Repository and Inventory of Human Renal Tumor Cell Lines for Urologic Oncology Research

-Establishment and Characterization of the First Fumarate Hydratase (-/-) Hereditary Leiomyomatosis Renal Cell Carcinoma Cell Line

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Abstract

The Human Renal Tumor Cell Line Repository is established by NCI-Urologic Oncology Branch (UOB) and provides scientists with the cell lines as both *in vitro* and *in vivo* models for studying kidney cancer at the preclinical, genetic, cytogenetic and molecular levels, for accelerating discoveries of the regulatory pathways and functions of disease genes.

The primary function of the resource is to establish and manage the cell lines from *in vivo*-derived human operative tumor specimen and *in vitro*-established tumor cell lines to assist investigators with cell culture-based research models. All cell lines in the repository are derived from patients' tissue specimen with informed consent according to the tissue procurement protocol approved by the Institutional Review Board (IRB). The repository is managed via *BioFortis* software. All the cell lines are described with UOB patient clinical annotations cited from CRIS and the repository is linked to Labmatrix™ database, which is part of a bioinformatics network with tissue bank recording system at the UOB tissue procurement core facility.

The repository not only provides extensively-multilevel-characterized, contaminant-free, immortalized cell lines, which is clearly an invaluable resource for the cancer research, but also ethical and legal documents associated with the cell lines and related clinical annotations, both having been subjected to rigorous quality controls by the UOB repository team.

Introduction

Under NCI's many initiatives, the Urologic Oncology Branch (UOB) has been focused on studying kidney cancer at the clinical, genetic and molecular levels. The research of more than two decades has resulted in the identification and characterization of critical genes - *VHL*, *Met*, *FH*, and *BHD* - each related to a different type of sporadic and hereditary renal cancer. Cell lines derived from these cancers are valuable tools for elucidating the mechanisms of the gene pathways.

Materials & Methods

- The repository is managed using BioFortis' Labmatrix (V3.5) software. Each individual cell line is described with patient's clinical annotations, including tumor size, histopathologic features, gene mutation analysis, which will be part of a bioinformatics network with matched tumor tissue database under UOB's Labmatrix database system.
- All cell lines in the repository are derived from patients' tissue specimen with informed consent according to tissue procurement protocols approved by the Institutional Review Board (IRB).
- An example of the characterizations of our recently-established first hereditary leiomyomatosis renal cell carcinoma (HLRCC) cell line, designated as UOK 262, is shown here, with all histopathologic, ultrastructural, physiologic, immunohistochemistry, molecular biochemistry and cytogenetics analysis conducted.

Results

Histopathologic and Ultrastructural analysis of HLRCC tumors and xenograft derived from UOK 262 cells

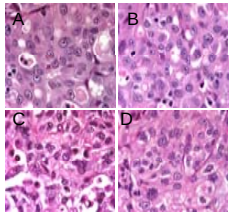


Fig. 1 Hematoxylin-eosin staining of tumor tissue from a patient with HLRCC and from a tumor xenograft: (A) Right renal tumor mass; (B) Right retrolcaval lymph node; (C) Xenograft derived from cells taken from patient's lymph node (UOK 262 LN-XRP); (D) Xenograft derived from UOK 262 cells ($3x10^6$).

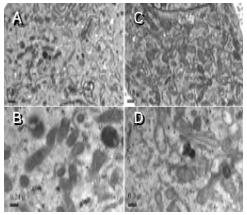
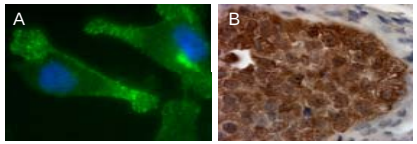


Fig. 2. EM images of mitochondria from normal human renal cortical epithelial (HRCE) cells (A & B), and from HLRCC tumor cells, UOK262 (C & D). Mitochondria of tumor cells are edematous and show disruption of internal membrane cristae. Magnification indicated as a scale bar on each image.

UOK 262 cells overexpress genes and proteins involved in energy metabolism



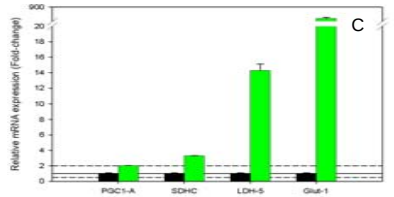


Fig. 3 Immunohistochemistry staining of UOK 262 cells and real time PCR analysis of gene expression. (A) GLUT-1 protein is strongly expressed at branching tumor cell membrane; (B) LDH 5 is strongly expressed in a xenograft of UOK 262 cells; (C) Comparison of gene expression in UOK262 cells with HRCE cells (left black columns).

Results

UOK 262 cell's proliferation and survival are glucose dependent

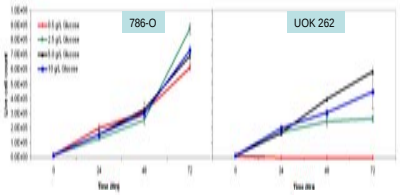


Fig.4.The *in vitro* growth of UOK 262 and 786-O cells in 0.5g/L, 2.5g/L, 5g/L, and 10g/L D-glucose. The cells' proliferation and survival were glucose dependent.

UOK 262 cells display higher Glycolysis rate and lost their mitochondrial respiration capability

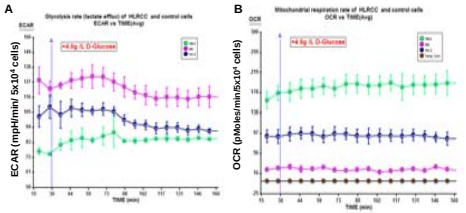


Fig.5. Glycolysis rate and mitochondrial respiration rate of UOK262 and 2 control cells. A: Basal extracellular acidification rate (ECAR). B: Basal cellular respiration rate (OCR). Results were express means ±SD; n=5. Both rates were normalized against cell number and expressed as rate per 5x104 cells.

Mutant *FH* gene is expressed and protein is present, but lacks catabolic activity in UOK 262 cells

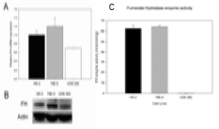


Fig. 6. RT-PCR (A) and Western blot (B) shows that both mutant *FH* gene and protein are present. Enzyme activity of FH in UOK 262 cells (C) is undetectable. Enzyme activity is expressed as 1 unit=1000 nmol/min/mg protein.

Characteristics of invasion of UOK 262 cells

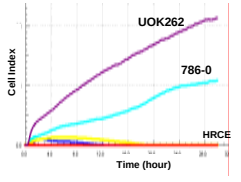


Fig. 7. *In vitro* real-time assay of the invasive potential of UOK 262 cells. UOK 262 is more invasive than 786-O, while normal HRCE cells are not invasive.

Results

UOK 262 cells harbor an *FH* germline mutation at exon 8

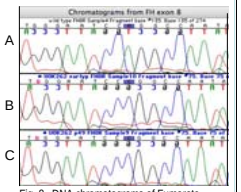


Fig. 8. DNA chromatograms of Fumarate Hydratase (*FH*) gene sequence surrounding nucleotide 1187. (A): Wild type control sequence; (B) and (C): Early (B) and late (C) passages of UOK 262 tumor cell DNA.

Mutant FH is primarily localized to mitochondria of UOK 262 cells

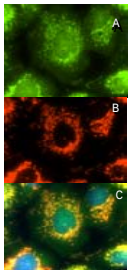


Fig. 9 Double immunofluorescence labeling for protein expression *in situ*. A: Alexa-Fluor®- 488 labeling with mouse monoclonal antibody to human FH; B: Alexa Fluor®- 594 labeling with rabbit antibody to human superoxide dismutase 2 (SOD2) which is with a marker of the mitochondria matrix. C: Overlap of signals obtained with both FH and SOD2 antibodies showing co-localization FH and SOD2.

Cytogenetics analysis: identification of isochromosome i(1)(q10) in UOK 262 cells

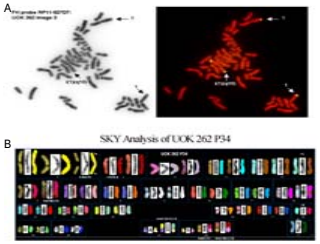


Fig.10. Gene-specific FISH and SKY analysis of UOK 262. (A): *FH* gene genomic probe RP11-527D7 (157 Kb) is present in both normal copies of Chromosome 1 and in an i(1)(q10), band 1q42.3 in sample UOK 262. Karyotyping by SKY (5B.) revealed clonal and multiple numerical and structural aberrations in UOK 262. Composite karyotype: 47, X, -X, +1, i(1)(q10), +5, der(21)t(15;21)(q15;p11.2), +22

Summary

We have maintained an efficient and stable production system by subculturing for the long-term use with fingerprinted genetic background and productivity. Many publications have cited UOB tumor cell lines (such as UOK 257) as unique source of materials. Recently molecular targeting studies have become valuable approaches, by using cell line as excellent model (such as UOK 262), both *in vitro* and *in vivo* xenograft for chemosensitivity and toxicity studies, which incorporates with imaging to immediately evaluate pre-clinical response to therapy. The cell line models could be the basis and ideal platform for identifying effective anticancer compounds that target tumor-specific, metabolic changes caused by cellular or genetic alterations. The repository not only provides extensively-multilevel-characterized, contaminant-free cell cultures of immortalized cell lines, which is clearly invaluable resource for the cancer research, but also ethical and legal documents associated with the lines and related clinical annotation, both subjected to rigorous quality controls by the UOB repository team.