Cytotoxic Differences of Anticancer Drugs on 2D and 3D Cancer Cell Lines

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Abstract

Cancer and multidrug resistance became increasingly a concern in health care outcomes, since it was founded that the monolayer of 2D cancer cell cultures lack many important features; 3D cell cultures have the advantage of resembling tumors in their characteristics of growth, proliferation, and in their response to treatment via enhancing cell-cell interaction mimicking the *in-vivo* microenvironment. This study was designed to first produce 3D models using different techniques to compare the cytotoxic effect of anticancer drugs on 2D and 3D models. This study involved the following cell lines: A549, H1299, MCF-7, MDA-MB-231, PC-3, and DU-145, treated under the same conditions by: colchicine, cisplatin, paclitaxel, and doxorubicin. For 2D cell culture, these cells were cultivated in 96 well plates for 24 hours, then treatments were applied in serially diluted quantities for 48 hours, and the IC_{50} values were determined using the MTT assay.

Afterwards, these IC_{50} values were used to study *PIK3CA/AKT1* genes expression by PCR. While 3D cell culture was achieved by two methods: the first one is by simple rotation technique, and the second one is by 3D molds, both methods aimed at generating spheroids to be treated and tested to determine the IC_{50} values and the changes in expressing the genes of interest when forming the cells in 3D structure. By 3D cell culture, the anticancer agents achieved the same inhibitory level as the 2D method but at higher concentrations, for example, H1299 cell line when treated with colchicine in 2D and 3D model had IC_{50} (21.4µM and 34µM, respectively), and when treated with PTX in had IC_{50}= 5µM in 2D model and IC_{50}=14.7µM in 3D model. Additionally, the results of *PIK3CA* gene expression levels in H1299 also showed high fold change in 3D model in comparison with its extent in 2D model when treated with doxorubicin. PTX didn't cause PIK3CA overexpression in 2D, while surprisingly it did in 3D model, which can be explained by needing higher doses to exert the needed effect and to trigger gene pathway overexpression, in addition, showing activity in 3D model suggests more possibility in exerting the same effect if studied *in vivo*. It is reasonable to conclude that 3D cell cultures would be more promising than traditional 2D cell culture methods to represent the *in-vivo* molecular changes in response to different potential treatments and multidrug resistance development. Further studies may be required to assess the effect and side effect of using higher IC_{50} values *in vivo*, targeting *PIK3CA/ AKT* pathway in 3D models.

Keywords: Anticancer drugs, *PIK3CA/AKT* pathway, inhibitory concentration (IC_{50}), three-dimensional (3D) cell culture, two-dimensional (2D) cell culture.