Three-dimensional (3D) co-culture system for organoids plus tissue infiltrating lymphocytes (TILs) derived from patient benign normal and hyperplastic proliferative ureter specimens

Lee, Sanghee, Muldong, Michelle, Kang, Sung Gu, Hsu, Jonathan, Kane, Christopher, Salmasi, Amirali, Jamieson, Christina

BACKGROUND: Three-dimensional (3D) co-culture systems recapitulates *in vivo*-like autocrine and paracrine signaling in culture systems which retain the cellular heterogeneity of the original tissue. However, such systems have not been established for benign inflammatory changes in ureter. We established a 3D organoids and tissue infiltrating lymphocytes (TILs) co-culture system to model host-immune interplay in normal ureter and ureter with benign proliferative changes in human.

METHODS: 10 mg of normal tissue and 10 mg of tissue with benign proliferative changes (abnormal) were harvested from upper ureter from a 78-year-old female patient. 3D organoid and TILs cultures were prepared and maintained for three weeks separately. Host-immune interplay of benign ureter was modeled in co-culture of 3D ureter organoids and 480,000 TILs. The TILs from abnormal tissues were not proliferating as much as the TILs from benign normal, therefore, TILs were added as a 1:1 mixture of TILs from benign normal and abnormal tissues to have total of 480,000 TILs, a pre-determined number for successful co-culture. Four areas within each co-culture plate were imaged by EVOS microscope at 0, 4, 28, 56, 84 and 91 hr. 3D organoids alone, co-cultured organoids plus TILs were processed for H&E staining and Immunohistochemistry using antibodies specific to Keratin 5 (CK5), P63 and Uroplakin III (UpkIII).

RESULTS: The abnormal tissues were later determined by the pathologist to be benign hyperplasia. 3D organoid cultures of benign normal vs benign hyperplastic proliferation of ureter contained a mix of cell masses. Strikingly, microscopic imaging of co-cultures revealed that TILs migrated into the matrigel dome and finally infiltrated ureter organoids. TILs retained their original ability to infiltrate host benign normal ureter organoids even after their surrounding environment had changed. Ureter organoids were CK5 and P63 positive but Upk III negative. A functional consequence of TILs infiltration of the organoids was a morphological change in organoids structure, an irregular boarder of organoids and an extrusion of luminal components. Both types of TILs infiltration were observed in both benign normal vs benign hyperplastic proliferation ureter.

CONCLUSIONS: Our study provides the first patient-derived model of benign ureter organoids plus TILs which maintained functional and cellular phenotype of urothelial cells and TILs.

Funding: The Leo and Anne Albert Charitable Trust Foundation, The JM Foundation