PRCC-TFE3 Dual-Fusion FISH Assay for Identifying PRCC-TFE3 Renal Cell Carcinoma in Paraffin-embedded Tissue

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Abstract

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Protocol

Step 1.
Design PRCC-TFE3 dual-fusion FISH probe:Suitable bacterial artificial chromosomes (BACs) for the dual-fusion FISH probe were selected at http://genome.ucsc.edu/. This probe consisting of 2 contigs that covered the entire TFE3 gene on the short arm of the X chromosome and the PRCC gene on the long arm of chromosome 1. The contig on the X chromosome consisted of 7 BAC clones (CTD-2311N12, RP11-416B14, CTD-2522M13, CTD-2516D6, CTD-2312C1, CTD-2248C21 and RP11-959H17) labeled with fluorescein-12-dUTP as green fluorescein, and the contig on chromosome 1 consisted of 4 BAC clones (CTD-2534H6, RP11-1047J23, RP11-730I22 and CTD-2547N15) labeled with tetramethylrhodamine-5-dUTP as red fluorescein.

Step 2.
FISH experimental procedure:After deparaffinization and washing, three-micrometer-thick paraffin-embedded sections were rehydrated in 100%, 85%, and 70% ethanol in turn for 3 minutes and digested with 10μL pepsin (4 mg/mL, 0.02M HCl; Sigma-Aldrich, Beijing, China) at 37℃ for 3 to 5 minutes, followed by subsequent dehydration. Then, the probe mixture was applied, and the slides were denatured at 85℃ for 5 minutes and target DNA simultaneously, followed by hybridization overnight at 37℃. The slides were immersed in 2×SSC for 10 minutes and in 0.1% NP-40/2×SSC for 5 minutes at 37℃. The nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). After hybridization, all slides were maintained at 4℃ in the dark.

Step 3.
FISH result evaluation:The slides were examined using an Olympus BX51TRF fluorescence microscope (Olympus, Tokyo, Japan) with the following three filters (DAPI/FITC/TexasRed) and the FISH analysis software (Imstar, Paris, France). For each case, a minimum of 100 nonoverlapping tumor cell nuclei were examined under fluorescence microscopy. Cells without the rearrangement presented split green and red signals (negative result), indicating intact Xp11 and 1q21. Dual fusion signal pattern (two fusion signals) was interpreted as the existence of reciprocal translocation of PRCC gene and TFE3 gene (positive result). We considered a yellow or closely approximated green-red signal as a fusion signal. Clear FISH signals should be observed in >100 nonoverlapping nuclei for each case.

Step 4.
Determine the cutoff:Normal renal tissues from 20 cases of non-PRCC-TFE3 renal cell carcinoma were randomly selected to determine the cutoff. We observed the signals of 50 nuclei in each case, and calculated the percentage of the cells with fusion signal. Subsequently, mean and standard deviation

of the percentages were figured out. The cutoff is the sum of mean and three times of standard deviation. Using this method, a positive result was reported when >2% of the tumor nuclei showed the fusion-signal pattern.