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VRIJE UNIVERSITEIT BRUSSEL

PhD in Medical Sciences
2018-2019

INVITATION to the Public defence of

Sumitava DASTIDAR

To obtain the academic degree of **'DOCTOR IN MEDICAL SCIENCES'**

Genome Editing for Myotonic Dystrophy Type 1.

Thursday 21 May 2019
Auditorium Piet Brouwer, 17:00
Faculty of Medicine and Pharmacy, Laarbeeklaan 103, 1090 Brussel

How to reach the campus :
http://www.vub.ac.be/english/infoabout/campuses

Summary of the dissertation

Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disorder with a prevalence ranging from 0.5 to 18.1 per 100,000 individuals. Typically, DM1 patients suffer from progressive myopathy and myotonia, cardiac conduction defects and cognitive impairments. DM1 is caused by the presence of expanded trinucleotide *CTG* repeats (CTG_{exp}) in the 3'*UTR* of *DMPK* gene on ch19q13.3. Expression of the *DMPK* gene with mutated 3'UTR gives rise to an expanded *CUG* repeat RNA transcript (CUG_{exp}), which is pathogenic to cells and is the primary factor in DM1 pathogenesis. For the treatment of DM1 patients there are numerous therapeutic strategies that have been proposed, but very few have been effective. Therefore, we intend to develop a novel therapeutic approach to correct the disease mutation by targeting the pathogenic mutated locus at the DNA level. In our study, we derived induced pluripotent stem cells from DM1 patients (DM1-iPSCs) and differentiated them into myogenic cells (DM1-iPSC-Myo). This represents an invaluable *in vitro* DM1 myogenic model mimicking the aberrant DM1 alternate splicing and biomarkers. Thereafter, we developed a dual guide RNA (gRNA) based CRISPR/Cas9 correction approach to excise the expanded *CTG* repeats and validated our correction approach in our DM1 myogenic model. The CRISPR/Cas9 correction approach yielded a relatively robust correction efficiency of up to 52% in DM1-iPSC-Myo obviating the need for any selection step, consistent with the disappearance of ribonuclear foci, reversal in alternate splicing of *SERCA* and redistribution of MBNL1 proteins. In anticipation of future clinical translation, we subsequently used a non-viral, non-DNA based "hit and go" approach to correct the DM1-iPSCs using CRISPR/Cas9 ribonucleoprotein (RNP) complexes, obtaining up to 90% efficiency of correction. The repeat excision was further confirmed by TP-PCR, Southern blot analysis and ribonuclear foci analysis of individual iPSC clones. Additionally, we evaluated the alternate splicing of the corrected DM1-iPSCs post cardiomyogenic differentiation, which showed a reversal in the splicing pattern in the corrected cells as compared to their non-corrected counterpart. Altogether, the proof of concept study demonstrates that CRISPR/Cas9 can mediate relatively efficient correction of the pathogenic DM1 mutation, which represents an essential step towards development of novel and clinically relevant therapeutic strategies for DM1 disease. Additionally, the *in vitro* DM1 myogenic and cardiomyogenic disease models generated in the current study provide invaluable tools for DM1 research for developing novel therapies and to further study the pathophysiological mechanisms of DM1.

Curriculum Vitae

Sumitava Dastidar was born on December 28th, 1986 in India. During his graduation in India, he obtained a Bachelor degree in Biotechnology followed by a Master degree in Stem Cell Biology & Regenerative Medicine in 2009 with highest distinction. Then, he was awarded a competitive Pre-doctoral fellowship in Biomedical Sciences at the Stem Cell Institute Leuven (SCIL) at KU Leuven for a period of 1 year. After successfully defending his pre-doctoral thesis, in 2011 he started his PhD research at the Department of Gene Therapy & Regenerative Medicine, Vrije Universiteit Brussel, under the supervision of Prof. Dr. Marinee K.L. Chuah and Prof. Dr. Thierry VandenDriessche. His research focused on the development & validation of novel genome editing technologies in order to correct DM1 disease mutation *in vitro*. Additionally he has worked on developing myogenic disease model for DM1 disease, which would provide invaluable tools for DM1 research for developing other novel therapies and to further study the pathophysiological mechanisms of DM1. He was also engaged in several other projects beyond his immediate PhD thesis subject, the research outcomes from which have been validated in several publications. He had stayed abroad for collaborative projects at ISTEM, (France) and University College London, (UK) during his PhD, which has fostered his scientific interactions. The scientific output from his research includes 6 publications in international peer-reviewed journals, 1 book chapter and 2 papers currently in revision. In addition, his first author paper from *NAR* (IF = 11.56) was selected for cover art of the journal issue. His work was also selected for an oral presentation and posters at international conferences at ESGCT-Berlin, ESGCT-Lausanne and ISSCR-Boston respectively.