Development of Three-Dimensional Lung Multicellular Spheroids for the Evaluation of Anti-Cancer Therapeutics

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ABSTRACT SUMMARY

Three-dimensional lung multicellular spheroids (MCS) in both air and liquid interface culture have been developed for the evaluation of anti-cancer therapeutics. The MCS were formed by seeding lung cancer cells on top of collagen where they formed spheroids after approximately one week. The effect of paclitaxel was evaluated for both the liquid covered culture (LCC) and air interface culture (AIC) conditions. LCC MCS were exposed to paclitaxel in media whereas AIC MCS were exposed using phospholipid particles containing paclitaxel which were designed for the treatment of lung cancer. The difference in 2D versus 3D culture viability was evaluated along with the effects of the particles on lung epithelium via transepithelial electrical resistance (TEER) measurements.

INTRODUCTION

People in the United States die from lung cancer more than any other cancer diagnosis, and there is an urgent need for more effective therapy options. In vitro models are frequently utilized in analyzing the effectiveness of treatment of anti-cancer therapeutics. In lung cancer specifically, there are many characteristic elements of a tumor that require an in vitro model to be designed in order to make the model as physiologically representative as possible. Applying in vitro outcomes to in vivo applications has limitations because conventional two-dimensional (2D) cell culture does not recreate a physiologically representative model for cells. This work investigated a three-dimensional (3D) cell culture technique to model lung tumors in vitro. This model allowed for 3D MCS to be grown in AIC conditions, which mimics the way tumors would grow in the body. This work also allowed for a direct comparison to conventional 2D cell culture as well as 3D LCC conditions to reveal the many factors that can affect the response of tumor cells in vitro.

EXPERIMENTAL METHODS

Cell seeding and MCS formation. Both H358 and A549 lung cancer cell lines were grown in DMEM with FBS. For 2D studies, H358 and A549 cells were seeded at 75,000 and 30,000 cells/ml, respectively. For 3D studies, they were seeded at 40,000 and 15,000 cells/ml, respectively. 3D MCS were formed on collagen gels (rat tail type I) formed from a 3 mg/ml solution. AIC conditions were initiated by growth in Transwells, removing the media on the apical side one day after seeding and replacing the basolateral media periodically. Both LCC and AIC MCS formed within 9 days. A schematic demonstrating these two models can be seen in Figure 1.

Drug Exposure. 2D and 3D LCC cells were exposed to paclitaxel in media at concentrations up to 1 µM for 72 hours. AIC cells were exposed to paclitaxel in 1 mg of phospholipid particles applied directly to the cell surfaces up to 0.2 mg paclitaxel to each well. A resazurin assay was used to evaluate viability in which corresponding IC50 values were calculated.
TEER. TEER was evaluated using an epithelial voltohmeter (EVOM²). Calu-3 lung cells were seeded into Transwells at 5 x 10⁵ cells/ml and were grown until the TEER values were steady. At this point, the cells were exposed to either paclitaxel in media (for LCC) or particles with or without paclitaxel (for AIC). The TEER was the evaluated after 4 and 24 hours.

RESULTS AND DISCUSSION

Lung MCS formed for both cell types after 9 days and were viable upon staining with calcein AM. A549 spheroids were 200-300 µm in diameter whereas H358 were 100-150 µm. Growth in LCC and AIC resulted in spheroids of similar size and morphology for both cell types as seen in Figure 2 demonstrating a robust in vitro MCS model using two different cell types. As seen in Table 1, IC50 values for LCC conditions increased for 3D MCS for both cell types, showing a significant difference in the way 2D versus 3D cultures will respond to paclitaxel. This is despite the fact that exposure to the paclitaxel for 72 hours dissociated many of the spheroids in culture as shown by the resulting diameters at day 12 in Figure 3. Initial AIC studies indicate a similar trend in viability upon exposure to phospholipid particles containing paclitaxel (data not shown).

Initial TEER results indicate that the particles do not negatively affect the Calu-3 monolayers when grown in AIC conditions indicating that the particles are safe upon delivery to the lung epithelium.

CONCLUSION

Overall, these results demonstrate a robust and easy model of lung multicellular spheroids comprised of two different types of lung cancer cells lines. They can be successfully formed in both LCC and AIC conditions for a 2D versus 3D comparison. Initial results indicate a significant difference in the response of 2D versus 3D cells and demonstrate that the model particles are safe for use in the delivery of anti-cancer therapeutics.

REFERENCES


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