Genetic causes and stem-cell-based therapeutic strategies in neuromuscular diseases

Chapter 7

Impact Paragraph

Neuromuscular disease is a large group of genetic disorders with complex symptoms and few treatment options, which places a heavy burden on society and health care. The work presented in this thesis will contribute immediately to improve and accelerate genetic diagnosis and will pave the way for future gene/stem cell therapy in these patients with the ultimate goal of precision diagnosis and personalized treatment.

WES in genetic diagnosis

Conventional genetic diagnosis of a patient in the past largely relied on sequential Sanger sequencing of known candidate genes inferred from the patient's phenotype, which is time-consuming, expensive and inefficient. On the contrary, WES enables massive parallel sequencing of the complete exome of a patient. The sequencing-by-synthesis platform used in this thesis, Illumina Hiseq 2000, is capable of efficiently sequencing up to 100 human exomes in a single run. WES was reported to solve 50% of a patient cohort with mitochondrial or mitochondrial associated disease, whereas the solving rate reduced to about 10% by Sanger sequencing in a heterogeneous patient cohort (1, 2). Moreover, with new sequencing platforms emerging, the average time and costs has rapidly declined over the last decade, making these ideal for identifying genetic defects in clinical diagnostics of heterogeneous mitochondrial disease (3). Next-generation sequencing has greatly accelerated the discovery of novel pathogenic genes in neuromuscular disease and facilitated the establishment of genotype-phenotype relationships (4). In 2013 alone, more than 180 novel disease-causing genes had been identified by WES with a broad variety of clinical manifestations (5). This can be exemplified by the identification for the first time of two heterozygous pathogenic mutations in an OXPHOS RNA homeostasis regulator *SLIRP* applying WES and functional validation. *SLIRP* turned out to be a novel gene, involved in mitochondrial encephalomyopathy and OXPHOS complex I and IV deficiency (chapter 2).

Variants detected by WES data should be analyzed with a comprehensive and structured approach to maximize the likelihood of discovering pathogenic variants. When no candidate gene is detected, matching the criteria and genetic model, a second-round examination should be performed to identify genes, in which for technical reasons or due to cut-off criteria variants have been missed (20-30% of all variants). As is shown in chapter 2, the initial WES data filtering only discovered one deletion variant in *SLIRP*. The second deep-intronic splicing variant was found by

reanalyzing the WES data in more detail based on the functional role of *SLIRP* in OXPHOS. Subsequent RNA analysis of *SLIRP* revealed an additional transcript in the patient compared to the control, thus making *SLIRP* the best candidate. Therefore, it is important to combine other approaches, like for example RNASeq as a general strategy, to prioritize the candidate list when analyzing WES data.

During WES data analysis, there might still remain a large number of variants after an initial step of allele frequencies screening in several public population databases (e.g., gnome, 1000G, EVS). Fortunately, a large variety of bioinformatic tools can be used to reduce and prioritize the candidate variants, such as cloud-based GENESIS and co-expression-based WeGET. In chapter 3, we applied these two *in silico* tools in analyzing WES data from a patient having progressive paralysis of the extraocular muscles and multiple deletions in mtDNA, and identified a homozygous deletion in *C1QBP*, encoding complement component 1 Q subcomponent-binding protein involved in mitochondrial homeostasis. GENESIS provided a comprehensive pathogenicity prediction score calculated from a set of prediction tools, which circumvented the conflicting results from each individual tool and made the prediction more reliable. WeGET evaluated the query gene set's co-expression within approximately 1000 multi-tissue datasets and ranked the variants by the co-expression level, which assessed the WES data from the co-expression perspective and complemented the other pathogenicity-based prediction methods.

Our data demonstrates that WES is a powerful tool to identify pathogenic mutations in neuromuscular disease, which not only has research values of understanding genetic causes and underlying pathophysiological mechanisms, but also helps clinical geneticists and physicians acquire accurate molecular diagnosis of patients, thus offering better health care to the patients and preventing disease transmission by prenatal and preimplantation genetic diagnosis.

Gene/stem cell therapy in neuromuscular disease

In recent years, a number of gene/stem cell therapies advanced to human clinical trial stage, some of them had even been approved and entered into the market (6). As described in this thesis, CRISPR-based gene editing and stem cells like mesoangioblasts can be developed into potential treatments for treating the dystrophic muscles in neuromuscular disease like myotonic dystrophy type 1 (DM1). In chapter 5, we designed and tested a novel *ex vivo* CRISPR/Cas9 editing approach in autologous mesoangioblasts from DM1 patients. The CRISPR/Cas9 component

is delivered in the form of ribonucleoprotein by electroporation, which can reduce the risk of off-target events and immune response. The approach turned out to be specific and efficient in skipping the transcription of CTG expansion with limited effect on the viability of the mesoangioblast. The underlying methodology is also suitable to correct the genetic defect in mesoangioblasts from patients with other neuromuscular diseases. As all methods and tools have been established in compliance with GMP, we expect to transfer this correction strategy towards clinical trials. In the future, once the safety and efficacy of CRISPR-edited mesoangioblasts is fully established, production of therapeutic mesoangioblasts can be further commercialized into a cell-based medicinal product, providing the possibility of a spin-off company with pharmaceutical and economic value.

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