muscular Atrophy

Richard Finkel (Florida, USA) reported on results from a Phase II clinical trial in which antisense therapy had been used to treat infantile-onset spinal muscular atrophy (SMA). SMA is caused by a genetic defect in the SMN1 gene, leading to reduced levels of SMN, a protein required for the survival of motor neurons. This results in a variety of symptoms comprising progressive muscle weakness and feeding and breathing failure in the most severe cases. SMA is the most common genetic cause of infant death. Although SMA patients are unable to generate fully functioning SMN protein from SMN1, they all retain at least one copy of the SMN2 gene. Due to a single nucleotide mutation, the majority of SMN2 transcripts are alternatively spliced producing a truncated protein that is rapidly degraded. However, a small amount of full-length protein is produced, allowing some neurons to survive and function to some extent. One therapeutic strategy is to find splicing modulators that can promote the inclusion of Exon 7 in SMN2, usually excluded during splicing, to produce more of the fully functional SMN protein. In theory, this approach would increase the total amount of functional SMN, and thereby preserve a greater proportion of motor neurons. To test this hypothesis, companies have used a high throughput approach to test splicing modulators that are able to achieve such a result, producing functional benefit in preclinical models. In an open label Phase II study sponsored by Ionis Pharmaceuticals, 20 pre-symptomatic newborns were treated with intrathecal administration of an antisense oligonucleotide (ASO) called Nusinersen (NCT01839656). Three babies died following enrollment. However, the remaining infants continuing the trial are now >2 years old, many on partial or no ventilation support, and display unprecedented improvement in motor milestones and function. These encouraging results have informed a Phase III trial in infants and children with SMA.