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

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ABSTRACT

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 play a role in inhibiting 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 hyperactivity of the mTOR cellular energy sensing pathway which is associated with increased cell survival. To assay mTOR activity, we performed immunoblotting for phospho- rp-S6, a downstream target of mTOR. We observed a dose-response between 0- 6.25 mM glucose. Upon glucose removal, phosphorylation of rp-S6 decreases rapidly, with a significant effect seen at one hour and abolished almost completely at 18 hours. To confirm that this is a reversible phosphorylation event, we deprived PC-3 cells of glucose for one hour and refed them with 25mM glucose. Restoration of rp-S6 phosphorylation levels occurred within 15 minutes of treatment. We then investigated if the effect seen at 18 hours was due to an increase in cell death and whether or not we could rescue phosphorylation status. We deprived cells of glucose for 18 hours and refed with 25mM glucose. The time required to restore phosphorylation of rp-S6 was increased as compared to the one hour fast. Physiologically, we see a decrease in cell viability only after 48 hours and lowest concentrations of glucose. In conclusion, glucose removal reduces the activity of the mTOR pathway in prostate cancer cells.

METHODS

Materials. Polycolonal phospho-rp-S6 and monocolonal rp-S6 antibodies were purchased from Cell Signaling Technology (Beverly, MA)Thiazolyal Blue Tetrazolium(MTT) was purchased from Sigma-Aldich(St.Louis, MA).

Cell culture. Human PC-3 prostate cancer cells were purchased from ATCC. Cells were maintained in Dulbecco's Modified Eagle (DMEM) growth medium supplemented with 10% fetal bovine serum, sodium pyruvate, glutamine and penicillin/streptomycin, and kept at 37º C in 5% CO2. For glucose treatment experiments, cells were seeded and allowed to attach overnight. All experiments were performed in serum free medium.

Immunoblotting. Proteins were separated using SDS-PAGE. The expression of rp-S6 and total rp-S6 were determined by immnunoblotting according to the manufacturer's instructions (Cell Signaling).

MTT- Cells were seeded in a 96 well dish and allowed to attach overnight. Prior to treatment, the cells were serum-starved for one hour. Treatments lasted for 18, 24 and 48 hours. After treatment, cell viability was measured using the MTT assay according to the manufacturer's instructions (Sigma-Aldrich).

INTRODUCTION

Prostate cancer is the most common malignancy among men. It is also the second leading cause of cancer morbidity 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.

Dietary Energy Restriction (DER) has been found 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. However, several studies have shown that the effects are independent of the growth factor axis(1). Thus, the mechanism behind the chemopreventive effects of DER remains elusive.

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.

PTEN tumor suppressor mutations are among the most common mutations seen in prostate cancer(2). Loss of PTEN is associated with increased levels of activated 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, its manipulation may be a key to prostate cancer chemoprevention. Therefore, we believe that DER will act through this pathway and we will measure key components of this regulatory pathway.

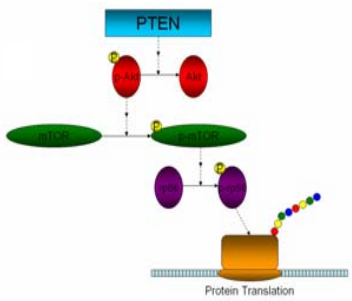
In this study, we altered glucose levels and glucose exposure time to simulate lowered plasma glucose levels that are associated with DER. We used human PC-3 cells which have PTEN mutations characterized by high basal mTOR activity. To examine the effects on the mTOR pathway, we examined phosphorylation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

OBJECTIVES

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

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

HYPOTHESIS



➢Glucose removal will result in a decrease in the activity of the mTOR pathway.

➢This will result in a decreased activation of rp-S6, a downstream target of mTOR.

➢rp-S6 phosphorylation is a tightly regulated reversible event that can respond rapidly and efficiently to glucose status.

➢Low glucose concentrations will decrease prostate cancer cell viability.

RESULTS

Glucose regulates rp-S6 phosphorylation in both a time- and dose-dependent manner

Figure 1-Glucose regulated dose-response increase in rp-S6 phosphorylation in PC-3 cells after 18 hours of treatment. Cells were serum-starved for 1 hour prior to treatment and the respective glucose concentration was added. Cell lysates were run on a 12% Tris-Glycine SDS-PAGE gel. Both phospo- and total rp-S6 were analyzed using immunoblotting.

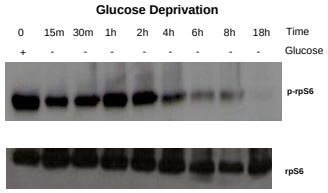


Figure 2- Time course for rp-S6 dephosphorylation in PC-3 cells after removal of glucose. Cells were serum-starved for 1 hour prior to treatment. Glucose was removed for the given time period. Cell lysates were run on a 12% Tris-Glycine SDS-PAGE gel. Both phospo- and total rp-S6 were analyzed using immunoblotting.

rp-S6 phosphorylation is a tightly regulated, reversible event

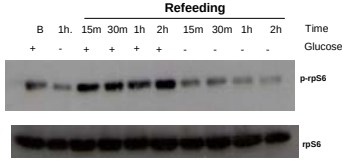


Figure 3- Glucose refeeding restores rp-S6 phsosphorylation that is lost as a result of glucose removal. Cells were serum-starved for 1 hour prior to glucose removal. B=baseline level. Cells were then glucose starved for 1 hour and then treated with 25mM glucose or PBS for 15 minutes, 30 minutes, 1 hour or 2 hours. Cell lysates were run on a 12% Tris-Glycine SDS-PAGE gel. Both phospo- and total rp-S6 were analyzed using immunoblotting.

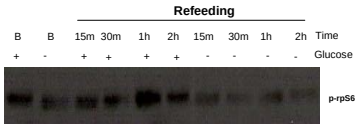


Figure 4- Glucose refeeding after an 18 hour glucose free period restores rp-S6 phosphorylation in PC-3 cells. The time required to restore rp-S6 phosphorylation was increased as compared to 1 hour of glucose deprivation. Cells were serum-starved for 1 hour prior to glucose removal. After 18 hours of glucose starvation, 25mM glucose or PBS was added for 15 minutes, 30 minutes, 1 hour or 2 hours. Cell lysates were run on a 12% Tris-Glycine SDS-PAGE gel. Phospho-rp-S6 were analyzed using immunoblotting

Low concentrations of Glucose decrease cell viability

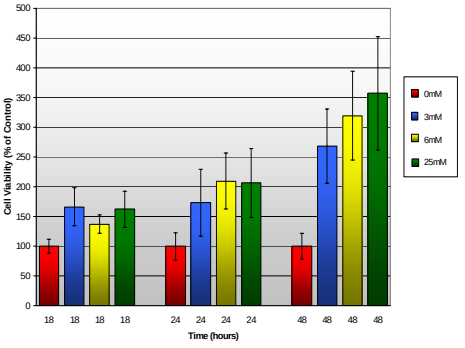


Figure 5- Low concentrations of glucose affect cell viability. As time progresses, with higher concentrations of glucose, cell number increases. This indicates that low glucose may be inhibiting the cell cycle. Cells were seeded in 96 well plates and allowed to attach overnight. They were serum-starved for 1 hour prior to treatment. Treatments were for 18, 24 and 48 hours. After these time periods, cell viability was assessed using the MTT assay according to the manufacturer's instructions (Sigma-Aldrich). n=6.

CONCLUSIONS

➢Glucose is able to modulate the mTOR pathway in both a time and dose dependent manner as evidenced by alterations in rp-S6 phosphorylation.

➢rp-S6 phosphorylation is a tightly regulated reversible event catalyzed by the addition and removal of glucose.

➢Glucose affects cell viability at low concentrations.

FUTURE DIRECTIONS

➢Investigate how nutrients affect the upstream pathways that activate mTOR.

➢Investigate the effects of nutrients on other downstream targets of mTOR including autophagy.

➢Manipulate other nutrients like lipids and investigate their effects on the mTOR pathway.

➢Perform Dietary Energy Restriction in a PTEN knockout mouse model of prostate cancer, examining the effects of DER on both tumorigenesis and the mTOR pathway.

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