Effects of androgen stimulus on glycolytic enzyme activity and anti-apoptosis in LNCap cells

Catalina Carrasco-Pozo, Kah Ni Tan, Tayner Rodriguez, Vicky M. Avery

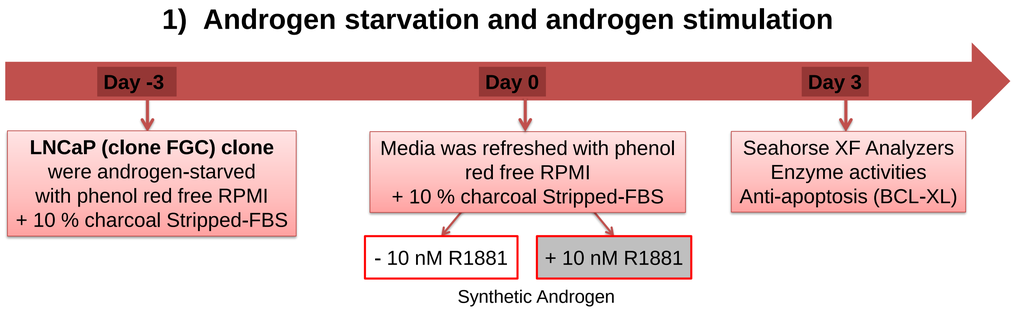
Discovery Biology, CRC for Cancer Therapeutics, Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, Australia

INTRODUCTION

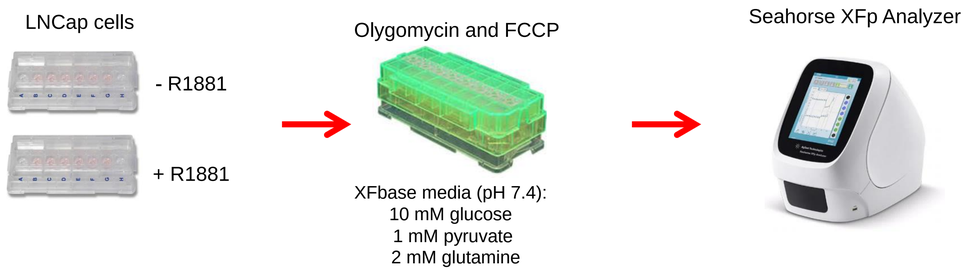
Metabolic reprogramming is a hallmark of “malignant transformation”. The production of biomass and energy are key points in the uncontrolled cell expansion that characterises cancer initiation, development and progression. In this aspect, metabolism stands out as a key biological process in understanding the conversion of a normal cell into a neoplastic precursor. Prostate cancer is the second most commonly diagnosed malignancy and the fifth leading cause of cancer mortality in men in Europe and worldwide. Prostatic carcinogenesis is initially androgen-dependent which is mediated primarily through androgen receptor (AR). The aim of this study was to evaluate the effects of androgen stimulus on glycolytic and mitochondrial functions and proliferation of LNCap cells.

METHODS

1) Androgen starvation and androgen stimulation

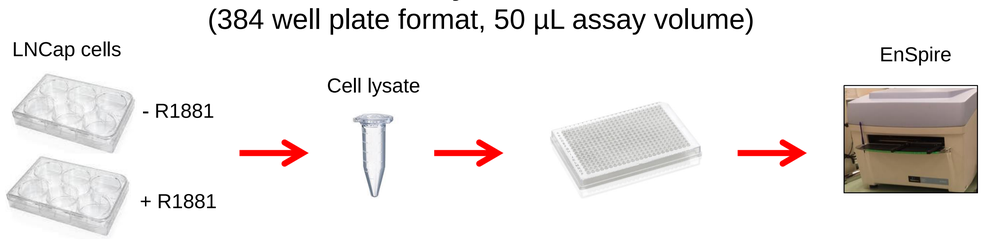


2) Extracellular flux analyser to determine extracellular acidification rate and metabolic phenotype (8 well plate format, 200 µL assay volume)



3) 340 nm spectrophotometry detection of the NAD(P)(H) redox kinetic to evaluate enzymatic activities.

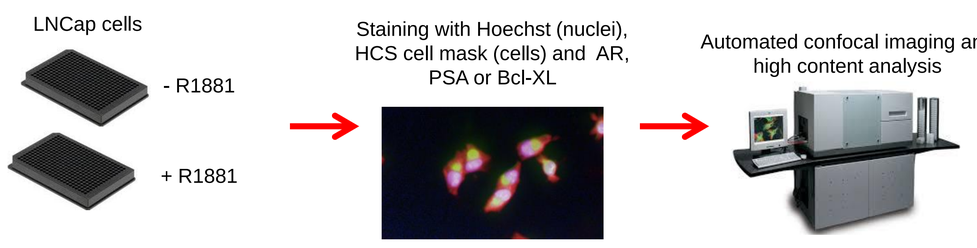
(384 well plate format, 50 µL assay volume)



Enzyme	Reaction Mix (final concentrations)	Initiating Substrate	Measurement
Hexokinase	Triethanolamine (pH 7.4), ATP, β-NADP, $MgCl_2$, Glucose 6-phosphate dehydrogenase	D-glucose	Reduction of NADP
Glucose 6-phosphate dehydrogenase	Tris HCl (pH 7.4), β-NADP, $MgCl_2$	Glucose 6-phosphate	Reduction of NADP
Pyruvate kinase	Imidazole (pH 7.2), ATP, $MgCl_2$, KCl, β-NADH, lactate dehydrogenase	Phosphoenolpyruvate	Oxidation of NADH

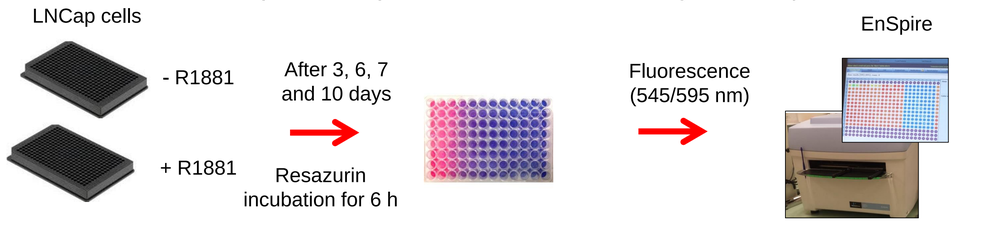
4) High content imaging assay to determine nuclear androgen receptor (AR), prostate specific antigen (PSA) and anti-apoptosis (Bcl-XL)

(384 well plate format, 50 µL assay volume)



5) Metabolic reduction of resazurin to determine proliferation

(384 well plate format, 50 uL assay volume)



REFERENCES

- Higgins *et al., Biochim Biophys Acta*. 2009; 1787(12):1433-43
- Sadeghi *et al., Tumour Biol. 2015*; 36(4):2893-905.
- Shiota *et al., Prostate*. 2010; 70(5):540-54.
- Sun *et al., Prostate*. 2008; 68(4):453-61.
- Tan, Carrasco-Pozo *et al., J Cereb Blood Flow Metab.* 2017; 37(6):2035-2048

ACKNOWLEDGEMENT

Griffith Universty Travel Grant
GRIDD Travel Grant contribution
CTx Travel Grant
C.C-P., K.N. T., T. R. and V.M. A. are members of the Cancer Therapeutics CRC

RESULTS AND DISCUSSION

1) Androgen stimulus enhances glycolytic function and switches the cells towards an energetic phenotype

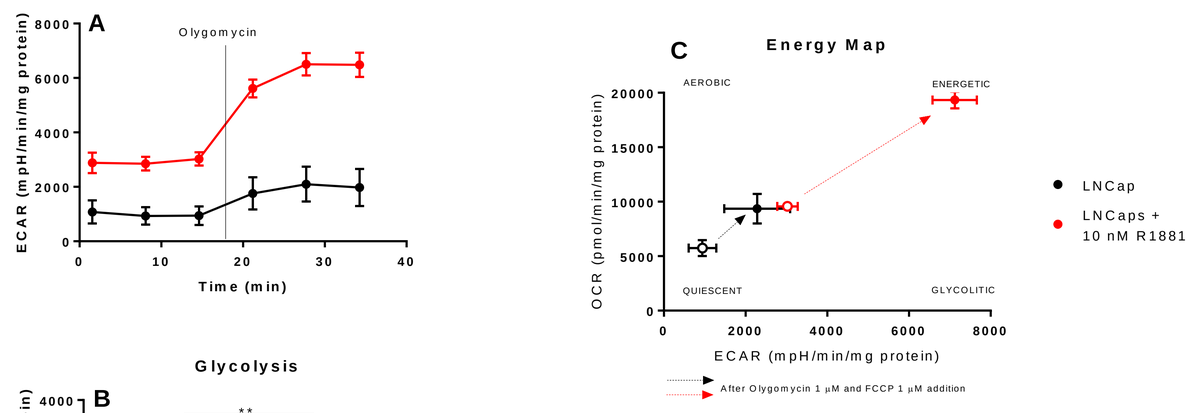


Fig 1.: Androgen increased the basal glycolytic function (Fig 1. A and B) and the glycolytic capacity, which was observed after inhibiting the ATP synthase by olygomycin (Fig. 1A). Androgen switched LNCap cells metabolism towards a more energetic and glycolytic phenotype (Fig. 1C). Mean ± SEM, N=3, t-test. ** p<0.01

2) Androgen stimulus increases the activity of metabolic enzymes

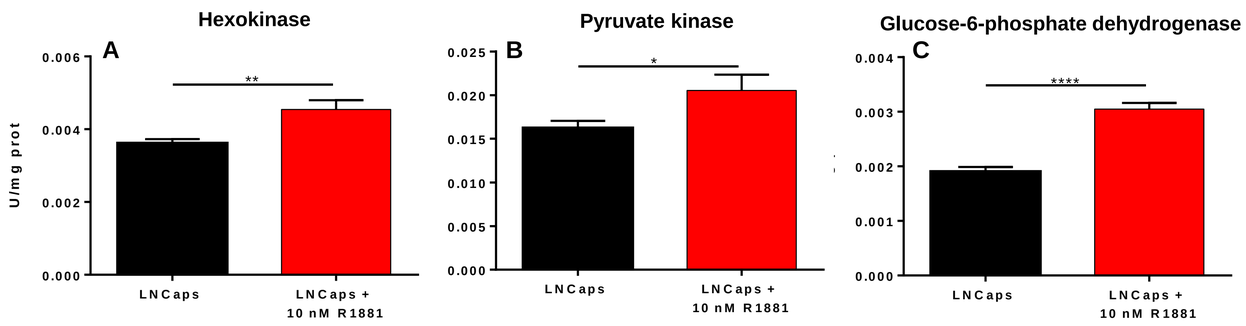


Fig 2.: Androgen stimulus increased the activity of hexokinase (Fig. 2A) and pyruvate kinase (Fig. 2B) by 26%, and the activity of glucose-6-phosphate dehydrogenase by 60% (Fig. 2C), indicating that androgen stimulates both metabolic pathways: glycolysis and the pentose phosphate pathways. Mean ± SEM, N=3, t-test. * p<0.05, ** p<0.01 and *** p<0.01

3) Androgen stimulus increases androgen receptor nuclear localisation and PSA levels

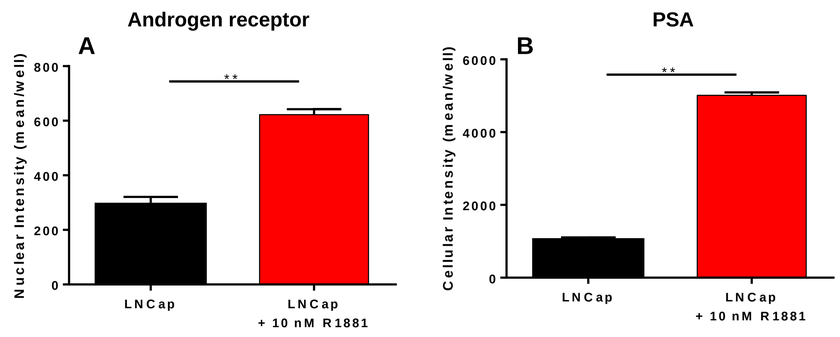


Fig 3.: Androgen stimulus doubled the AR localisation in the nucleus (Fig. 3A). Consistently, androgen also increased the intracellular PSA levels by 3-fold (Fig. 3B). Mean ± SEM, N=3, t-test. ** p<0.01

4) Androgen stimulus promotes cells proliferation

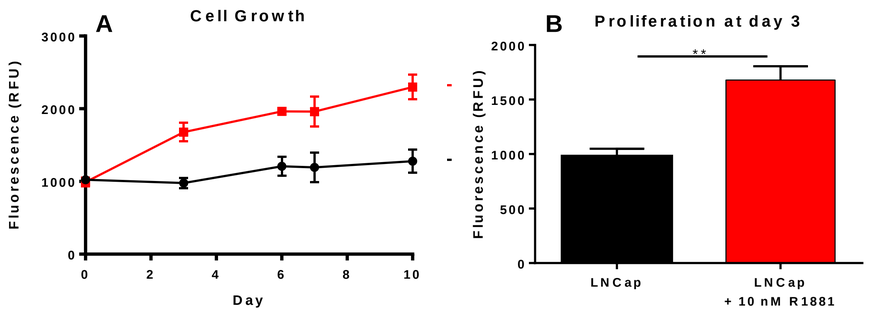


Fig 4.: Cell proliferation was stimulated in the presence of androgen (Fig. 4A). After 3 days incubation, androgen increased the cell proliferation by 66% (Fig. 4B). Mean ± SEM, N=3, t-test. ** p<0.01

5) Androgen stimulus promotes anti-apoptosis

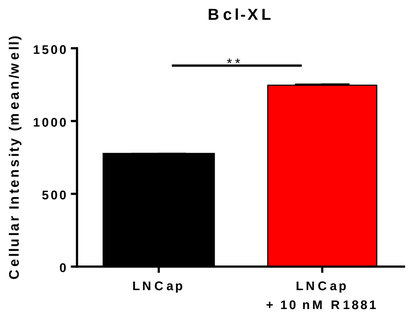


Fig 5.: Androgen stimulus increased intracellular Bcl-XL levels by 62%, which is consistent with the activation of the androgen receptor. Mean ± SEM, N=3, t-test. ** p<0.01

CONCLUSION AND FUTURE EXPERIMENTS

- The stimulation of LNCap cells proliferation induced by androgen, may be explained by the ability of androgen to increase the activities of metabolic enzymes in glycolytic and pentose phosphate pathways, which is translated to an enhanced glycolytic function.
- In addition to an increased androgen receptor localisation in the nucleus, androgen also increased the intracellular PSA and Bcl-XL levels. Both androgen receptor activation and the anti-apoptotic effect induced by androgen are consistent with the enhanced cell proliferation observed.

This new knowledge, namely glycolytic enzyme reprogramming results in increased anti-apoptotic biomarker and augmented proliferative rate, will help to develop new therapeutic strategies that targets cancer metabolism.