Abstract

The immunobiology of sarcomatoid renal cell carcinoma (RCC) and how it has been transformed or progressed from clear cell-RCC are currently poorly understood. We analyzed a sarcomatoid renal carcinoma cell line (RCC52) derived from a primary clear cell renal carcinoma with intensive sarcomatoid differentiation, with respect to its immunophenotypic, molecular cytogenetic features and xenotransplantability. RCC52 cells grown as monolayers consisted of mostly small-sized epithelioid cells along with a small proportion of spindle/fibroblast-like cells, suggestive of growth of the sarcomatoid, but not clear cell component of the tumor. Cytogenetic analysis revealed a phenotype of cell surface HLA-A, B, C, E-CAM, N-CAM, EpCAM, CD44+, CD54+, URO-1 and URO-10, and cytoplasmic AE-1, E-CAM, N-CAM, Vimentin, S100 and VEGF.

Spectral karyotyping (SKY) show the chromosomal abnormalities in 22 metaphases examined to be 40-42;21,X;1;21, der(1;21)(q11;q21), der(4;14)(q11;p11), der(17)(q17); Y[22] and Y[14] (22[16]; 14[15]; 13[14] (22[16]; 14[15]; 13[14] (22[16]). Numerical imbalances were assessed by comparative genomic hybridization (CGH), which are found to be consistent with the findings determined by banding and SKY. These results along with the documented genotype have established the identity of the RCC52 cell line. Nude mouse xenografts resulting from RCC52 cell s.c. injection and the original tumor shared similar histopathology with mostly sarcomatoid elements, suggestive of potential cell line of this RCC52 of sarcomatoid RCC cells to undergo transdifferentiation or dedifferentiation. The total HLA class I loss caused by the two distinctive mutations in the β₂-microglobulin gene and loss of heterozygosity (LOH) in chromosome 15 observed recently (Cancer Immunol Immunother, 58: 395-408, 2009) did not prevent sarcomatoid RCC52 cells from undergoing such transdifferentiation. Overall our results is of immunological (evasion of the host immunosurveillance) and pathological (progression/differentiation/transdifferentiation) significance, which forms the basis of further investigations with additional cell lines/ clones and tissues of clear cell-RCC with sarcomatoid differentiation to confirm (i) loss HLA class I expression in most, if not all, sarcomatoid RCCs, and (ii) the proposed sequence of clear cell-RCC → fibroblast-like sarcomatoid RCC → epithelioid sarcomatoid RCC, which could in turn transdifferentiate into clear cell RCC.

Materials and Methods

1. RCC52 cell is an aggressive sarcomatoid clear cell RCC established by Drs. Cheng-Kung Chuang and Shuen-Kuei Liao. This cell line was completely negative for the expression of surface HLA class I antigens. (Cancer Immunol Immunother, 58: 395-408, 2009)

2. CGH and SKY were conducted to detect if there is any chromosome copy number changes and structural rearrangements in RCC52 cell line.

3. Flow cytometry and immunohistochemistry were used for immunophenotyping of cells and tissues, respectively.

4. Nude mice and NOD/SCID mice were used to assay the tumorigenicity in RCC52 cells and RCC52 subpopulations.

5. Anchorage-independent cell growth assay (soft agar assay) was used to determine the colony forming ability of RCC52 cells and RCC52 subpopulations.

6. Immunophenotyping, xenotransplantability and molecular cytogenetic features of the sarcomatoid renal carcinoma cell line RCC52: Pathobiological significance

Figure 1. H&E staining (A, B) and immunohistochemical staining (C, D, E) of the clinical specimen from which the RCC52 cell line was established. (A) A majority of the area of the microphotograph shows clear cell component, while the major area of the section in (B) reveals sarcomatoid component exclusively. Staining with anti-β₂-microglobulin mAb L368 (C), and with anti-HLA class I heavy chain-specific mAb HC-10 (D). Negative staining with normal mouse IgG (E).

Figure 2. Chromosome alterations of RCC52. (A) A digital image of a CGH. Chromosomal regions that are in a predominant green represent “gains”, whereas regions that are conspicuous red represent “losses”. (B) CGH profiles demonstrate variations in chromosome region. Green bars showed gains (c) of the region and red bar showed losses (d) of the region. (C) Classified chromosome colors of a typical SKY karyotype of RCC52 cell line with the major findings listed on the left are shown.

Table 1. Surface / cytoplasmic antigen expression on sarcomatoid RCC52 cells determined by flowcytometric analysis

Table 2. Surface / cytoplasmic antigen expression on sarcomatoid RCC52 cells determined by flowcytometric analysis

Table 3. Surface / cytoplasmic antigen expression on sarcomatoid RCC52 cells determined by flowcytometric analysis

Table 4. Surface / cytoplasmic antigen expression on sarcomatoid RCC52 cells determined by flowcytometric analysis

Figure 3. Morphology of RCC52 cell and the representative sublines (A) Parental cells. (B) Epithelioid subline. (C) Fibroblastic subline.

Figure 4. Xenotransplantation experiments of RCC52 cells, epithelioid sublines (E3G5, E3D4 and E4F5) and fibroblastic sublines (M1D6, M1D6 and M1G7) in nude mice (A) and NOD/SCID mice (B) were independently carried out (n=4 for each subline). The results showed the mean tumor volume of the different groups. (i.e. injection with 5x10⁶ cells at day 0; tumor volume (mm³) = 0.2 x a² x b², where a is the major tumor diameter and b is the minor diameter perpendicular to the major one as measured by a caliper) (* p < 0.05).

Figure 5. Anchorage-independent cell growth by soft agar assay. Soft agar assay was used to compare the colony forming ability of RCC52 cells, epithelioid sublines (E3G5 and E4F5) and fibroblastic sublines (M1D6 and M1G7) in complete RPMI-1640 medium. 5000 cells were seeded in 6-well plates. The colonies were counted after 28 days of culture. The results showed the mean number of colonies of different groups.

Figure 6. Histopathology (H&E stained) of a xenograft in a nude mouse resulting from s.c. injection of 5x10⁶ RCC52 cells/site (A) and that of the original tumor lesion from which the RCC52 cell line was originated (B). In the images of A and C, the typical sarcomatoid RCC histology with spindle-shaped pleomorphic cells was exhibited, while in the images of B (left side) and D (most of the areas), clear cell components were clearly separated from the sarcomatoid component.

Figure 7. Immunohistochemical staining (A, C, E) and H&E stain (B, D, F) of a xenograft in a nude mouse resulting from s.c. injection of 5x10⁶ RCC52 cells/site. The upper panel (A, C, E) is the staining with anti-PAK 2 antibody. The lower panel (B, D, F) is the relative field to the upper panel with H&E stain.

Summary

1. SKY shows the chromosomal abnormalities in 22 metaphases examined to be 40-42;21,X;1;21, der(1;21)(q11;q21), der(4;14)(q11;p11), der(17)(q17); Y[22] and Y[14] (22[16]; 14[15]; 13[14] (22[16]). Numerical imbalances were also assessed by CGH, and found to be consistent with the findings determined by banding and SKY analysis.

2. Xenotransplantation studies in both nude mice and NOD/SCID mice revealed that epithelioid sublines had greater capability to produce tumors than fibroblastic ones. Interestingly, the parental RCC52 cells were far more tumorigenic than epithelioid and fibroblastic sublines. These results suggest (i) the possible synergetic effects among epithelioid and fibroblastic sublines, and/or (ii) exclusive of greater tumorigenic sublines in the xenotransplantation studies.

3. Nude mouse xenografts resulting from RCC52 cell subcutaneous injection and the original patient tumor shared similar histopathology with mostly sarcomatoid elements with occasional appearance of clear cell areas. This result strongly indicates that the sarcomatoid RCC cell line RCC52 has the capability to undergo transdifferentiation or dedifferentiation to a clear cell type in the xenografted tumors.

4. SARK-2, a kidney-specific transcription factor, was found to be expressed with a high frequency in most renal tumors, but not in sarcomatoid RCC. Immunohistochemical staining of a nude mouse xenograft resulting from RCC52 cell showed PAX2-2 in the sarcomatoid component, but PAX2 in the clear cell areas. This result confirms the validity of the presence of clear cells in the xenograft as a result of sarcomatoid RCC cell inoculation.