

TECHNICAL ABSTRACT

Type 2 diabetes mellitus (T2DM) affects 28 million people in the United States and more than 80 million are at a high risk to develop T2DM¹. Hypomagnesemia contributes the risk of T2DM and is a common problem affecting up to 30% of T2DM patients^{2-8,10}. Urinary Mg²⁺ wasting occurs more frequently in T2DM but the cause is unclear¹⁰. While lower serum Mg²⁺ levels double the risk for T2DM, oral Mg²⁺ therapy improves diabetic control³³⁻³⁵. However, gastrointestinal side effects from oral Mg²⁺ therapy are common, cause non-adherence, and therefore mandate new approaches to address hypomagnesemia. The kidney is the primary organ for regulating systemic Mg²⁺ homeostasis, and urinary Mg²⁺ wasting contributes to hypomagnesemia⁸³. In the kidney, the apical Mg²⁺ channel TRPM6 determines the final urinary Mg²⁺ concentration³⁸. However, TRPM6 regulation is not well understood. Our research showed that urinary proteins Mucin-1 (MUC1) and Uromodulin (UMOD) upregulate TRPM6 from the luminal side of the tubule. In *Umod*^{-/-} mice, we found renal Mg²⁺ wasting and, in contrast to wild-type (WT) animals, impaired glucose tolerance when fed a low Mg²⁺ diet. Moreover, we identified insulin receptor substrate 4 (IRS4) as an interaction partner of TRPM6 which is required for TRPM6 stimulation by insulin. The rationale of this project is to study the combined effects of (i) MUC1 and UMOD, and (ii) IRS4 on TRPM6 channels and the risk for T2DM if this regulation is dysfunctional.

Our hypothesis is that MUC1 and UMOD secretion into the tubular lumen increase TRPM6 channel current density in the distal nephron from the luminal side by impairing TRPM6 endocytosis, thereby enhancing tubular Mg²⁺ reabsorption. As urinary secretion of MUC1 and UMOD is reduced with specific *MUC1* or *UMOD* single nucleotide polymorphisms (SNPs) in humans, carriers of these SNPs are at risk for urinary Mg²⁺ wasting, hypomagnesemia, and T2DM^{48,49}. Such individuals may be incapable to compensate for low Mg²⁺ states by enhancing TRPM6 cell surface abundance by secreting more UMOD or MUC1 in a compensatory fashion. Therefore, low urinary UMOD and MUC1 concentration may represent novel risk factors for T2DM. In addition, we postulate that IRS4 also stimulates tubular Mg²⁺ absorption by TRPM6 activation and that the TRPM6-IRS4 interaction affects glucose metabolism by linking Mg²⁺ and insulin signaling.

We will test our hypothesis with these three aims: First, we will examine how MUC1 regulates TRPM6 applying patch-clamp recording and protein biochemistry. We will determine if MUC1 enhances TRPM6 channel activity *in vitro* by impairing TRPM6 channel endocytosis, increases channel forward trafficking, amplifies channel expression, or enhances single channel conductance/open probability. We will study if TRPM6 N-glycosylation is required, and if MUC1 and UMOD physically interact to regulate TRPM6. In the second aim we will study *in vivo* TRPM6 regulation by MUC1 and UMOD and their effect on renal Mg²⁺ and systemic glucose homeostasis applying whole animal physiology in *Muc1*^{-/-}, *Umod*^{-/-}, combined *Muc1*^{-/-}/*Umod*^{-/-}, *Trpm6*^{+/-}, and WT mice. To study the effect of Mg²⁺ deficiency and T2DM, we will challenge these murine models with a low Mg²⁺/high caloric diet. We will then study if Mg²⁺ supplementation improves glucose homeostasis in our mouse models when fed a high Mg²⁺/high caloric diet. Animals will be tested with hyperinsulinemic-euglycemic clamp studies. In a third aim we will test for the effect of IRS4 on TRPM6 channels by confirming the TRPM6-IRS protein interaction and testing *Irs4*^{-/-} mice for renal Mg²⁺ and glucose homeostasis.

Our experiments have relevance to the FY18 PRMRP Topic Area of Diabetes by providing innovative insight into the role of (i) urinary proteins MUC1 and UMOD, and (ii) IRS4 on renal Mg²⁺ homeostasis, TRPM6 regulation, and the systemic risk of T2DM due to hypomagnesemia. Long-term impact of these experiments will be the identification of urinary Mg²⁺ wasting and its causes as a T2DM risk. This will affect guidelines regarding blood and urinary Mg²⁺ monitoring and Mg²⁺ therapy in hypomagnesemic T2DM patients. We expect that early Mg²⁺ therapy in this cohort will prevent or ameliorate the complications of T2DM. Mg²⁺ therapy is readily available, FDA approved, and could be instantly used for therapy. For patients who do not tolerate oral Mg²⁺ therapy our studies may provide novel treatment targets such as specific MUC1 or UMOD domains as future therapeutic options to enhance TRPM6 activity and improve T2DM outcome. The short-term impact includes the identification of renal Mg²⁺ wasting, hypomagnesemia and low urinary MUC1 and UMOD concentrations as new, sensitive biomarkers for early detection of T2DM at-risk individuals to initiate Mg²⁺ therapy pre-emptively. Variable urinary secretion of MUC1 and UMOD may also explain the heterogeneity in T2DM causing renal Mg²⁺ wasting. This represents a new mechanism for urinary Mg²⁺ wasting and T2DM.