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 T2DM[1]. Hypomagnesemia contributes the risk of T2DM and is a common problem affecting up to 30% of T2DM patients[2-8,10]. Urinary Mg^{2+} wasting occurs more frequently in T2DM but the cause is unclear[10]. While lower serum Mg^{2+} levels double the risk for T2DM, oral Mg^{2+} therapy improves diabetic control[33-35]. However, gastrointestinal side effects from oral Mg^{2+} therapy are common, cause non-adherence, and therefore mandate new approaches to address hypomagnesemia. The kidney is the primary organ for regulating systemic Mg^{2+} homeostasis, and urinary Mg^{2+} wasting contributes to hypomagnesemia[83]. In the kidney, the apical Mg^{2+} channel TRPM6 determines the final urinary Mg^{2+} concentration[38]. 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 Mg^{2+} wasting and, in contrast to wild-type (WT) animals, impaired glucose tolerance when fed a low Mg^{2+} 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 Mg^{2+} 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 Mg^{2+} wasting, hypomagnesemia, and T2DM[48,49]. Such individuals may be incapable to compensate for low Mg^{2+} 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 Mg^{2+} absorption by TRPM6 activation and that the TRPM6-IRS4 interaction affects glucose metabolism by linking Mg^{2+} 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 Mg^{2+} and systemic glucose homeostasis applying whole animal physiology in *Muc1*$^{-/-}$, *Umod*$^{-/-}$, combined *Muc1*$^{-/-}$*/Umod*$^{-/-}$, *Trpm6*$^{+/-}$, and WT mice. To study the effect of Mg^{2+} deficiency and T2DM, we will challenge these murine models with a low Mg^{2+}/high caloric diet. We will then study if Mg^{2+} supplementation improves glucose homeostasis in our mouse models when fed a high Mg^{2+}/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 Mg^{2+} 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 Mg^{2+} homeostasis, TRPM6 regulation, and the systemic risk of T2DM due to hypomagnesemia. ***Long-term impact*** of these experiments will be the identification of urinary Mg^{2+} wasting and it's causes as a T2DM risk. This will affect guidelines regarding blood and urinary Mg^{2+} monitoring and Mg^{2+} therapy in hypomagnesemic T2DM patients. We expect that early Mg^{2+} therapy in this cohort will prevent or ameliorate the complications of T2DM. Mg^{2+} therapy is readily available, FDA approved, and could be instantly used for therapy. For patients who do not tolerate oral Mg^{2+} 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 Mg^{2+} wasting, hypomagnesemia and low urinary MUC1 and UMOD concentrations as new, sensitive biomarkers for early detection of T2DM at-risk individuals to initiate Mg^{2+} therapy pre-emptively. Variable urinary secretion of MUC1 and UMOD may also explain the heterogeneity in T2DM causing renal Mg^{2+} wasting. This represents a new mechanism for urinary Mg^{2+} wasting and T2DM.