American Journal of Biomedical Science & Research

www.biomedgrid.com

ISSN: 2642-1747

Mini Review

A New Protocol to Obtain 80-Passage Lncap Androgen-Independent Cells

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To Cite This Article: *Huifeng Wang* and Die Zhang. A New Protocol to Obtain 80-Passage Lncap Androgen-Independent Cells. Am J Biomed Sci & Res. 2023 20(5) AJBSR.MS.ID.002758,* DOI: 10.34297/AJBSR.2023.20.002758

Received: November 23, 2023; **Published:** December 06, 2023

Abstract

The cell culture assay has been widely used for many subjects, especially in life science. Most researchers used to do it and failed by neglected tiny details. Lncap androgen-dependent cells (Lncap-AD), one of typically human prostate cancer cells, will be converted to Lncap androgen-independent cells (Lncap-AI) with androgen deprivation condition (ADT) and sustained culture 60-80 passages. Androgen receptor is one of the most important reagents to stimulate prostate cancer proliferation. The ADT just simulate clinical therapy to decrease proliferation. Androgen receptor (AR) sustained in culture condition and produced by prostate cancer itself. AR can be inhibitor by chemotherapeutic agents flutamide. The cells with long passage culture will be facing multiple damages, including trypsin digest, chemical reagent, and others. Multiple protocol details cannot be neglected to obtain suitable Lncap-AI cells. Therefore, this essay tries to supply pure, efficient, and suitable experiment conditions to help to adjust strict culture conditions. Also, we try to supply a repeatable protocol to sustained cell culture passage. Therefore, this essay tries to supply pure, efficient, and suitable experimental conditions to help to adjust strict culture conditions. Also, we try to supply a repeatable protocol to sustained cell culture passage.

Keywords: Prostate cancer, CRPC, Androgen independent, ADT, Androgen resistant

Background

In most nations, prostate cancer (PCa), one of the top 10 cancers that affect men, is the main reason why males die from cancer [1]. With the widespread use of early prostate-specific antigen (PSA) screening and magnetic resonance imaging techniques, the earlier stage of PCa can be identified. Currently, the utilize of surgery and radiation therapy has become an effective treatment for PCa [2]. In the early stage of PCa, androgen deprivation treatment (ADT) showed a produced positive therapeutic effects [3]. However, PCa patients may change from androgen-dependent to androgen-independent state after ADT 6 to 18 months of androgen deprivation therapy, giving patients with castration-resistant prostate cancer (CRPC) a worse prognosis [4]. Cancer cells are able to withstand ADT-induced tumor inhibition once PCa transitions into a hormone-independent condition. For CRPC people, there is no cure that can extend their lives [5].

In addition to androgen-dependent and androgen-independent models, there are various prostate cancer cell lines. Because the PCa cell lines PC3 and DU145 could not produce androgens, which can develop in an environment absence of hormones. Only the Lncap cell line, which secretes PSA, PsMA, and androgens while maintaining steroid hormones, still exhibits PCa androgen-dependent traits in PCa. Therefore, the prostate cancer cell line most frequently utilized is Lncap. However, it is challenging to investigate how the process of prostate cancer develops in a state of reliant

progression as opposed to a condition of non-dependent androgen progression. Despite the fact that some studies offer useful details on the ways in which testosterone works, PCa is not entirely reliant on it [6-8]. However, little is known about the cellular biology of PCa's shift from an androgen-dependent to an androgen-independent state. The absence of a perfect cell model is a significant study barrier. In a setting devoid of androgen, a PCa cell model should exhibit androgen-dependent characteristics that produce androgen receptors and change into androgen-independent characteristics.

In this protocol, we aimed to establish an androgen-independent Lncap (LncapAI) cell subsystem from an androgen-dependent Lncap (LncapAD) cell line during a castration androgen environment and to explore a more suitable method to improve the culture protocol and enhance the efficiency to establish the Lncap-AI cell line.

Materials and Reagents

Human Lncap cell line (the Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences).

RPMI Medium 1640(gibco, catalog number: 11835-030).

Flutamide (Yuanye, ShanghaiyuanyeBio-Technology™, catalog number: H20A9Z68074).

GlutaMAX-I (gibco, catalog number: 35050-061).

Sodium Pyruvate (gibco, catalog number: 11360-070).

Certified Foetal Bovine Serum (FBS) Charcoal Stripped (Biological Industries Pricelist, catalog number: 04-201-1A).

Penicillin-Streptomycin Liquid (Solarbio, catalog number: P1400).

Phosphate buffered saline (Solarbio, catalog number: P1020-500).

Dulbecco's phosphate buffered saline (Solarbio D, catalog number: 1040-500).

Trypsin Digestion solutions with EDTA (Solarbio, catalog number: T1350).

Ethanol (Keshi, catalog number: CAS 64-17-5).

Equipment

Cell culture dish (Corning Lncorporatcd, catalog number: 430167).

Pipet tips (Axygen, catalog number: T-300; T-200-Y, T-1000-B; BS-5000-TL).

Centrifuge Tube (Corning, catalog number: 430790, 430828).

Cell culture incubator: 37°C and 5% CO_2.

Rainin pipet tips (1 ml) (Mettler-Toledo, catalog number: GPS-L1000).

Procedure

Grow cells in 1640 supplemented with 10% FBS, 1% GlutaMAX-I, 1% Sodium Pyruvate, 0.1% Penicillin-Streptomycin Liquid.

Seed cells into 100 mm culture dish at a density that after 48 h of growth, they should reach ~70-80% confluence as a monolayer.

The culture dish was taken and pour the old culture medium.

Use the D-PBS to wash culture cells mixture the affluence of Fetal bovine serum by once.

Tips: D-PBS was performed by a 50 mL centrifuge tube. Pour the D-PBS without pipette to leave a bit of medium. Use hand instead of a pipette to make sure the D-PBS is trapped in the culture dish and then will dilute trypsin concentration.

Use 1 mL 0.25% trypsin (with EDTA) to digestion adherent cells.

Tips: Trypsin was used before the temperature rise to 37°C by water-bath.

Pour the trypsin after moist all the cells mildly and put the empty culture dish into an incubator for 40-50 seconds.

Use 10 mL 1640 medium to stop the digestion.

Blow and beat until the cells float.

Draw 5 mL cell suspension to a new culture dish.

Add the match drug concentration of Flutamide 5μl (Table 1) to each dish.

Table 1: Different passage to join different drug concentration.

Lncap Passage	Concentrated Solution	Ingredient Condition	Drug Concentration	Culture Condition	Terminal drug Concentration
0-20	0.2762g Flutamide +10ml Ethyl Alcohol (100mM/ml)	1μl CS*+1ml 1640	0.1mM	1μl DS* + 1ml 1640	0.1μM
20-40		5μl CS+1ml 1640	0.5mM		0.5μM
40-60		25μl CS+1ml 1640	2.5mM		2.5μM
60+		50μl CS+1ml 1640	5mM		5μM

Note*: *CS is short for concentrated solution.

*DS is short for diluted solution.

Incubator until the cells get reach ~70-80% confluence as a monolayer to cell freezing or cell passage.

Acknowledgment

This protocol was developed in the Department of Life and science, Guangxi Medical University. This work was supported by grants from the National Natural Science Foundation of China (8216110292), Guangxi Medical University Youth Science Foundation (GXMUYSF202307), First-class discipline construction-preclinical medicine (DC2300011025).

Competing Interests

The authors declare no competing interests.

References

1. American Cancer Society Cancer Statistics 2021 Report. J Nucl Med 62(3): 12N.
2. Chen M, Ma T, Li J, Zhang HJ, Li Q, et al. (2021) Diagnosis of Prostate Cancer in Patients with Prostate-Specific Antigen (PSA) in the Gray Area: Construction of 2 Predictive Models. Med Sci Monit 27: e929913.
3. Siegel RL, Miller KD, Fuchs HE, Jemal A (2021) Cancer Statistics, 2021. CA Cancer J Clin 71(1): 7-33.
4. Shi XB, Ma AH, Tepper CG, Xia L, Gregg JP, et al. (2004) Molecular alterations associated with LNCaP cell progression to androgen independence. Prostate 60(3): 257-271.
5. Buck SAJ, Koolen SLW, Mathijssen RHJ, de Wit R, van Soest RJ (2021) Cross-resistance and drug sequence in prostate cancer. Drug Resist Updat 56: 100761.
6. Abd Wahab NA, Lajis NH, Abas F, Othman I, Naidu R (2020) Mechanism of Anti-Cancer Activity of Curcumin on Androgen-Dependent and Androgen-Independent Prostate Cancer. Nutrients 12(3): 679.
7. Cai F, Zhang Y, Li J, Huang S, Gao R (2020) Isorhamnetin inhibited the proliferation and metastasis of androgen-independent prostate cancer cells by targeting the mitochondrion-dependent intrinsic apoptotic and PI3K/Akt/mTOR pathway. Biosci Rep 40(3): BSR20192826.
8. Cha S, Shin DH, Seok JR, Myung JK (2017) Differential proteome expression analysis of androgen-dependent and -independent pathways in LNCaP prostate cancer cells. Exp Cell Res 359(1): 215-225.