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Poster

Establishment of Patient-Derived Organoid Model from Hydrothorax and Ascites

Research Field

Novel Organoid Model

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Background & Objective

Organoids are miniaturized organs cultured in vitro, exhibiting self-renewal and self-organization capabilities. In comparison to traditional 2D cell cultures, organoids can reproduce the spatial structure and functionality resembling corresponding tissues or organs, providing highly physiologically relevant in vitro systems.

Methods

Consequently, they can be utilized for simulating disease occurrence, testing the tumor drug sensitivity and co-culturing with other cells . This study aims to establish a Patient-Derived Organoid (PDO) model using hydrothorax and ascites samples. To ensure cell viability, it is essential to store the collected malignant fluid at 4°C. Upon arrival at the laboratory, the fluid should be divided into centrifuge tubes for subsequent centrifugation, allowing the collection of cell precipitates. These cell precipitates need to be washed multiple times with DPBS until the supernatant appears clear. If the pellet contains red blood cells, they should be lysed, followed by cell counting. Depending on the desired seeding density, cells should be resuspended in Matrigel and plated as domes in a culture plate. The plate should then be inverted and placed in a 37°C incubator for 30 minutes to allow the Matrigel to solidify. Once solidified, complete culture medium should be added, and the culture plate should be placed in a 37°C incubator for further incubation.

Results

Organoid growth can be monitored using microscopy to assess their development and progression. Once the organoids have reached a stable growth state, immunohistochemistry can be performed to identify specific cell types or markers within the organoids.

Conclusion

Furthermore, the drug responsiveness of the organoids can be evaluated through cell viability assays, enabling researchers to determine the effects of different drugs or treatments on organoid viability and function.

Limitations

The organoids derived from thoracoabdominal fluid could not simulate the immune microenvironment.

Funding for this study

This study was supported by National Natural Science Foundation of China (Grant/Award Number: 82172679), and Zhejiang Provincial Medicine and Health Science Foundation (Grant/Award Number: 2021KY010).

Figures

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Establishment of Patient-Derived Organoid Model from Hydrothorax and Ascites

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Abstract

Organoids are miniaturized organs cultured in vitro, exhibiting self-renewal and self-organization capabilities. In comparison to traditional 2D cell cultures, organoids can reproduce the spatial structure and functionality resembling corresponding tissues or organs, providing highly physiologically relevant in vitro systems. Consequently, they can be utilized for simulating disease occurrence, testing the tumor drug sensitivity and co-culturing with other cells . This study aims to establish a Patient-Derived Organoid (PDO) model using hydrothorax and ascites samples. To ensure cell viability, it is essential to store the collected malignant fluid at 4℃. Upon arrival at the laboratory, the fluid should be divided into centrifuge tubes for subsequent centrifugation, allowing the collection of cell precipitates. These cell precipitates need to be washed multiple times with DPBS until the supernatant appears clear. If the pellet contains red blood cells, they should be lysed, followed by cell counting. Depending on the desired seeding density, cells should be resuspended in Matrigel and plated as domes in a culture plate. The plate should then be inverted and placed in a 37℃ incubator for 30 minutes to allow the Matrigel to solidify. Once solidified, complete culture medium should be added, and the culture plate should be placed in a 37℃ incubator for further incubation. Organoid growth can be monitored using microscopy to assess their development and progression. Once the organoids have reached a stable growth state, immunohistochemistry can be performed to identify specific cell types or markers within the organoids. Furthermore, the drug responsiveness of the organoids can be evaluated through cell viability assays, enabling researchers to determine the effects of different drugs or treatments on organoid viability and function.

Acknowledgment

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Figures and Legends

Figure1. The sample should be stored at 4℃. When transported to the laboratory , divide it into centrifuge tubes for centrifugation to collect cell precipitates

Figure2. Wash these cell precipitates multiple times with DPBS until the supernatant appears clear.

Figure3. If the pellet contains red blood cells, lyse erythrocytes by RBC lysis buffer. After lysis , fill the Falcon tube with DPBS, centrifuge and discard the supernatant.

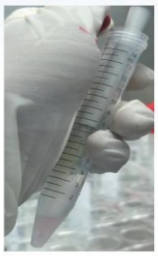

Figure4. Count cells ,resuspend the pallet of cells in ice-cold complete organoid medium , and add twice the volume of Matrigel. *Matrigel needs to be kept ice-cold at all times.

Figure5. Plate cell-Matrigel drops in a 24-well culture plate(50μL per drop).Place the plate in a 37℃ incubator for 30 min ,to let the Matrigel solidify.

Figure6. Place plate in upright position and add complete organoid medium . Fill other wells with DPBS to prevent evaporation of the culture medium . Placed plate in a 37℃ incubator for further incubation .

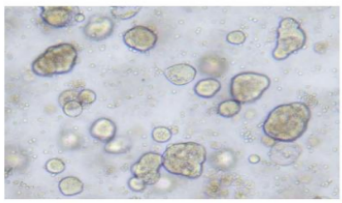

Figure7. Organoid growth can be monitored using microscopy to assess their development and progression. Replace the medium with complete organoid medium twice per week .