

VHL-mutant renal cell carcinomas contain cancer cells with mesenchymal phenotypes

Craig Gedye, Danylo Sirskyj, Nazleen Lobo, Andrew Evans, Neil Fleshner, Michael Robinette, Alexandre Zlotta, Robert Hamilton, Girish Kulkarni, Antonio Finelli, Brenda L. Gallie, Michael A.S. Jewett, Laurie Ailles.

Background: To study “cancer stem cells” it is imperative to account for all stromal cell populations within the tumour. The existence of “cancer stem cells” in clear cell renal cell carcinoma (ccRCC) has not been examined in *ex vivo* patient samples.

Methods: We established a multiplex flow cytometry (FC) antibody panel in ccRCC, which reliably identified stromal lineages including CD45+ immune, CD31+/CD144+ endothelial and fibroblast-marker-positive subpopulations, thus allowing isolation of “lineage-negative” tumor cells. To verify the identity of tumour-derived populations as either cancer cells or normal stromal cells, we took advantage of the fact that mutations in *VHL* occur early during ccRCC tumorigenesis and are found in two-thirds of patients.

Results: We sequenced 18 patient tumor samples, 12 of which had *VHL* exome mutations. Targeted re-sequencing of FC sorted subpopulations from these patients’ samples revealed that while CD45+ immune cells and CD31+/CD144+ endothelial cells were genetically normal, a population of *VHL*-mutant fibroblast-marker positive cells was consistently identified in every patient’s tumour.

Immunohistochemistry showed that fibroblast marker-positive *VHL*-mutant cells do not have the large “clear cell” morphology typical of the majority of the cancer cells in these tumours. When purified and cultured, these fibroblast marker-positive *VHL*-mutant cells proliferate extensively under mesenchymal culture conditions, but displayed different morphologies to lineage-negative *VHL*-mutant tumor cells. Functional characterization of these FC sorted cell subpopulations is ongoing, including proliferation, migration, invasion, differentiation and treatment resistance.

Conclusions: The phenotype and preliminary functional characterization of these *VHL*-mutant fibroblast-marker positive cells suggests a mesenchymal differentiation program in ccRCC, with implications for the ontogeny, biology and clinical management of *VHL*-mutant renal cancer.