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ADP-dependent Glucokinase – a novel regulator of ER-mediated glucose handling

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The present thesis established 2 new model systems to further elucidate the role of ADP-dependent glucokinase in eukaryotic metabolism, particularly in T- cellular immunity. Alleviated contact hypersensitivity reaction in ADPGK- *knockout* mice showed for the fist time that ADPGK exhibits *in vivo* relevance in adaptive immunity.

The bulk of experiments, however, was conducted using 4 different clones of Jurkat T Cells exhibiting ADPGK-*knockout* generated via CRISPR/Cas9. These Jurkat cells showed an intriguing and complex phenotype, exhibiting induced cell death, especially upon activation, diminished Actin ring formation indicative of impaired immunological synapse formation, a tendency to accumulate in S- phase of cell cycle, deranged activation-induced Warburg metabolism as well as reduced GlcNAc-levels and disturbed N-glycosylation. Validation experiments in ADPGK-*overexpressing* Jurkat cells showed protection against cell death and accelerated Actin ring formation. Major metabolic changes upon ADPGK-depletion could be summarized as an “Anti-Warburg-like phenotype” and included reduced glucose uptake, lack of lactic acid increase upon activation, reduced glycolytic activity, reduced respiratory chain activity under base- line, but not stimulatory conditions and a drop of AMP- and NADPH-levels, concomitant with a rise of malonyl-CoA. This failure to provide the appropriate metabolic changes upon activation to support cell proliferation could very well be the reason why ADPGK-depleted cells exhibited a higher rate of cell death and has previously been titled “metabolic catastrophe”. Fittingly, later analyses of cell death and associated pathways rather revealed that several compensatory pro-survival pathways are being activated in ADPGK-*knockout* cells, such as ER-stress, unfolded protein response and chaperone-mediated autophagy, but ultimately fail to maintain homeostasis and survival. The observed cell death was later verified to be a form of apoptosis as opposed to other forms of cell death as it was inhibitable by pan-caspase-inhibitor zVAD.

Aberrant GlcNAc-formation and N-glycosylation in ADPGK-*knockout* cells as well as electron-microscopy-based immunostainings providing evidence for ER- luminal localization of ADPGK suggest a regulative role of ADPGK in glucose handling within the Endoplasmic Reticulum. The hypothesis emerged that ADPGK could function as a ‘phosphate trap’ to retain glucose-6-phosphate with- in the ER for posttranslational modifications such as glycosylation. Silencing of ADPGK would therefore cause glucose-starvation within the ER and affect numerous intracellular pathways via perturbing glycosylation. This could possibly explain the complex phenotypical changes upon ADPGK-depletion including induced cell death, diminished Actin ring formation and the observed metabolic derangement, which cannot be sufficiently explained by the loss of ADPGK’s comparatively small glucose-phosphorylating capacity. Future experiments will have to reveal glycosylation patterns of key proteins involved in the respective pathways in correlation to ADPGK-expression and develop a way to dynamically measure glucose- and glucose-6-phosphate-levels within the ER to verify the presented hypothesis.

Taken together, this study provides experimental evidence that ADPGK’s relevance is certainly not limited to archaea, but exhibits an intriguing role in T- cellular activation by

regulating glucose handling and glycosylation within the ER. It furthermore proves *in vivo* relevance for the first time.