

Cellular studies of neuromuscular disorders related to the sarcomeric proteins

Akademisk avhandling

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av

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The thesis is based on the following papers:

- I. Homa Tajsharghi, Trond P Leren, **Saba Abdul-Hussein**, Mar Tulinius, Leif Brunvand, Hilde M Dahl, Anders Oldfors. Unexpected myopathy associated with a mutation in *MYBPC3* and misplacement of the cardiac myosin binding protein C. *J Med Genet* 2010;47:575-577
- II. **Saba Abdul-Hussein**, Peter F.M. van der Ven, Homa Tajsharghi. Expression profiles of muscle disease-associated genes and their isoforms during differentiation of cultured human skeletal muscle cells. Submitted
- III. **Saba Abdul-Hussein**, Karin Rahl, Ali-Reza Moslemi, Homa Tajsharghi. Phenotypes of myopathy-related beta-tropomyosin mutants in human and mouse tissue cultures. Submitted
- IV. Montse Olivé, **Saba Abdul-Hussein**, Anders Oldfors, Dieter O. Fürst, Peter F. M. van der Ven, José Gonzalez-Costello, Laura Gonzalez-Mera, Benjamin Torrejón-Escribano, Josefina Alió, Adolf Pou, Isidro Ferrer, Homa Tajsharghi. MuRF1 and MuRF3 mutations cause a new protein aggregate myopathy and cardiomyopathy. Manuscript



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Sarcomere is the basic unit of cardiac and skeletal muscle contraction and its proper function requires an invariant organization of this structure. Mutations in sarcomeric proteins are known to cause increasing number of different cardiac and skeletal muscle diseases. The front line in research on muscle diseases is at present to define the genetic background and pathogenesis of these diseases. The potential for development of effective therapies depends on elucidation of the molecular and cellular impact of the mutations on morphological abnormalities and muscle weakness that accompany pathogenesis.

In paper I we identified an unexpected skeletal muscle myopathy in an infant with fatal cardiomyopathy due to a homozygous mutation in *MyBPC3*. The ectopic expression of cardiac MyBPC was restricted to abnormal type 1 muscle fibres, indicating that the muscle pathology was caused by a dominant-negative effect of mutant *MyBPC3*.

In paper II we addressed the expression profile of a panel of sarcomeric components during myogenesis, with a focus on proteins associated with a group of congenital disorders. The analyses were performed in cultured human skeletal muscle myoblasts and myotubes. We identified early expression of certain isoforms involved in congenital diseases, suggesting the possibility of an early role for these proteins as constituent of the developing contractile apparatus during myofibrillogenesis.

In paper III we used human tissue-culture cells as a model to investigate the primary trigger for β -tropomyosin-related myopathies and the basis for the histological changes seen in muscle biopsies of patients. Protein localization and pathobiology caused by dominant *TPM2* mutations were investigated by transfecting human myoblasts and C2C12 with WT and mutant EGFP-fusion β -TM constructs. Abnormal aggregation of β -TM variants and their localization within the thin filaments was observed in myoblasts and differentiated myotubes. We demonstrated that histopathological phenotypes associated with β -TM mutants might be accounted for the variable response to the cellular environment influenced by physiological context, in combination with the time course of expression of mutant protein rather than the alteration of amino acid itself. Our results confirmed that cell cultures of human skeletal muscle are an appropriate tool and environment closer to the reality in human skeletal muscle and more reliably mimic the disease conditions.

In paper IV we identified and characterized a new human protein aggregate myopathy and cardiomyopathy associated with combined mutations in isogenes *TRIM63* and *TRIM54*, encoding muscle specific ring finger proteins, MuRF1 and MuRF3, respectively. Our morphological and cellular investigation suggested that the disease is caused through impaired organization of the microtubule network and sarcomeric M-band proteins.

The results from this study have deepened the understanding of pathogenesis of a group of sarcomeric myopathies, which is an essential step towards identifying new therapeutic targets.

Key words: Myogenesis, myoblast, sarcomeric myopathy, *TPM2*, *TRIM54*, *TRIM63*

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