Impact of anticancer drug combinations on small cell lung cancer cell lines using 2D and 3D models.

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Abstract

Research on drug combinations contributes to the development of novel lung cancer treatment approaches. The cultivation of cancer cells in 2D and (tumor spheroids) 3D cultures have an impact on the way cancer cells respond to chemotherapeutic treatments as well as gene expression. Tumor spheroids (3D) are more realistic models than conventional (2D) cultures for analyzing cellular responses to chemotherapy treatments. As spheroid cell is more closely mimic the real *in vivo* tumor microenvironment, as it facilitates cell-cell and cell-matrix interactions, in addition to provide nutrient, oxygen gradient and natural cell structure. Our objectives in this study to investigate the effect of BOR on solid tumors as lung cancer, since it is only approved for myeloma by FDA, but there are current studies on solid tumors. In addition to Discuss the use of DOX and BOR

in lung cancer cell lines as well as an emphasis on the multidrug resistant H69AR. Also to emphasize the importance of using 3D cell culture over 2D in terms of sensitivity, resistance, genetic alternations, and drug response. Moreover, combining DOX with BOR for the first time using *in vitro* models may demonstrate if these combination regimens can offer additive/synergistic effects compared with the single treatments, or if they could increase resistance or improve sensitivity of the A549, H69 and/or H69AR cell lines.

In this study 2D and 3D models assays of different lung cancer cell lines were done; namely small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) cell lines. SCLC cell lines used were the H69 (sensitive to doxorubicin (DOX)) and H69AR (resistant to DOX) along with A549 NSCLC cell line. The anti-proliferative effects of Bortezomib (BOR) and DOX were investigated separately as single agents and in combination using 2D and 3D cell culture models on those cell lines. Since the FDA has only approved BOR for non-solid tumors, research is now being done to determine its impact on solid tumors such as lung cancer.

The effect of the drugs on the proliferation of lung cancer cells was determined by performing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Resazurin assay in 2D and 3D cells. Polymerase Chain Reaction (PCR) was performed to explore and quantify the fold change in the expression of apoptotic genes, such as *PIK3CA, AKT, BCL-2, NFκB1* and *PTEN*. Drugs combination demonstrated significant synergistic effects in sensitive H69 and A549 cell lines at both 2D and 3D models, whereas in resistant H69AR cell line synergistic effects was seen at 3D model only, based on combination index (CI) calculations. Furthermore, combination therapy provided significantly greater efficacy as measured in comparison with treatment with BOR or DOX alone. Moreover, it was found that the gene expression of proapoptotic genes was notably upregulated, whereas the gene expression of the anti-apoptotic *NFκB1* gene was

significantly downregulated in both lung cancer models (2D and 3D) treated with BOR with p values (p**<0.01 for H69 and A549, p***< 0.001 for H69AR) for 2D cells, and (p**<0.01 for H69AR, p****<0.0001 for A549) for 3D cells, and cells treated with the combined therapy with p values of (P****<0.0001, p***<0.001, p*<0.05 for H69AR, H69 and A549 cell lines ,respectively) for 2D cells and (P**<0.01 for H69AR, p***<0.001 for A549) for 3D cells. PCR implicated the *BCL2* and *NFκB1* expression as a potential mechanism for this enhanced combination effect. It is reasonable to conclude that 3D cell cultures would be more promising than 2D cell culture methods to represent the *in-vivo* cellular and molecular changes. Further studies may be required to assess the efficacy *in vivo*. It also determined that compared to using BOR and DOX alone, the combination therapy increases the efficacy of both drugs at lower concentrations. This combination was used for the first time for this type of cancer and showed very promising results.

Keywords: 3D cell culture, Bortezomib, *BCL2*, Combined therapy, Doxorubicin, gene expression, Lung cancer, *NFκB1*.