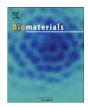
Biomaterials 33 (2012) 9087-9096

Contents lists available at SciVerse ScienceDirect

Biomaterials



journal homepage: www.elsevier.com/locate/biomaterials

Influence of a three-dimensional, microarray environment on human Cell culture in drug screening systems

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ARTICLE INFO

Article history: Received 17 August 2012 Accepted 29 August 2012 Available online 19 September 2012

Keywords: 3D cell culture Microarray High-throughput Drug resistance

ABSTRACT

We have used a modified 3D cellular microarray platform for the high-throughput analysis of growth, cytotoxicity, and protein expression profile of a human hepatocellular carcinoma cell line, HepG2, in alginate. The results obtained were compared to analogous studies in 2D and 3D environments at the microtiter scale. The antiproliferative effects of four drugs, tamoxifen, 5-fluorouracil, doxorubicin, and amitriptyline, were studied as a function of seeding density in the three different culture platforms. The chemosensitivity of HepG2 cells to all four compounds decreased substantially with increasing cell number in the 2D and 3D microtiter-based cultures, while no seeding density dependence was observed in the IC_{50} values obtained in the 3D microarray culture platform. These results can be rationalized based on the development of confluence-dependent resistance in cultures where proliferation is restricted by cell-cell contacts and nutrient availability, as is the case for both of the microtiter-based cultures. Additionally, further development of an on-chip, in-cell immunofluorescence assay provided quantitative data on the levels of specific target proteins involved in proliferation, adhesion, angiogenesis and drug metabolism, and was used to compare expression profiles between 2D and 3D environments. The upregulation of several CYP450 enzymes, β1-integrin and vascular endothelial growth factor (VEGF) in the 3D microarray cultures suggests that this platform provides a more in vivo-like environment allowing cells to approach their natural phenotype.

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1. Introduction

A drug candidate in Phase I clinical testing often requires a decade of discovery followed by preclinical evaluation, yet still has only an 8% chance of reaching the bedside [1]. A leading cause for the failure of drug candidates during clinical trials, and even after a drug has been introduced into the market, is adverse toxicity that was not predicted by animal models [2]. Moreover, the ethical issues and financial constraints surrounding the use of animalbased models in drug screening and toxicity testing have placed increasing pressure to transition testing to in vitro, human cell-

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based assays that are inexpensive, faster, and potentially more predictive than the current animal testing paradigm [2–4].

Cell-based assays can facilitate evaluation of a target molecule in a cellular context at an early stage in drug discovery by simultaneously providing information on multiple biochemical and biological end-points, such as proliferation, chemoresistance, motility, differentiation, cell shape, drug absorption, metabolism, and protein expression and localization [5]. These assays are not only rich in information, but are often amenable to automated, highthroughput (HT) screening, reducing screening cost and time, and improving accuracy. However, there remain significant challenges in developing in vitro cell-based models that can recapitulate the in vivo tissue environment to evaluate biologically complex processes. One step toward developing more realistic culturing models is to constrain cells to a more in vivo-like, three-dimensional (3D) environment. This should facilitate cell-cell communication. and can be useful in determining how the cells perceive, interpret. and respond to cues from their microscale environment.



^{0142-9612/\$ -} see front matter © 2012 Elsevier Ltd. All rights reserved. http://dx.doi.org/10.1016/j.biomaterials.2012.08.065