Phenotypic Screening Using AMIDA Identifies Different Drug Responses in Breast and Prostate Cancer Cell Lines in an Organotypic Cell Culture Model

AACR 2018
1158

Mervi Toriseva[1], Katja M. Fagerlund[2], Jesse Mattsson[1], Tiina E. Kähkönen[2], Ilmari Ahonen[3], Malin Åkerfelt[1], Jenni Bernoulli[2], Jussi M. Halleen[2], Matthias Nees[1] and Jenni H. E. Mäki-Jouppila[2]
[1]HCSLab, Institute of Biomedicine, University of Turku, Finland; [2]Pharmatest Services, Finland;
[3]Department of Mathematics and Statistics, University of Turku, Finland

Email correspondence to katja.fagerlund@pharmatest.com

Turun yliopisto
University of Turku
HCSLab

pharmatest

Introduction

In patients, tumor cells are always exposed to the surrounding extracellular matrix (ECM) and their three-dimensional (3D) microenvironment. Since these factors are known to affect cellular functions such as gene expression, motility, proliferation, differentiation and drug response, 3D cell culture models are interesting platforms to study drug efficacy in addition to conventional 2D cultures (1). Organotypic 3D cell culture models combined with screening modalities and automated high-content image analyses provide simultaneously a spectrum of biologically relevant information (2,3,4). This includes information about cell growth, cell death, differentiation and tumor cell invasion. Here, we present results from a phenotypic drug screen using prostate cancer cell lines PC-3 and LNCaP, and breast cancer cell lines MDA-MB-231 (SA) and MCF-7 cultured in a miniaturized, Matrigel-based 3D organotypic system.

The aim of the study was to demonstrate that the use of the organotypic 3D cell culture technology, combined with AMIDA automated morphometric image data analysis tool, provides a solid method to study drug responses of cells in a phenotypic, high-content screening platform.

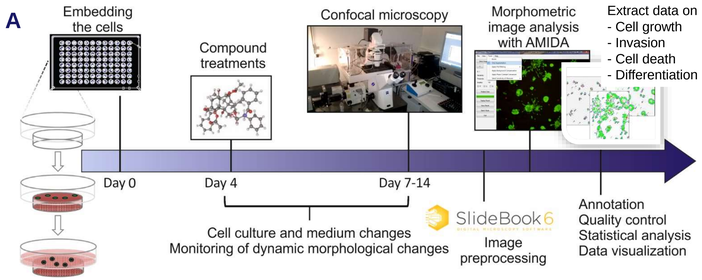
Materials and Methods

Single cells were seeded in 3D Matrigel matrix. The emerging multicellular tumor organoids were treated with the cytostatic drugs doxorubicin, docetaxel and paclitaxel, a selective inhibitor of matrix metalloproteinase-13 (WAY170523), and with Rho-associated protein kinase (ROCK) inhibitors RKI-1447 and Y-27632 starting from the day 4 of culture. Treatments were continued at seven different drug concentrations for 4-10 days. At the end point, confocal live cell images were captured and analyzed using AMIDA. Among others, the numerical data representing cell growth (Area) and cell invasion (Appendages) were visualized and used for statistics

References

1. Elliott NT, Yuan F. A review of three-dimensional in vitro tissue models for drug discovery and transport studies. J Pharm Sci. (2011) 100: 59–74
2. Härmä V, Virtanen J, Mäkelä R, Happonen A, Mpindi JP, Knuuttila M, Kohonen P, Lötjönen J, Kallioniemi O, Nees M. A comprehensive panel of three-dimensional models for studies of prostate cancer growth, invasion and drug responses. PLoS One 2010, 5:e10431.
3. Härmä V, Schukov HP, Happonen A, Ahonen I, Virtanen J, Siitari H, Åkerfelt M, Lötjönen J, Nees M. Quantification of dynamic morphological drug responses in 3D organotypic cell cultures by automated image analysis. PLoS One 2014, 9:e9642.
4. Åkerfelt M, Bayramoglu N, Robinson S, Toriseva M, Schukov HP, Härmä V, Virtanen J, Sormunen R, Kaakinen M, Kannala J, Eklund L, Heikkilä J, Nees M. Automated tracking of tumor-stroma morphology in microtissues identifies functional targets within the tumor microenvironment for therapeutic intervention. Oncotarget. 2015 6(30): 30035-56.

Results



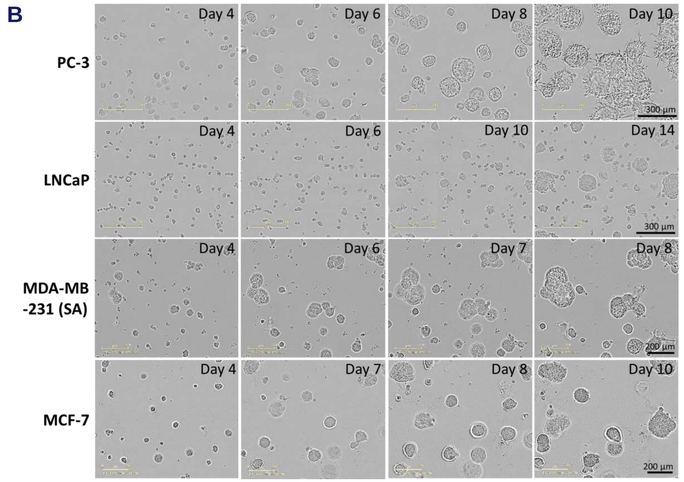


FIGURE 1. Organotypic 3D cultures and phenotypic analysis of tumor cell lines. A) Working pipeline for phenotypic analysis of multicellular organoids in 3D cell culture. The single cells were seeded inside Matrigel and cultured up to 14 days. The phenotypic high-content analysis is based on microscope imaging and morphometric analysis. **B**) The development of cell organoids. PC-3 cells initially form round and well differentiated structures but around day 9 start to show strong epithelial string-like invasion. A subset of MDA-MB-231 (SA) cells developed strong extensions representing collective cell invasion by day 7-8.

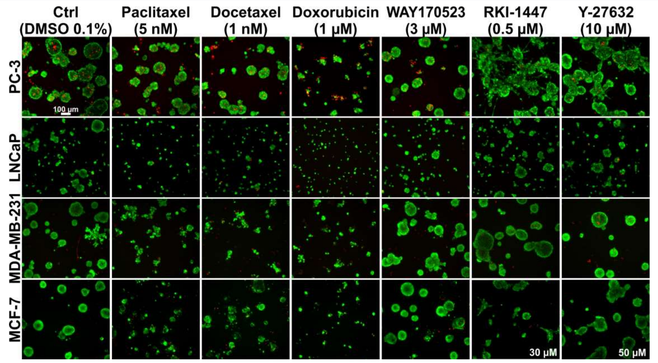


FIGURE 2. Drug-treatments induce different morphologies in prostate and breast cancer cell lines, which can be quantitatively analyzed with AMIDA software. Organotypic cultures were treated with drugs up to 10 days, stained with live-cell dyes, imaged using confocal microscope, and the images were subjected for phenotypic analysis with AMIDA. Green = CalceinAM, living cells; Red = EthD2, dead cells.

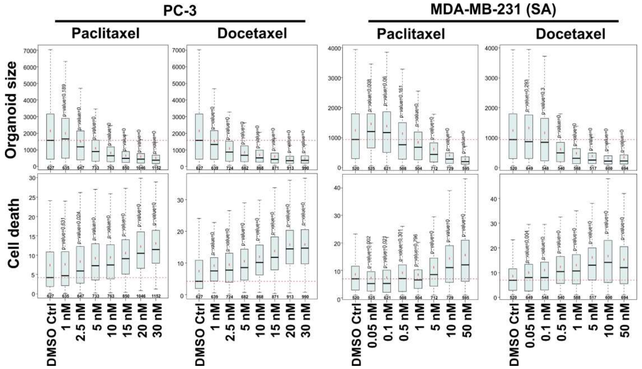
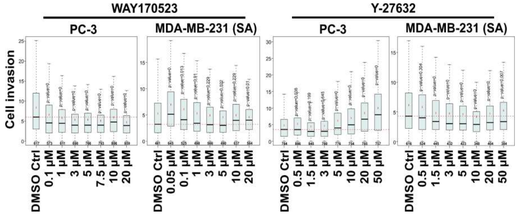


FIGURE 3. Quantitative phenotypic data from PC-3 and MDA-MB-231 (SA) cells, reflecting cell viability. Organoid size (area) was reduced and cell death (red signal) was increased in a concentration dependent manner in organotypic cell cultures treated with Paclitaxel and Docetaxel. Similar effects were seen in LNCaP and MCF-7 cells.

TABLE 1. EC50 values. EC50 calculations were performed for the normalized Area-values using the nonlinear 4PL curve modelling.

	PC-3 (6 days)	MDA-MB-231 (SA) (4 days)
Doxorubicin	91 nM	268 nM
Docetaxel	2.1 nM	0.3 nM
Paclitaxel	4.1 nM	2.7 nM



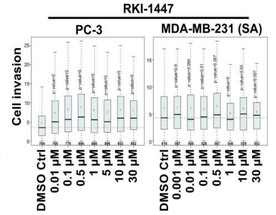


FIGURE 4. Quantification of cell invasion by AMIDA. MMP-13 inhibitor WAY170523 reduced invasive cell extensions of PC-3 cells but had no effect on MDA-MB-231 (SA) cells. ROCK-inhibitors RKI-1447 and Y-27632 both enhanced invasive morphology of PC-3 cells, whereas in MDA-MB-231 (SA) cells Y-27632 reduced invasion. At high concentrations, both inhibitors induced formation of invasive cell extensions in normally non-invasive MCF-7 cells (see Fig. 2).

Conclusions

- **Organotypic cultures and high-content phenotypic screening provide valuable information on variety of drug effects in prostate and breast cancer cell lines.**
- **MDA-MB-231 (SA) cells are more sensitive to taxanes than PC-3 cells.**
- **PC-3 and MDA-MB-231 (SA) cells show different responses to an MMP-13 inhibitor and ROCK-inhibitors, demonstrating fundamental differences in their invasion mechanisms.**