Glucose moderates the activity of the mTOR signaling pathway in human prostate cancer cells.

Justin B. Smolinski and Russell D. Klein
Dept. of Human Nutrition and Comprehensive Cancer Center, Cancer Chemoprevention Program, The Ohio State University, Columbus, OH 43210.

ABSTRACT

Glucose is able to modulate the mTOR pathway in both a time and dose dependent manner as evidenced by alterations in rp-56 phosphorylation. rp-56 phosphorylation is a tightly regulated reversible event catalyzed by the addition and removal of glucose. Glucose affects cell viability at low concentrations.

INTRODUCTION

Prostate cancer is the most common malignancy among men. It is also the second leading cause of cancer mortality in men. Prostate cancer is characterized by a long latency period for cancer progression and development of metastasis. Since the side effects of surgery and radiotherapy are associated with significant morbidity, other treatment options are desirable during this period. This presents an excellent chemopreventive opportunity.

OBJECTIVES

To investigate the effects of reduced plasma glucose seen with Dietary Energy Restriction on the mTOR pathway in human prostate cancer cells.

RESULTS

Since tumors grow rapidly, they rely heavily on nutrient supply for energy to fuel this growth process. It is possible that decreasing cellular energy levels will halt the tumor growth and will lead to regression. On a molecular level, this may be accomplished through decreased flux through several pathways that govern cell growth and proliferation.

HYPOTHESIS

Dietary Energy Restriction (DER) has been shown to be chemopreventive against several types of cancer including mammary and prostate in animal models. There is evidence that DER acts through modulation of hormones and growth factors like IGF-1 and insulin. There is evidence that DER acts through modulation of hormones and growth factors like IGF-1 and insulin. Since tumors grow rapidly, they rely heavily on nutrient supply for energy to fuel this growth process. It is possible that decreasing cellular energy levels will halt the tumor growth and will lead to regression. On a molecular level, this may be accomplished through decreased flux through several pathways that govern cell growth and proliferation.

METHODS

Materials. Polyclonal rabbit-p56 and rabbit-p62 antibodies were purchased from Cell Signaling Technology (Beverly, MA) and Thermo Scientific (Rockford, IL). Cells were purchased from ATCC. Cells were maintained in Dulbecco’s Modified Eagle Medium (DMEM) growth medium supplemented with 10% fetal bovine serum, sodium pyruvate, L-glutamine, penicillin, and streptomycin, and kept at 37°C in 5% CO2. For glucose treatment experiments, cells were serum starved and glucose removal will result in a decreased activation of Akt. Akt will then activate a downstream target, mTOR. Its activation is associated with apoptosis evasion, increased cell growth and angiogenesis(3). These features are associated with poor prognostic outcome. mTOR has been implicated as a sensor of cellular energy status(4). Inhibition of mTOR leads to a decrease in total protein synthesis, a decrease in cell growth and an increase in autophagic cell death. This mimics a starvation-like response. It has also been shown that mTOR responds to manipulation of certain nutrients(5). Since this pathway is important in prostate cancer chemoprevention, therefore, we believe that mTOR will act through this pathway and we will investigate key components of this regulatory pathway.

In this study, we altered glucose levels and glucose exposure time to simulate lowered plasma glucose levels that are associated with DER. We used human PC-3 cells which have PTEN mutations characterized by high basal mTOR activity. To examine the effects on the mTOR pathway, we examined phosphorylation status of ribosomal protein S6, which is active when phosphorylated and indicative of protein translation(6).

RESULTS

Glucose regulates rp-56 phosphorylation in both a time- and dose-dependent manner. Glucose refeeding restores rp-S6 phosphorylation in PC-3 cells after removal of glucose. This indicates that low glucose may be inhibiting PI3K/Akt/mTOR signaling in the cell cycle progression of human prostate cancer. Biochemical and Biophysical Research Communications. 310:1124-1132.

FUTURE DIRECTIONS

Investigate how nutrients affect the upstream pathways that activate mTOR.

Glucose Deprivation

MATERIALS AND METHODS

RESULTS

CONCLUSIONS

Since tumors grow rapidly, they rely heavily on nutrient supply for energy to fuel this growth process. It is possible that decreasing cellular energy levels will halt the tumor growth and will lead to regression. On a molecular level, this may be accomplished through decreased flux through several pathways that govern cell growth and proliferation.

mTOR activity, we performed immunoblotting in response to glucose. PC-3 cells exhibit hyperactivity of the mTOR cellular energy sensing pathway which is catalyzed by the addition and removal of glucose. Glucose affects cell viability at low concentrations.

HYPOTHESIS

Dietary Energy Restriction (DER) has been shown to be chemopreventive against prostate cancer although the mechanism remains elusive. We hypothesized that reduced plasma glucose levels resulting from DER might provide increased control over prostate cancer cell growth and cell signaling. To address this hypothesis, we used human PC-3 cells to study the effects of glucose on these parameters. PC-3 cells exhibit high activity of the mTOR cellular energy sensing pathway which is associated with increased cell survival. To assess mTOR activity, we performed immunoblotting in response to glucose.

OBJECTIVES

To investigate the effects of reduced plasma glucose seen with Dietary Energy Restriction on the mTOR pathway in human prostate cancer cells.

RESULTS

To investigate the effects of varying glucose concentration and/or time without glucose on cell proliferation and survival.

HYPOTHESIS

To investigate the effects of nutrients on other downstream targets of mTOR including autophagy.

CONCLUSIONS

FUTURE DIRECTIONS

To investigate how nutrients affect the upstream pathways that activate mTOR.

Conclusions: Glucose is able to modulate the mTOR pathway in both a time and dose dependent manner as evidenced by alterations in rp-56 phosphorylation. rp-56 phosphorylation is a tightly regulated reversible event catalyzed by the addition and removal of glucose. Glucose affects cell viability at low concentrations.

Acknowledgments: Thank you to the Klein lab- Kristin Keatley, Xingya Wang, Rob Rangel for technical support.