Name: Mitra Forouhan
Email: mitra_fourouhan@yahoo.com

Title of thesis: The role of ATF6α and ATF6β in the UPR associated with an ER-stress induced skeletal chondrodysplasia.

Home Institute: Isfahan Genetic Center
Number 5, Sheikh Mofid St,
Sheikh Sadoogh Shomali St,
Isfahan,
Iran

Host institute: The University of Manchester,
Oxford Rd,
Manchester
M13 9PL

Abstract:

Mutations in the *COL10A1* gene cause metaphyseal chondrodysplasia type Schmid (MCDS) by triggering ER stress and unfolded protein response (UPR). MCDS is characterised by a mild short-limb dwarfism accompanied by expansion of the cartilage growth plate hypertrophic zone (HZ) and altered differentiation of hypertrophic chondrocytes (HCs). ATF6 is one of the UPR mediators, which exists in two isoforms, ATF6α and ATF6β. Activation and up-regulation of ATF6α was a prominent biochemical sign of ER stress in a mouse model of MCDS, *COL10a1* p.N617K. Although ATF6β is induced and activated in response to ER stress in a similar fashion to ATF6α, the role and significance of ATF6β in the pathology of many ER stress-associated diseases including MCDS is unknown. Here we utilized a combination of *in vitro* and *in vivo* approaches to define the precise role of each isoform of ATF6 in MCDS.

To investigate the functions of ATF6α and ATF6β *in vitro*, we developed a MCDS cell model system (expressing either the wild type collagen X or one of the following MCDS-causing mutant forms of the protein: p.N617K, G618V, Y598D, and NC1del10) in which the expression of either ATF6α or ATF6β was efficiently silenced using siRNAs. ATF6α knockdown in HeLa cells expressing different MCDS-causing mutations suppressed the increased expression of UPR-associated genes such as BiP leading to an elevated ER stress, based on increased XBP1 splicing and/or ATF4 protein. In contrast, ATF6β knockdown did not significantly affect the mutant collagen X-induced increased expression of UPR-associated genes. Furthermore, the ER stress levels were significantly reduced in the ATF6β knockdown MCDS mutant cells based on the lower levels of XBP1 splicing and/or ATF4 protein detected.

We then crossed the ATF6α/β knockout mice models with *COL10a1* p.N617K mouse model of MCDS to investigate the function of ATF6α and ATF6β *in vivo*. Ablation of ATF6α in MCDS mice further- reduced the endochondral bone growth rate, further expanded the growth plate hypertrophic zone, and disrupted differentiation of HCs. Therefore, ATF6α appeared to play a chondroprotective role in MCDS as its deficiency caused an increase in the severity of the disease. Of particular note, the level of ER stress was further increased in the absence of ATF6α in MCDS, based on enhanced activities of PERK and IRE1

signalling pathways in compensation for the ATF6α loss. Paradoxically, ablation of ATF6β in MCDS mice reduced the intracellular retention of collagen X protein, and alleviated the ER stress as judged by the attenuated activities of PERK and IRE1 signalling pathways. The reduced ER stress resulting from deficiency for ATF6β in MCDS mice restored the expression of collagen X mRNA towards normal and improved the differentiation of HCs, causing a mark decrease in the expansion of HZ.

The results presented within this thesis greatly increased our understanding of the function of ATF6α and ATF6β and their interplay in the pathogenesis of MCDS. We demonstrated an indispensable beneficiary role for ATF6α but a detrimental role for its closely related isoform, ATF6β, in pathology of MCDS. We also showed that the role of ATF6β should not be ignored. These findings may be used to develop a potential therapeutic strategy for MCDS through targeting and enhancing ATF6α-dependent and/or attenuating/blocking of ATF6β-dependent signalling pathways.