HUMAN TUMOR CELL LINES REPOSITORY WITH CLINICAL AND MOLECULAR CHARACTERIZATION FOR UROLOGIC ONCOLOGY RESEARCH

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Abstract

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.

The primary function of the renal cell carcinoma (RCC) Cell Lines Repository is to establish and manage the RCC cell lines from in vivo-derived human surgical 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 tissue procurement protocols approved by the Institutional Review Board (IRB). The repository is managed through BioFortis' Labmatrix (V3.5) software. The individual cell lines are described with UOB clinical annotations in both FreezerWorksUL2 and FileMaker Pro 8.1 database, which will be part of a bioinformatics network with other renal tumor tissue database at the UOB under the Labmatrix database system. Many publications have cited UOB RCC cell lines as unique source of materials. Recently, tumor cell lines have been used as models in molecular targeting studies, in vitro and in vivo (xenograft) drug sensitivity and toxicity studies, which combines with imaging technology to immediately evaluate pre-clinical response to therapy.

The repository contains not only contaminant-free immortalized cell lines but also extensively-characterized DNA/RNA/Protein samples derived therefrom, both with "high quality" both in terms of biological quality of the samples and in terms of the status of ethical and legal documents associated with the samples and related clinical and molecular annotations, and both subjected to rigorous quality controls by the repository team.

Background

- 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.
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Materials & Methods

- 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).
- The repository is managed through BioFortis' Labmatrix (V3.5) software. The individual cell lines are described with UOB clinical annotations in both FreezerWorksUL2 and FileMaker Pro 8.1 database, which will be part of a bioinformatics network with other renal tumor tissue database at the UOB under the Labmatrix database system.
- An example of the characterization of one of our recently established cell lines is shown below, with all histologic, ultrastructural, immunologic and genetic analysis conducted.

Results

Histologic analysis of the primary tumors

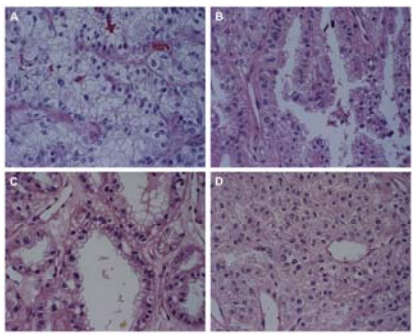

Fig. 1. Light microscopic appearance of different histologic types (A-D), kidney tumor mass samples from a patient with Birt-Hogg-Dube (BHD) syndrome: (A) large areas composed of nests of clear cells with enlarged, round to irregular nuclei with prominent nucleoli and abundant vacuolated cytoplasm, scattered atypical naked nuclei (X400); (B) papillae lined by atypical epithelial cells with granular eosinophilic cytoplasm (X400); (C) atypical lining of the tubular structures (X400); and (D) histologically reminiscent foci of chromophobe renal cell carcinoma (X400).

Results

Histologic analysis of Xenografts

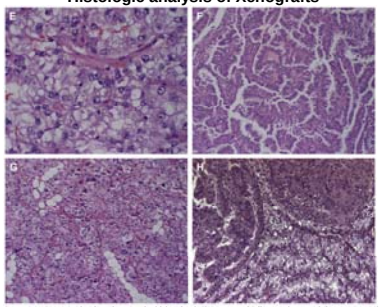

Fig. 1. Tumor xenografts (E-H) revealed histologic features similar to those of the case pathologic report: (E) clear cell areas throughout the tumor (X400); (F) papillary architecture (X200); (G) areas with histologic resemblance to chromophobe (X200); and (H) solid areas of cells with eosinophilic cytoplasm, junction with complex of different histologic type (X200).

Phase contrast microscopy and flow cytometry analysis

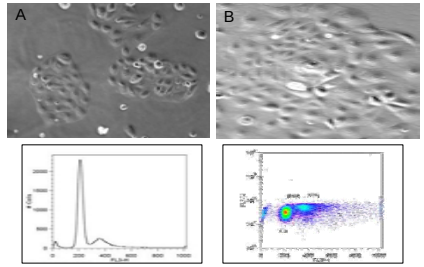

Fig. 2. Morphology of UOK 257 at 50% confluence exhibits small patched islands (A); at 90% confluence, loss of contact inhibition is evident (B). From flow cytometry, the scatterplot (C) and its one-dimensional histogram projection depict the cell cycle distribution of the tumor cell population.

Ultrastructural (Transmission EM) analysis

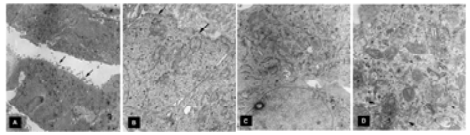

Fig. 3. (A-D) Ultrastructural features of findings from cultured UOK 257 tumor cells (90-nm thin section from fixed cell pellet) (6,500). (A) Arrows indicate microvilli on one (apical) surface and basal lamina on the opposite surface. (B) Arrows indicate basal lamina-like material, focally abundant in between tumor cells. (C) Abundant mitochondria have appeared, consistent with oncocytic change. (D) Cytoplasmic microvesicles (arrowheads) were present in chromophobe renal cell carcinoma and rare tumor cells.

Results

Genetic analysis

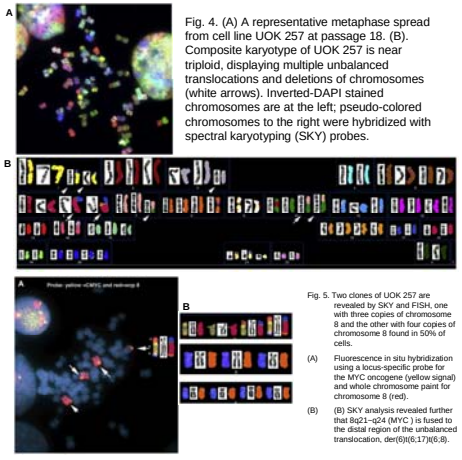

Fig. 4. (A) A representative metaphase spread from cell line UOK 257 at passage 18. (B). Composite karyotype of UOK 257 is near triploid, displaying multiple unbalanced translocations and deletions of chromosomes (white arrows). Inverted-DAPI stained chromosomes are at the left; pseudo-colored chromosomes to the right were hybridized with spectral karyotyping (SKY) probes.

Fig. 5. Two clones of UOK 257 are revealed by SKY and FISH, one with three copies of chromosome 8 and the other with four copies of chromosome 8 found in 50% of cells.

(A) Fluorescence in situ hybridization using a locus-specific probe for the MYC oncogene (yellow signal) and whole chromosome paint for chromosome 8 (red).

(B) (B) SKY analysis revealed further that 8q21→q24 (MYC) is fused to the distal region of the unbalanced translocation, der(6)t(6;17)t(6;8).

Summary

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Discussion

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