Effects of High Glucose on IGF-I–mediated Growth Signaling and Metabolic Responses in Renal Cell Carcinoma

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ABSTRACT

Louisiana has the highest annual incidence of renal cancer for both men and women in the United States. Additionally, the state has the second highest mortality from type-2 diabetes in the nation, with a steadily increasing prevalence of 10.3% for the adult population as of 2010. Previous studies have shown that metabolic factors commonly seen in type 2 diabetic patients, including obesity, hypertension and hyperglycemia, are associated with an increased risk of renal cancer, and type 2 diabetes has been independently associated with an increased risk of renal cell cancer in women. However, studies disagree over the degree of excess risk involved, and the reason for an increased risk of renal cell cancer (RCC) associated with type-2 diabetes is not well understood. This study aims to shed light on the relationship between these two diseases that both greatly impact the health of the Louisiana population.

Specifically, this study examines the extent to which elevated glucose affects growth, survival, signaling and metabolism of renal cancer, using the murine renal cancer model (Renca). Our results demonstrate that serum stimulated monolayer growth of the Renca cells is almost 2-fold higher when cells are maintained in the presence of high glucose (40mM) in comparison to normal glucose conditions (5mM). This increase in cell number was caused by a shift from G1 to S/G2/M phase of the cell cycle, rather than by an increase in cell survival. In agreement with these growth-promoting effects of high glucose, Renca cells also demonstrated a metabolic shift from mitochondrial respiration to glycolysis and demonstrated slightly higher glycolytic capacity in the presence of high glucose.

To further investigate which growth-promoting pathway is affected by the high glucose in Renca cells, we first evaluated the insulin-like growth factor 1 (IGF-I) signal transduction pathway. We have selected this pathway since previous reports indicate that kidney tumors are often characterized by dysregulated metabolic signaling between insulin, IGF-I and glucose. Moreover, elevated glucose content is frequently observed in clear cell carcinoma of the kidney. Insulin or IGF-I stimulate the phosphorylation of the insulin receptor substrate IRS-1. Phosphorylation of IRS-1 is important for cell survival, proliferation and glucose metabolism, perhaps by facilitating cell cycle progression and inhibiting apoptosis. In agreement with these growth-promoting effects of high glucose, IRS-1 was constitutively phosphorylated on tyrosine in Renca cell cultures exposed to normal glucose (5mM). However, in contrast to IRS-1, the level of IRS-2 did not change in response to high glucose.

In vitro experiments demonstrated that in serum starved Renca cells, IRS-1 is constitutively phosphorylated on tyrosine residues. Next, we evaluated the effects of high glucose concentrations on IGF-I induced signaling responses. Interestingly, two major signaling branches, which originate from the activated IGF-1 receptor (IGF-IR) i.e. Akt and MAP kinases, Erk1/2 were highly activated when stimulation with IGF-I was administered in the presence of 40mM glucose in comparison to 5mM glucose.

IN CONCLUSION

Our results indicate growth-promoting properties of high glucose in Renca cells, which may depend on hyper-activation of the IGF-IR signaling. Further experiments are required to verify if indeed a positive correlation exists between high-incidence of renal cell carcinoma and elevated blood glucose levels.

1.1. Respiration to Glycolysis and glucose metabolism in the presence of high glucose

Effects of different glucose concentrations and IGF-I on mitochondrial mass and function in Renca cells. A) Mitochondrial inner transmembrane potential (A) was evaluated by a cationic dye JC-1, which indicates mitochondrial membrane potential. Fluorescence dependent upon mitochondrial membrane depolarization. Renca cells were cultured in serum free media in the presence of NG, HG. In addition to the effect of glucose, we assessed the protective role of IGF-I. Following, 4 days of serum starvation in the presence of NG and HG cells were exposed to IGF-I for 1 additional 72 hours. After the total incubation time cells were harvested by trypsinization, lysed with 1% triton x-100 and immediately analyzed by Guava EasyCyte flow cytometer using MicroPotential software. The cell population in the first quadrant represents healthy unaffected cells with polarized mitochondria (purple population); the second and third quadrants comprise cells with compromised and lost mitochondrial membrane potential respectively. In all groups serum starvation significantly decreased mitochondrial potential, which was for NG conditions 47.1% and HG 40.19%, the addition of IGF-I showed improvements of 26%; consequently, the effect of IGF-I treatment was significantly greater (2.6 fold) on cells maintained in high glucose than normal glucose.

- Figure 3: Effect of different glucose concentrations and IGF-I on mitochondrial mass and function in Renca cells. A) Mitochondrial inner transmembrane potential (A) was evaluated by a cationic dye JC-1, which indicates mitochondrial membrane potential. Fluorescence dependent upon mitochondrial membrane depolarization. Renca cells were cultured in serum free media in the presence of NG, HG. In addition to the effect of glucose, we assessed the protective role of IGF-I. Following, 4 days of serum starvation in the presence of NG and HG cells were exposed to IGF-I for 1 additional 72 hours. After the total incubation time cells were harvested by trypsinization, lysed with 1% triton x-100 and immediately analyzed by Guava EasyCyte flow cytometer using MicroPotential software. The cell population in the first quadrant represents healthy unaffected cells with polarized mitochondria (purple population); the second and third quadrants comprise cells with compromised and lost mitochondrial membrane potential respectively. In all groups serum starvation significantly decreased mitochondrial potential, which was for NG conditions 47.1% and HG 40.19%, the addition of IGF-I showed improvements of 26%; consequently, the effect of IGF-I treatment was significantly greater (2.6 fold) on cells maintained in high glucose than normal glucose.

THE EFFECTS OF DIFFERENT GLUCOSE CONCENTRATIONS ON PROLIFERATION (A) AND CELL CYCLE DISTRIBUTION (B) OF RENCA CELLS.

Effects of High Glucose on IGF-I in Renca cell morphology (A) and survival in suspension cultures (B). Contrast phase images show morphology of exponentially growing monolayer cultures of Renca cells cultured with high glucose concentration (5mM and 40mM) and images were taken using camera equipped Olympus microscope (original magnification 10x). No morphological differences were observed between Renca cells exposed to normal (5mM) and high (40mM) glucose concentrations. (B) Cell viability of Renca cells growing in suspension on Poly-riboflavin plates were evaluated by Trypan Blue staining based on membrane integrity also for the use with the GuavaEasyCyte flow cytometer (Milipore). The Guava/VideoCyte Software were used for data analysis and quantification according to the manufacturer recommendations (Milipore). We expected increase of survival in the presence of IGF-I and HG based on the growth advantage observed in Figure 1. However, the percent of viable cells was similar across all conditions, indicating that elevated glucose and IGF-I do not promote increased survival. Histogram represent data from 3 experiment of ViCyte assay.

- Figure 4: Immunohistochemical evaluation of insulin receptor substrates p IRS-1 (51, 46 kDa) and pGSK3 (40 kDa) in normal and renal kidney clinical samples. A) Immunohistochemical evaluation of IRS-1 in normal kidney cortex and renal cancer tissue sections. B) IRS-1 expression in normal and kidney cancer tissue sections. C) IRS-1 expression in normal and kidney cancer tissue sections. In all sections IRS-1 expression significantly increased in tumor areas compared to normal areas. D) IGF-I receptor phosphorylation in normal and kidney cancer tissue sections. IRS-1 expression was significantly increased in all normal and tumor areas.

High glucose has been shown to be upregulated in several kidney tumors, it is thought to be associated with the loss of normal glucose transport and to increase the production of glucose from the extracellular matrix. In addition, high glucose has been shown to be upregulated in several kidney tumors, it is thought to be associated with the loss of normal glucose transport and to increase the production of glucose from the extracellular matrix. In addition, high glucose has been shown to be upregulated in several kidney tumors. It is thought to be associated with the loss of normal glucose transport and to increase the production of glucose from the extracellular matrix.

- Figure 5: Effect of high glucose on proliferation of Renca cells. To evaluate the effect of different glucose concentrations we employed XF24 Extracellular Flux Analyzer (Seahorse Bioscience) with plate reader 

William H. Goss, 1.1. Effects of glucose concentration on proliferation (A) and cell cycle distribution (B) of Renca cells. (A) Renca cells were cultured in full serum conditions for five days in the presence of normal (5mM, NG) or high glucose (40mM, HG) concentrations. Cell populations were maintained in the presence of high glucose (40mM) and images were taken using camera equipped Olympus microscope (original magnification 10x). No morphological differences were observed between Renca cells exposed to normal (5mM) and high (40mM) glucose concentrations. (B) Cell viability of Renca cells growing in suspension on Poly-riboflavin plates were evaluated by Trypan Blue staining based on membrane integrity also for the use with the GuavaEasyCyte flow cytometer (Milipore). The Guava/VideoCyte Software were used for data analysis and quantification according to the manufacturer recommendations (Milipore). We expected increase of survival in the presence of IGF-I and HG based on the growth advantage observed in Figure 1. However, the percent of viable cells was similar across all conditions, indicating that elevated glucose and IGF-I do not promote increased survival. Histogram represent data from 3 experiment of ViCyte assay.

- Figure 6: Effects of high glucose concentration on IGF-I signaling pathways in Renca cells. Western blot analysis shows levels of phosphorylated downstream signaling molecules IGF-I receptor (IR), Akt, GSK3, and Erk1/2. Renca cells were cultured in serum-free media for 48 hrs, and treated with IGF-I (100ng/ml) at indicated time points. The cells without treatment were used as controls (cont). Renca cells are characterized by the presence of constitutive mitogenic signaling (IGF-I receptors). IGF-I stimulation activates the Akt/GSK3/Erk1/2 pathway in Renca cells cultured with normal glucose. (A) Levels of phosphorylated Akt, GSK3 and Erk1/2. (B) All downstream signaling molecules are stimulated by high glucose. (C) Effects of IGF-I stimulation on Akt/GSK3/Erk1/2 activity in Renca cells cultured with normal glucose. IGF-I stimulation results in increased IGF-I receptor (IR) phosphorylation, which may depend on hyper activation of the IGF-IR signaling. Further experiments are required to verify if indeed a positive correlation exists between high incidence of renal cell carcinoma and elevated blood glucose levels.

- Figure 7: Effects of high glucose concentration on IGF-I signaling pathways in Renca cells. Western blot analysis shows levels of phosphorylated downstream signaling molecules IGF-I receptor (IR), Akt, GSK3, and Erk1/2. Renca cells were cultured in serum-free media for 48 hrs, and treated with IGF-I (100ng/ml) at indicated time points. The cells without treatment were used as controls (cont). Renca cells are characterized by the presence of constitutive mitogenic signaling (IGF-I receptors). IGF-I stimulation activates the Akt/GSK3/Erk1/2 pathway in Renca cells cultured with normal glucose. (A) Levels of phosphorylated Akt, GSK3 and Erk1/2. (B) All downstream signaling molecules are stimulated by high glucose. (C) Effects of IGF-I stimulation on Akt/GSK3/Erk1/2 activity in Renca cells cultured with normal glucose. IGF-I stimulation results in increased IGF-I receptor (IR) phosphorylation, which may depend on hyper activation of the IGF-IR signaling. Further experiments are required to verify if indeed a positive correlation exists between high incidence of renal cell carcinoma and elevated blood glucose levels.