THE ROLE OF ANDROGEN RECEPTOR IN GLUCOSE TRANSPORTERS EXPRESSION IN PROSTATE CANCER CELLS

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INTRODUCTION AND OBJETIVES

Cancer cells have different metabolic requirements compared to normal cells. Thus, aerobic glycolysis is mostly used by tumor cells for cell proliferation, phenomenon known as "Warburg Effect". In tumor cells, changes in protein levels or membrane translocation of glucose transporters are related to glycolytic pathways. An increased in glucose uptake has been associated mainly with GLUT1 overexpression but may also involve an increment of other glucose transporters.

In prostate cancer, glycolytic metabolism profile differs in androgen-sensitive and insensitive cells. Androgen Receptor (AR) regulates many genes required for glucose consumption and biomass production. Moreover, an increase of glucose concentration in medium downregulates AR in LNCaP cells through NF-κB activation. In reference to GLUT transporters, GLUT1 expression in androgen-sensitive LNCaP seems to be regulated by androgens and GLUT4 expression was recently described in our laboratory. In addition, AR presence in androgen-insensitive PC-3 cells modifies the regulation of GLUT1/4 expression by natural compounds that block glucose uptake.

Thus, the aim of this work was to study the regulation of GLUT1/4 by androgen receptor in prostate cancer cells in order to relate the protein levels of these transporters with the AR presence and glucose uptake status.

MATERIAL AND METHODS

Cell culture: Androgen-sensitive LNCaP cells were cultured in complete RPMI-1640 medium, androgen-insensitive PC-3 cells were grown in complete DMEM/F12 medium. PC-3^{AR}, $LNCaP^{GLUT1}$, $LNCaP^{GLUT4}$, PC-3^{GLUT1} and PC-3^{GLUT4} clones were obtained by stable transfection. All experiments were applied 48 hours after seeding.

Glucose uptake: A non-radiolabeled 2-deoxyglucose (2-DG) uptake was used.

Immunoblot: After treatments, cells were lysed in RIPA buffer and proteins were transferred to PVDF membranes. For cytoplasmatic and nuclear fractions, low-salt and high-salt buffer, respectively, were used. Then they were incubated with anti-GLUT1, anti-GLUT4, anti-AR and anti-β-actin (internal and cytoplasmatic standard) or HDAC2 (nuclear standard). After adding the appropiate secondary antibodies, membranes were developed using a chemiluniniscent reagent.

Immunocytochemistry: Cells were seeded on Thermanox coverslips. Cells were fixed in 3% paraformaldehyde, permeabilized with 0.1% TritonX-100, bloqued with 0.5% BSA and reacted with anti-AR. Then, phycoerythrin-conjugated antibody was used. Nuclei were counterstained with DAPI. Coverslides were then mounted using fluoromont G and observed with confocal microscopy.

PSA measurement: PSA (Prostate Specific Antigen) was measured in culture medium by ELISA.

Cell cycle analysis: Fixed cells ($5x10^5$) were pelleted, resuspended in PBS-1% glucose and stained with 100 mg/ml propidium iodide containing 1000 U/ml ribonuclease A. Flow cytometry analysis was perfomed using a Cytomics FC500 flow cytometer (Beckman Coulter).

Detection of GLUT1 protein by flow cytometry: GLUT1 was detected by flow cytometry using AlexaFluor488-conjugated antibody.

RESULTS

1. GLUT1/4 levels depend on culture medium renewal in androgen-sensitive and insensitive prostate cancer cells

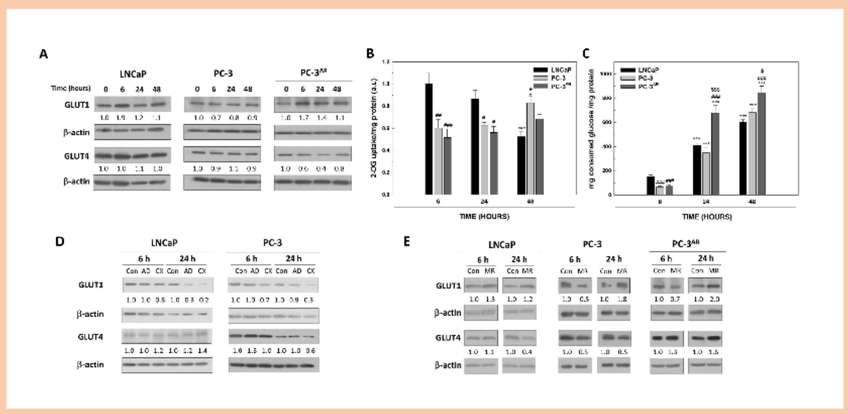


Figure 2. GLUT1 and GLUT4 regulation in prostate cancer cells along culture time. GLUT1 and GLUT4 protein expression in LNCaP, PC-3 and PC-3^{AR} cells after 6, 24 and 48 hours after medium renewal was determined by western-blot (A). Arbitrary 1.0 value was given to cells at time 0. 2-DG uptake at the same time was evaluated in LNCaP, PC-3 and PC-3^{AR} cells (B). Arbitrary 1.0 value was given to LNCaP at 6 hours. Consumed glucose was obtained measuring glucose concentration at each time. (C). Arbitrary 1.0 value was given to LNCaP cells at 6 hours. The effect of 1 µg/mL Actinomycin D (AD) and 5 µg/mL Cicloheximide (CX) at 6 and 24 hours on GLUT1 and GLUT4 protein expression was studied in LNCaP and PC-3 cells (D). Arbitrary 1.0 value was given to control cells at each time. Constant medium renewal (MR) was established to study GLUT1/4 protein levels in LNCaP, PC-3 and PC-3^{AR} cells (E). *p < 0.05 vs. 6 hours; ***p < 0.001 vs. 6 hours; #p < 0.05 vs. LNCaP; ##p < 0.01 vs. LNCaP; ###p < 0.001 vs. LNCaP; $p < 0.05 vs. PC-3; $$$p < 0.001 vs. PC-3.

2. The involvement of GLUT4 transporter in glucose uptake is more relevant in androgen-insensitive cells

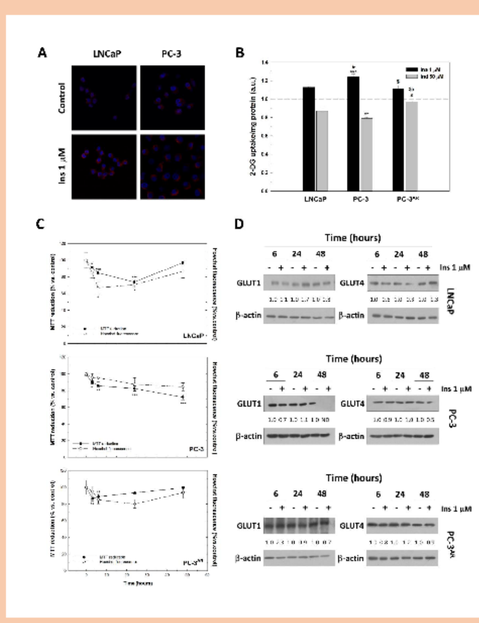


Figure 3. Response to insulin of prostate cancer cells. Immunocytochemical analysis of GLUT4 showing its subcellular location in LNCaP and PC-3 cells was made after 1 hour of treatment with 1 µM insulin (Ins) (A). Cells were incubated with a secondary antibody conjugated with phycoerythrin (red fluorescence) and then counterstained with DAPI (blue florescence). Effect of 1 µM Ins and 50 µM indinavir (Ind) in LNCaP, PC-3 and PC-3^{AR} cells after 1 hour of treatment was evaluated by 2-DG uptake (B). Results are expressed as mean ± SEM (n=3) and standardized to protein concentration. Cell proliferation and viability were assayed after 3, 6, 24 and 48 hours of treatment with 1 mM Ins (C). Results are expressed as mean ± SEM (n=6). Effect of 1 mM Ins at 6, 24 and 48 hours on GLUT1 and GLUT4 protein expression in LNCaP, PC-3 and PC-3^{AR} was determined by western-blot Arbitrary 1.0 value was given to control cells at each time. Experiments were repeated at least 3 times and a representative experiment is shown. *p < 0.05 vs. control; **p < 0.01 vs. control; ***p < 0.001 vs. control #p < 0.05 vs. LNCaP; $p < 0.05 vs. PC-3; $$p < 0.01 vs. PC-3.

3. AR expression causes an increment of GLUT1 levels at low concentration of glucose

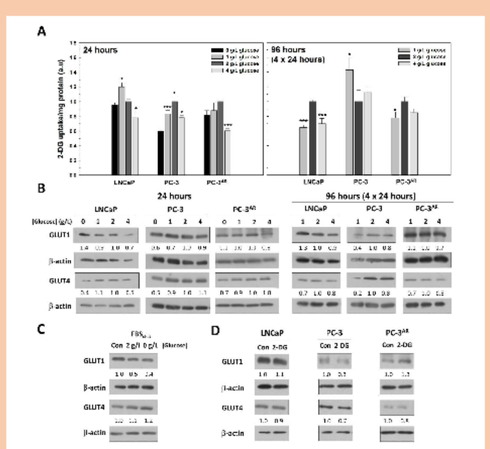


Figure 3: GLUT1 and GLUT4 regulation by glucose concentration in prostate cancer cells. The effect of glucose concentration in 2-DG uptake was evaluated at 24 and 96 hours in LNCaP, PC-3 and PC-3^{AR} cells (A). GLUT1/4 protein levels were determined by western-blot (B). GLUT1/4 protein expression in LNCaP cells cultured in charcoal/dextran-stipped FBS (FBS_{chst}) for 24 hours was analysed by western-blot (C). GLUT1 and GLUT4 levels in LNCaP, PC-3 and PC-3^{AR} cells treated with 10 mM 2-DG were also evaluated (D). *p < 0.05 vs. 2 g/L of glucose; **p < 0.01 vs. 2 g/L glucose; ***p < 0.001 vs. 2 g/L glucose.

4. AR expression avoids cell cycle arrest by glucose deprivation in prostate cancer cells

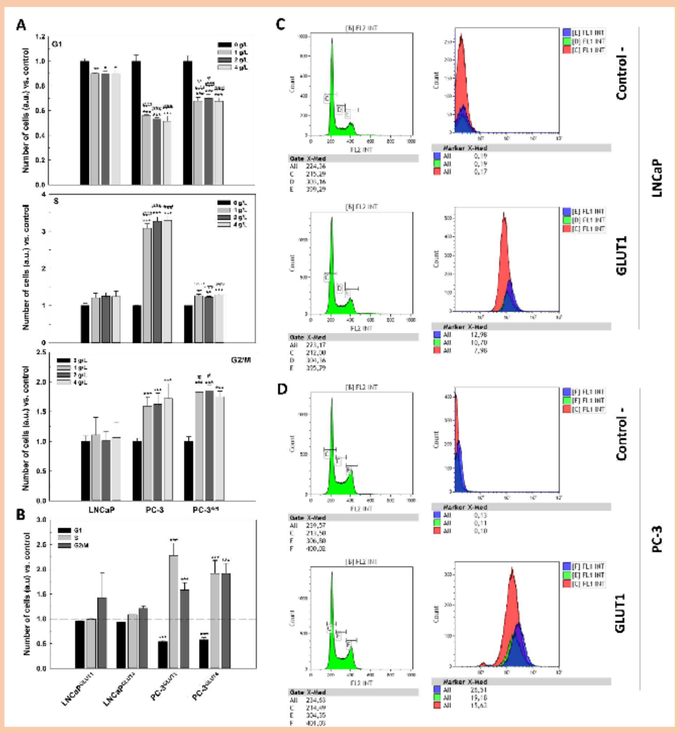


Figure 4: Effect of glucose concentration in culture medium in cell-cycle distribution. LNCaP, PC-3 and PC-3^{AR} cells were grown with 0, 1, 2 and 4 g/L of glucose for 24 hours. The relation between cells in each phase with cells in absence of glucose was calculated (A). The effect of glucose concentration in cells that overexpressed GLUT1 or GLUT4 transporters was analyzed with 0 and 2 g/L of glucose for 24 hours (B). Results are expressed as mean ± SEM (n=3). *p < 0.05 vs. 0 g/L glucose; **p < 0.01 vs. 0 g/L glucose; ***p < 0.001 vs. 0 g/L glucose; ###p < 0.001 vs. LNCaP; ψp < 0.05 vs. PC-3; ψψψp < 0.001 vs. PC-3. Cell cycle analysis using propidium iodide was combined to determinate GLUT1 protein levels using an AlexaFluor488-conjugated antibody in LNCaP (C) and PC-3 cells (B). Negative controls wthout anti-GLUT1 was used to characterize cells in each cell-cycle phase. Experiments were repeated three times and one representative experiment is shown.

5. Nuclear AR correlates with GLUT1 levels while GLUT4 overexpression decreases its nuclear translocation

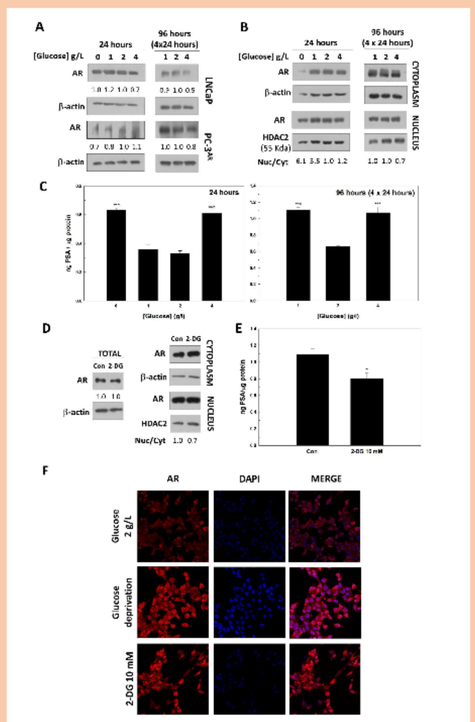


Figure 5: AR regulation by glucose concentration in prostate cancer cells. AR protein levels in LNCaP and PC-3^{AR} cells were determined by western blot (A). Cells were grown with 0, 1, 2 and 4 g/L of glucose for 24 hours or with 1, 2 and 4 g/L of glucose at 96 hours.Nuclear and cytoplasmic AR protein levels were determined by western-blot using the same glucose concentrations at the same times (B). PSA levels in culture medium of LNCaP cells were determined (C). Western-blot and PSA measurement were also performed in LNCaP cells treated with 10 mM 2-DG (D, E). Immunocytochemical analysis of AR showing the subcellular location in LNCaP cells at 24 hours (F). *p < 0.05 vs. control; ***p < 0.001 vs. 2 g/L glucose.

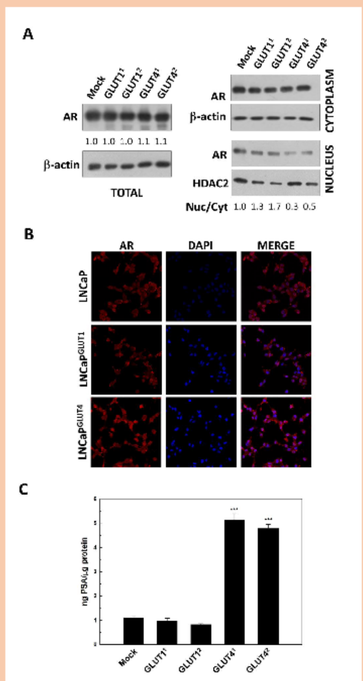
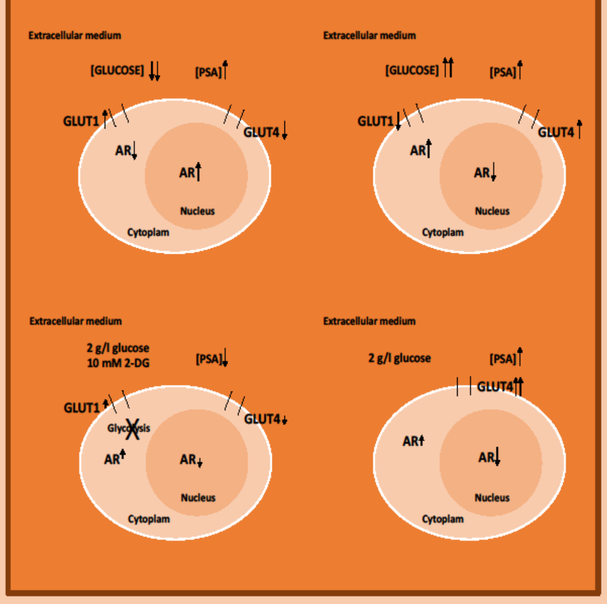


Figure 6: AR regulation in $LNCaP^{GLUT1}$ and $LNCaP^{GLUT4}$ cells. AR protein levels in LNCaP cells, $LNCaP^{GLUT1}$ clones and $LNCaP^{GLUT4}$ clones were determined by western-blot (A). Cells were grown with 2 g/L of glucose for 24 hours. Nuclear and cytoplasmic AR protein levels were also determined by western-blot Immunocytochemical analysis of AR showing the subcellular location was performed to confirm western-blot results (B). PSA levels in culture medium were determined (C). ***p < 0.001 vs. LNCaP control.

CONCLUSIONS

1) Under hypoglycemic conditions, AR nuclear translocationis enhanced, correlating with an increase of GLUT1

2) GLUT4 overexpression seems to drive to androgen-insensitivity



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