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AMPK Activation Attenuates Albumin-induced Alterations in Renal Tubular Cells In Vitro

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Background

Chronic kidney disease (CKD) is characterized by progressive decline in renal function; if left untreated, it ultimately results in end-stage renal disease (ESRD), a condition that demands either dialysis or kidney transplant for survival. CKD and ESRD are associated with a multitude of complications ranging from increased hospitalization to accelerated cardiovascular events and mortality. Currently, type-2 diabetes and hypertension are the two major risk factors for CKD. With the increasing incidence and prevalence of these conditions globally, the patient population with CKD is expanding worldwide. According to local sources, CKD affects about 13% of Qatar's population, and the prevalence of ESRD, the advanced phase of CKD, in Qatar was found to be 212 per million patients. The increased risk of complications associated with CKD in conjunction with its high prevalence in Qatar and in the rest of the world, necessitates its prevention and management as a high national and international priority. Elevated urinary albumin excretion (commonly referred to as proteinuria) is not only a hallmark of renal disease, but also strongly associated with the development and progression of CKD. Albuminuria is thought to induce endoplasmic reticulum (ER) stress, consequently triggering AKT pathway and resulting in inhibition of AMP-activated kinase (AMPK). AMPK, a fuel sensor present in cells, is primarily involved in the regulation of fatty acid oxidation and ATP synthesis. Inactivation of AMPK was found to trigger mTOR (mammalian target of rapamycin) pathway, and subsequently inhibit autophagy (a defense mechanism) and induce epithelial-to-mesenchymal transition (EMT). These signaling changes eventually accelerate renal cell apoptosis, and manifest into CKD. Thus, the objectives of this study are: 1) to standardize and characterize an in vitro model of albumin-induced renal cell injury using normal rat kidney proximal tubular (NRK-5E) cells, and 2) to explore the effect of AMPK activation on ER stress, AKT, mTOR, EMT, autophagy and apoptosis that are thought to mediate renal cell injury during proteinuria using the developed in vitro model of albumin-induced renal cell injury.

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Methods

NRK-52E cells were grown to 60% confluency and then serum-starved for 24 hours to arrest cell proliferation. Cells were then exposed to albumin, at concentrations ranging from 1 to 30 mg/ml, for 24 to 72 hours. At specific endpoints, cells were assessed for induction of ER stress and alterations in the status of AKT, AMPK, mTOR and autophagy and changes in cellular senescence via x-galactosidase (an enzyme that is expressed in senescent cells) staining. Following standardization of albumin-induced renal cell injury model, studies were performed in the presence and absence of AMPK activator metformin (1 mM) for 24 to 72 hours. Cells were then assessed for alterations in the status of AMPK, AKT and mTOR, and the markers of ER stress, EMT, autophagy and apoptosis.

Results

Exposure to albumin for 72 hours caused a dose-dependent increase in cellular senescence in NRK-52E cells. In contrast, cells exposed to albumin for 24 and 48 hours did not reveal any marked changes in cellular senescence. A 4-fold induction in ER stress marker CHOP and the EMT marker a-SMA was noted. Moreover, higher concentrations of albumin, particularly 30 mg/ml, caused severe induction of ER stress and EMT, marked by 20-fold increase in CHOP and 6-fold increase in a-SMA respectively. Similarly, the phosphorylation of AKT and P70S6K (a downstream target of mTOR) was increased by more than 1.5-fold in cells subjected to albumin treatment. In addition, albumin treatment caused a dose-dependent reduction in AMPK phosphorylation and about 66% decrease in the expression of autophagy marker LC3-II. The above changes were observed in conjunction with prominent dose-dependent induction of apoptotic markers - caspase-3 and caspase-12 ranging between 1.5 to 3.5-fold and 3 to 5-fold respectively in cells exposed to albumin. In contrast, metformin co-treatment restored the levels of phosphorylated AMPK, and suppressed activation of AKT and P70S6K in NRK-52E cells exposed to albumin. Notably, metformin also prevented albumin-induced EMT; this was marked by a 50% decrease in a-SMA and a 60% increase in E-cadherin expression. In addition, 2.5-fold increase in LC3-II expression was noted. Intriguingly, the pro-apoptotic protein CHOP was induced following treatment with metformin; nonetheless, the expression of apoptotic markers caspase-12 and caspase-3 was reduced by 80% and 70% respectively, indicating that metformin protected the cells against albumin-induced apoptosis.

Conclusion

Albumin treatment induces ER stress, and activates AKT, EMT and apoptosis, with concomitant decreases in autophagy and inactivation of AMPK in renal tubular cells. Activation of AMPK via metformin treatment suppresses AKT and mTOR activation, and prevents EMT and apoptosis, but increases autophagy and ER stress in renal tubular cells. Further studies are required to understand the mechanisms by which metformin differentially modulates ER stress and apoptosis in renal cells under proteinuria. Together, our findings suggest that AMPK activation via metformin could serve as a potential therapeutic strategy to prevent and/or treat the development of CKD in patients with established proteinuria.