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**The generation of renal cell carcinoma - kidney assembloids: A preclinical platform for anticancer drug screening and biomarker assessment in tumor microenvironment**

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**Objectives :** Tumor microenvironment (TME) is a complex and continuously evolving entity. It includes not only the tumor cells, but also diverse supporting cell types, such as activated fibroblasts, blood vessels, infiltrating immune cells, and extracellular matrix. We will implement this. RCC-kidney assembloids will provide a robust preclinical tool for drug screening and biomarker assessment in line with alternative ex vivo methods like tumor tissue slice culture or in vivo xenograft models.

**Methods :** We generated the 3D complex RCC spheroids by co-culturing RCC, endothelial cell (EC) and fibroblast. And then we assembled the complex RCC spheroid with kidney organoids, which is the RCC-kidney assembloids. RT-PCR and immunofluorescence analysis were performed. and the feasibility of drug testing for the assembloid was confirmed through treatment with anticancer drugs. Then, it was transplanted into the kidney capsule of a NOD-SCID mouse, and engraftment and RT-PCR histological analysis were performed.

**Results :** The expression of assembloids mRNA was significantly increased ccRCC standard included CA9, PECAM1, ENPP3, FABP7, KISS1R, OCT4, and SIX1 except for LOX and EGLN3 compared with ccRCC spheroid with or without LF+EC. It was confirmed that more cells died in the RCC spheroid position than in the kidney organoid position when treated with anticancer drugs. After transplantation into NOD-SCID mice, kidney organoid and cancer marker staining revealed successful engraftment, and RT-PCR results indicated an increase in the expression level of cancer markers.

**Conclusions :** As a result of treating the assembloid we produced with anticancer drugs, a greater number of dead cells were detected in the RCC spheroid compared to the kidney organoid. When transplanted into NOD SCID mice, the engraftment was successful, and RCC-related markers increased. Therefore, assembloids can serve as powerful preclinical tools for drug screening and biomarker evaluation, aligning with alternative in vitro methods such as tumor tissue slice culture or in vivo xenograft models