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Viability effects on cell cycle synchronization of different prostate cancer cell lines: A brief report

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ABSTRACT

Aim: Serum deprivation is often used to synchronize cells in G0/G1 phase for comparative *in vitro* studies. Here we aimed to investigate the effect on viability of the three commonly used prostate cancer-cell lines PC3, LNCaP and DU145 after serum deprivation.

Methods: The cell lines were cultured in standard culture medium (controls) and under serum deprivation for 48 and 72 h. Then the proportion of cells in G0/G1 phase was analysed by flow cytometry and cell morphology was microscopically investigated. The cells were allowed to recover for three days in standard culture condition before cell viability (using MTT assay) was analysed.

Results: In comparison to control cells, significant effects on cell cycle arrest in G0/G1 phase were noted for DU145 and PC3 cells and the cell morphology was negatively affected in a time-dependent manner. These parameters were unaffected in LNCaP cells. After three days of recovery, the viability of DU145 and PC3 cells was significantly reduced compared to LNCaP.

Conclusions: Serum deprivation showed different effects on the prostate cancer cells, probably due to differences in growth rate. Such effects on viability should be considered as an obstacle for comparative studies.

Key words: Serum deprivation, synchronization, cell cycle, viability.

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Introduction

The stage of aggressive, metastatic prostate cancer is incurable and the major challenge is therapeutic resistance to androgen-deprivation therapy (ADT) and chemotherapy [1-3]. Thus, there is a great demand for development and much research is underway to overcome the resistance through e.g. improvements of cytotoxic drugs, inconsistent cell cycle signals, or development of death signals [4]. For this kind of studies human prostate cancer (PCa) cell lines are commercially available and it is crucial to include different biological characteristics, represented by e.g. the commonly used PC3, DU145, LNCaP cell lines.

The PC3 cell line is of epithelial origin and was isolated from bone metastases with low degree of differentiation [5]. The DU145 cell line is of primary prostate adenocarcinoma origin and isolated from a central nervous system metastasis [6]. Androgen receptors are expressed [7] but the cell line is not androgen sensitive and has a lower metastatic potential than PC3 cells [8]. The LNCaP cell line was established from metastasis

in lymph node [9] and represents early androgen dependent PCa.

For comparative or kinetic studies of cell cycle progression, or to distinguish cell cycle stage sensitivity it is highly desirable to synchronize the cells. Serum deprivation has long been known to arrest cells in G0/G1 phase by withdrawing growth factors and other related components from cultured cells [10]. However, as reviewed by Davis et al. [11] consequences on the overall physiology of the cell should be better explored since tumour cells may have different requirements of serum content. Here, we intended to compare the response to synchronization in G0/G1 phase in the PCa-cell lines PC3, DU145 and LNCaP and possible consequences on their viability.

Materials and Metods

Prior to the serum deprivation experiment, the cell lines were cultured in standard cell culture conditions. PC3 and DU145 were maintained in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) and LNCaP in Roswell Park Memorial Institute 1640 (RPMI 1640; Life Technologies) supplemented with 10% fetal calf serum (FCS; Life Technologies) and 1% penicillin-streptomycin (PenStrep; 100 U/ml; Sigma–Aldrich) [12]. For the experiment they were subcultured into 35- or 60-mm culture dishes without antibiotics (Polystyren, Sarstedt) until reaching 90% confluence. Then, the culture medium was replaced with specified serum concentrations during different culture times (as indicated in the Figure 1) and control cells were maintained in standard culture conditions (10% serum).

Cell morphology was documented with light-microscopy and cell cycle phases were analysed with flow cytometry. Briefly, cells were fixed with 70% ice-cold ethanol and treated with 50 mg/ml RNase A (Roche) and 50 mg/ml PI (Sigma-Aldrich) for 30 min. The cells were then subjected to flow cytometry (Novocyte™; ACEA Bioscience, San Diego, CA, USA). Data acquisition and cell-cycle distribution (Watson model) were carried out using NovoExpress (Novocyte™; ACEA) [12]. After recovery in standard culture conditions for three days, cell viability was analysed using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Cell Proliferation Kit I, Roche) according to the manufacturer´s instructions. In brief, cells were seeded into 96-well plates (Sarstedt) at a cell density of 2 000 cells/well with standard culture conditions. After three days of recovery 0.5 mg/ml MTT was added and the optical density was measured in a microplate reader after solubilization (Victor™ X4, # 2030 Multilabel Reader; Perkin Elmer Inc., Akron, OH, USA) at wavelength of 595 nm. Data analysis as described in the Figure 1.

Results

LNCaP cells showed no obvious morphological changes and no significant changes of the proportion of cells in G1/G0 phase (Figure 1a). This proportion was high (~75%) even in those not exposed to deprivation. After 48 h deprivation of the DU145 and PC3 cell lines morphological differences were noted and after 72 h a lot of rounded, detached cells were shown (Figure 1b and c). The proportion of cells in G0/G1 phase increased significantly in a time dependent manner (Figure 1b and c).

After three days of recovery, the viability of LNCaP was not affected for those starved for 48 h but slightly reduced after 72 h treatment (Figure 1d). The viability of DU145 and PC3 cells was significantly reduced after serum deprivation for 48 and 72 h respectively, particularly for PC3 cells.

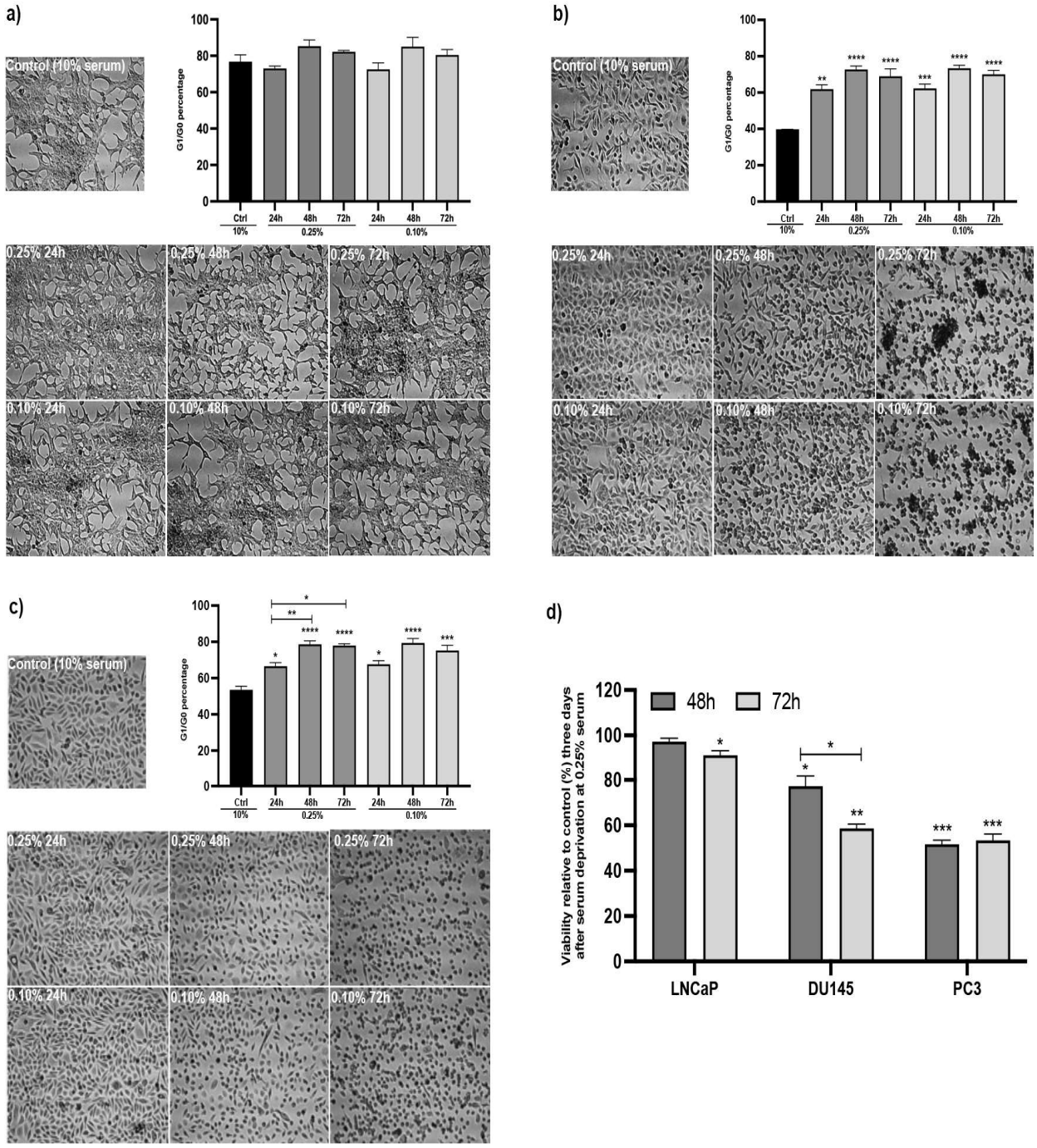

Figure 1. Morphology and flow cytometric analysis of cell cycle after serum deprivation for either 48 or 72 h a) LNCaP, b) DU145 and c) PC3. Cell viability (MTT-assay) was analysed after three days of recovery d). Data are expressed as mean ± SEM of at least three independent experiments for cell cycle analysis and two independent experiments performed in triplicates for viability after recovery. Shapiro-Wilk test was used to analyse normal distribution and ordinary one-way ANOVA with Tukey's test for multiple comparison was used to analyse statistical significance using Prism 9.00. Statistically significantly differences compared to controls are indicated as: *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$.

Discussion

The results showed different response upon serum deprivation between the three classical cell lines commonly used for *in vitro* experiments in PCa research. LNCaP is a slow-growing PCa cell line with a doubling time of 60-72 h depending on serum concentration [13] which could explain the insignificant effect after 72 h of deprivation. The faster doubling time (33-34 h) [13] of PC3 cells is more energy demanding and a likely reason for the lower viability compared to that for LNCaP. It has been reported that PC3 and DU145 after serum deprivation and G1 arrest were more sensitive to anandamide compared to that of LNCaP [14]. However, according to the results of the present study it is difficult to determine whether the difference is due to LNCaP not being affected by the deprivation and thus coping better with the toxic treatment [14]. Thus, time of serum deprivation should be adapted to the doubling time of each cell line to obtain comparable, viable cells after cell cycle synchronization.

Conclusion

Impaired condition in response to serum deprivation is important to take into account in experimental studies where e.g. treatments using different PCa cell lines are compared. Effects of cell cycle synchronization on cell viability should thus always be considered prior to the experiments.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement*: Ethics committee decision was not taken as it was a cell culture study.*

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References

[1]Crawford ED, Petrylak D, Sartor O. Navigating the evolving therapeutic landscape in advanced prostate cancer. Urol Oncol. 2017;35S:S1-S13.

[2]Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017;67(1):7-30.

[3]Wade CA, Kyprianou N. Profiling Prostate Cancer Therapeutic Resistance. Int J Mol Sci. 2018;19(3).

[4]Vaux DL, Strasser A. The molecular biology of apoptosis. Proc Natl Acad Sci U S A. 1996;93(6):2239-44.

[5]Tai S, Sun Y, Squires JM, et al. PC3 is a cell line characteristic of prostatic small cell carcinoma. Prostate. 2011;71(15):1668-79.

[6]Stone KR, Mickey DD, Wunderli H, et al. Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer. 1978;21(3):274-81.

[7]Alimirah F, Chen J, Basrawala Z, et al. DU-145 and PC-3 human prostate cancer cell lines express androgen receptor: implications for the androgen receptor functions and regulation. FEBS Lett. 2006;580(9):2294-300.

[8] Pulukuri SM, Rao JS. Matrix metalloproteinase-1 promotes prostate tumor growth and metastasis. Int J Oncol. 2008;32(4):757-65.

[9] Horoszewicz JS, Leong SS, Chu TM, et al. The LNCaP cell line--a new model for studies on human prostatic carcinoma. Prog Clin Biol Res. 1980;37:115-32.

[10] Brooks RF. Regulation of fibroblast cell cycle by serum. Nature. 1976;260(5548):248-50.

[11] Davis PK, Ho A, Dowdy SF. Biological methods for cell-cycle synchronization of mammalian cells. Biotechniques. 2001;30(6):1322-6, 8, 30-1.

[12] Hernroth B, Holm I, Gondikas A, et al. Manganese Inhibits Viability of Prostate Cancer Cells. Anticancer Res. 2018;38(1):137-45.

[13] Cunningham D, You Z. In vitro and in vivo model systems used in prostate cancer research. J Biol Methods. 2015;2(1).

[14] Mimeault M, Pommery N, Wattez N, et al. Anti-proliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. Prostate. 2003;56(1):1-12.