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Developing robust heterotypic 3D lung cancer cultures for drug screening: preliminary results

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Abstract

Background: To propel advancements in cancer treatment, it is imperative to conduct initial drug testing using *in vitro* models. In laboratory settings, the efficacy of most compounds is rigorously evaluated through 2D cell cultures. However, many compounds showing promise in these models fail to perform similarly in preclinical or clinical trials, prolonging the timeline for developing effective drugs for human use [1,2]. Therefore, substantial research efforts are focused on creating *in vitro* models that better mimic the *in vivo* tumor microenvironment, as demonstrated by the exploration of 3D cell culture systems [3]. **Objective:** to implement a standard protocol for heterotypic 3D lung cancer cultures, aiming to provide a more effective alternative for anticancer drug screening. **Methods:** Monocytes were polarized into macrophages 72 hours prior seeding by adding phorbol-12-myristate-13-acetate. A549 lung cancer cells were then co-cultured with polarized macrophages (THP-1), lung fibroblasts (IMR-90), and lung endothelial cells (HPMEC) on ultralow attachment plates and monitored for 10 days. Spheroids were photographed on days 2, 4, and 6 post-seeding. **Results:** During the initial protocol standardization, different cell ratios were tested: i) 10,000 cells/well with a ratio of 1:3:3:10 for A549, THP-1, IMR-90, and HPMEC, respectively; ii) 8,000 cells/well with a ratio of 3:3:3:10; and iii) 10,000 cells/well with a ratio of 3:3:3:10. Surprisingly, none of the ratios consistently generated a single spheroid. To address this issue and aid spheroid compaction, a centrifugation step was introduced immediately after plating at either 1000 RPM for 10 minutes at 22°C or 4000 RPM for 10 minutes at 22°C. Notably, centrifugation at 4000 RPM for 10 minutes at 22°C proved most effective in producing single, compact, robust spheroids with an appropriate diameter (>350 nm). **Conclusions:** Our initial findings indicate successful development of single heterotypic 3D lung cancer spheroids. Standardization of histological analyses is ongoing, and further experiments will be undertaken to characterize this novel *in vitro* model of lung cancer.

Keywords: 3D heterotypic cell culture; lung cancer; drug screening; protocol standardization

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