= BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Assessing the Efficacy of Anti-Cancer Drugs on Organoid Models Derived from Prostate Cancer

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Abstract—It was proven that tumor organoids effectively mirror the phenotypic and genetic traits of the original biomaterial. It was reported that outcomes from drug testing in organoid cultures can accurately represent the clinical response observed in patients. In this study, an organoid culture was derived from biopsy material of prostate cancer (PC). Subsequently, clinical practice drugs, docetaxel and enzalutamide, were tested on this organoid culture. Various techniques for evaluating the efficacy of drugs in vitro were compared. The half-maximal inhibitory concentration of docetaxel was found to be markedly lower compared to that of enzalutamide. However, when tested at clinically relevant concentrations and incubation times, enzalutamide was more effective than docetaxel. Therefore, it is crucial to optimize the testing conditions for drugs on in vitro cultures for their subsequent application in clinical practice.

Keywords: organoids, prostate cancer, docetaxel, enzalutamide, drug test **DOI:** 10.1134/S1607672923700692

INTRODUCTION

According to statistics, more than 1.4 million new cases of prostate cancer (PC) are registered annually in the world, with a mortality rate of approximately 375 thousand [1]. In 112 countries, this type of cancer is the most commonly diagnosed in men [1]. In Russia, prostate cancer ranks second in terms of most common cancer types in men and third in mortality [2].

Currently, there are several approaches and a number of drugs for the treatment of prostate cancer. The choice of a particular treatment approach is determined mainly by the level of disease progression. First of all, when choosing a treatment strategy, castration sensitivity or resistance of prostate cancer, as well as the presence or absence of metastases, are taken into account [3]. Docetaxel and enzalutamide are among the most common drugs used in clinical practice for the treatment of prostate cancer. The mechanism of action of docetaxel is the inhibition of cell division by disrupting the normal function of microtubules [4]. This drug is used for treatment of not only various forms of prostate cancer but also other types of cancer, such as breast cancer, stomach cancer, etc. [4]. Enzalutamide is an antiandrogen, its mechanism of action is to block androgen receptors, thereby preventing the important effect of hormones such as testosterone and dihydrotestosterone on prostate cells, including tumor cells [5]. Enzalutamide, similarly to docetaxel, is effective in the treatment of different types of PC, such as metastatic and non-metastatic castration-resistant PC, as well as metastatic castration-sensitive PC [6].

Today, given the diversity of drugs available for treatment and the problem of drug resistance, individualized approaches to choosing a treatment regimen are becoming increasingly popular. In recent years, 3D patient-derived tumor organoids have become popular models for in vitro cancer research [7]. This became possible due to the fact that tumor organoids effectively preserve the genetic and morphological characteristics of the original patient's biomaterial, making it fairly easy to screen drugs and even identify their mechanisms of action [8, 9]. It was also shown that such cultures can be successfully used to predict patient's response to drugs in clinical practice [10]. All these features make tumor organoids derived from particular patients a promising model for studying sensitivity to certain drugs in order to select an individually tailored treatment plan.

MATERIALS AND METHODS

A primary organoid culture of prostate tumor cells was derived from tumor tissue using the technology described previously [11]. Before performing the cyto-

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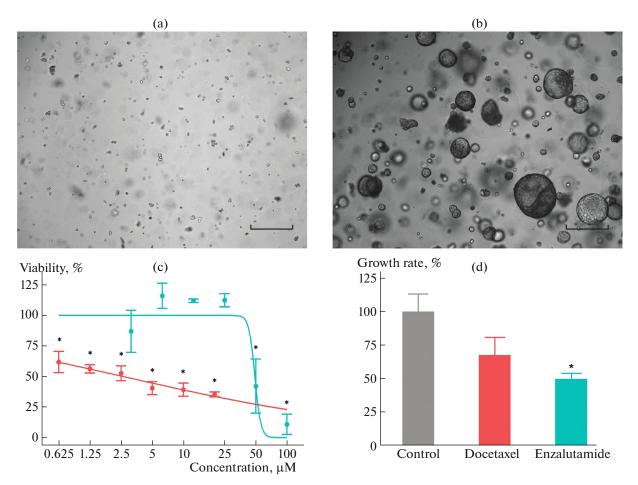


Fig. 1. Micrographs of prostate cancer patient-derived organoids, 2nd passage immediately (a) and 14 days (b) after passing (scale bar 400 μ m), viability of organoids after exposure to docetaxel (red) and enzalutamide (blue) in the concentration range from 0.625 to 100 μ M (c), and growth rate of organoids after exposure to clinical concentrations of docetaxel and enzalutamide (d). * Statistical significance compared to the control (p < 0.05).

toxicity test, organoids were treated with the TrypLE Express dissociating solution, resuspended, transferred into a 1.5-mL tube, and incubated in a thermostat at 37°C for 2–3 min. Then, an equal volume of DPBS was added, organoids were resuspended and centrifuged at 300 g for 4 min, and the supernatant was collected. Matrigel extracellular matrix was added to the pellets, the cells were resuspended, and $10-\mu$ L aliquots of the suspension were transferred into the wells of a 96-well culture plate. The plate was placed in a cell incubator until the gel solidified. Thereafter, $100 \,\mu\text{L}$ of nutrient medium was added to the wells, and the plate was incubated in the cell incubator for 24 h. To assess the cytotoxic effect of docetaxel and enzalutamide, titration with drugs in the concentration range from 0.625 to 100 μ M was performed. After adding the test drugs, the cells were incubated in the cell incubator for 72 h. To assess the inhibition of the growth rate of organoids, docetaxel was added to the culture medium at a concentration of 5.47 μ M, which corresponds to clinical data on pharmacokinetics [12]. The cells were incubated with the drug for 3 h, after which the medium was replaced with a fresh one, and incubation was continued for another 72 h. Similarly, enzalutamide was added at a concentration of 24.55 μ M, and the cells were incubated with it for 72 h [13]. The experiment was performed in triplicate. Cell viability was assessed using the MTS test according to the manufacturer's protocol. Absorbance was measured using a SpectraMax iD3 multifunctional microplate reader. The background absorbance was measured in wells without cells. Viability was calculated using the formula:

$$(A - O)/(A(0) - O) \times 100\%$$
,

where A is the absorbance in the test wells after incubation with the drug; A(0) is the absorbance in the control wells after incubation with the drug, and O is the background absorption. The growth rate was calculated using the formula

 $O_{1}^{[\log_2((A-O)/(A_0-O))]/[\log_2((A-O)/(A_0-O))]} - 1) \times 100\%$

found that the half-maximal inhibition concentration for docetaxel is almost 10 times lower compared to enzalutamide. However, to obtain clinically relevant results, it is important to take into account the fact that the clinical concentration of enzalutamide is almost 5 times higher than the corresponding concentration of docetaxel [12, 13]. Determination of a more sensitive parameter, growth inhibition efficiency, showed that only enzalutamide at clinical concentrations statistically significantly inhibits the growth of tumor organoids. Thus, tumor organoids derived from patients with prostate cancer have the potential for determining the sensitivity of a particular patient to a particular drug if the testing technique is chosen correctly. It should be noted that there are few reports in the literature that the results of organoid tests may correspond to the responses of patients with PC in clinical practice [18]. All this makes organoid models of PC a promising in vitro system that can be useful in deter-

where A_0 is the absorbance in the wells with cells

immediately before adding the drug. The statistical

significance of the observed differences was assessed

RESULTS AND DISCUSSION

with prostate cancer. Microphotographs of organoids

are shown in Figs. 1a and 1b. It should be noted that

derivation of organoid cultures from prostate tumor

cells is technically more difficult compared to many

other types of cancer [14, 15], and the cultivation of

prostate cancer organoids is often limited to only sev-

determine the half-maximal inhibition concentra-

tion (Fig. 1c): $49 \pm 25.8 \ \mu\text{M}$ for enzalutamide and

 $2.6 \pm 0.9 \,\mu$ M for docetaxel. Therefore, it can be concluded that, when standard methods for assessing drug efficacy are used, docetaxel is a more effective anti-

cancer drug. However, the assessment of the growth

rate of organoid cultures under the influence of drugs

in conditions close to clinical showed that enzalut-

amide inhibited the growth rate of tumor organoids

more effectively than docetaxel (Fig. 1d). Enzalut-

amide slowed down the culture growth rate by almost

50% (p < 0.05), whereas docetaxel did not show statis-

tically significant inhibition. It is worth noting that

growth inhibition is considered a more sensitive

parameter because it can be measured independently

of parameters such as cell density or growth rate,

with prostate cancer showed different sensitivity to the

Thus, the organoid culture derived from a patient

which can bias viability measurements [17].

Analysis of the viability of the derived organoid lineages under the treatment with the anticancer drugs docetaxel and enzalutamide made it possible to

In this work, we successfully established an organoid culture derived from a biopsy material of a patient

using analysis of variance (ANOVA).

eral passages [16].

FUNDING

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the local ethics committee of the Herzen Moscow Research Oncology Institute, branch of the National Medical Research Center of Radiology of the Ministry of Healthcare of Russia (no. 488 of March 27, 2020). The patient signed informed consent to participate in the study.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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