The R106H mutation (residue 316 G to A change) in the TIP30 gene was identified in the genomic DNA isolated from a HCC specimen (HLC#576) by a PCR amplification/sub-cloning-sequencing method as described previously (1). To confirm the presence of this mutation in this HCC specimen, we performed a mutant-PCR-amplification assay (2-4) to detect a TIP30-R106H mutation in the genomic DNA isolated from HCC specimens. This assay is based on the inability of Taq polymerase to amplify DNA if the nucleotide at 3’ end of a given primer does not match the sequence of template DNA. Therefore, the forward primer in this assay matches only templates containing the R106H mutation. As demonstrated in the supplemental data Figure 1a, the specific primers are able to amplify a specific DNA fragment only from a positive control plasmid template containing TIP30\textsuperscript{R106H} cDNA (Fig. 1a, Lane 4) but not from a negative control plasmid template containing wild type TIP30 cDNA (Fig. 1a, Lane 3). Figure 1b shows that the primers amplify a strong DNA band (Lane 3) and a week DNA band (Lane 4) from the genomic DNA templates that were isolated from two different tissue sites in the same HCC sample. As a control, the same primers could not amplify any specific DNA fragment (Lane 5) from the genomic DNA isolated from the HCC specimen (HLC#488) in which TIP30 mutation was not detected by the PCR amplification/sub-cloning-sequencing method (1). This result demonstrates that the R106H mutation is indeed present in HCC specimen.

Materials and Methods: Genomic DNA was extracted from two locations of HLC specimen #576. As a control, genomic DNA from HLC specimen #488 was also isolated in an identical condition as described previously (1). TIP30 exon 3 was first amplified from genomic DNA with Pfu polymerase (Stratagene) and primers as described previously (1). Then, one microliter of amplified products from the first PCR reaction was used as templates for the second PCR amplification with Taq polymerase
The forward primer: 5'-CTTAGAGGGATTTGTTCA-3', and reverse primer: 5'-CCATGCGCTTTCCAGGTCA-3' amplified a 200-bp fragment. As a control, plasmids containing TIP30 cDNA or TIP30R106H cDNA were used as templates in the PCR reaction, in which the same forward primer: 5'-CTTAGAGGGATTTGTTCA-3', and reverse primer: 5'-GGAGCCAAAGAACTTTCTAAC-3' amplified a 400bp DNA fragment. The PCR conditions were as follows: 1 cycle of 94°C for 3 minutes, 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 60 seconds followed by 1 cycle of 72°C for 8 minutes. The PCR products were electrophoresed in 1.8% agarose gel and stained with EB.

References:


Figure Legend.

Figure 1. R106H mutation is detected in the HCC sample by a specific mutant-amplification assay. a. Amplified PCR products from plasmid DNA templates are electrophoresed on 1.8% agarose gel. Lane
1, 1 kb DNA Ladder; Lane 2: Water as the template; Lane 3: the plasmid containing wild type TIP30 as the template; Lane 4: the plasmid containing TIP30$^{R106H}$ cDNA as the template. b. Amplified PCR products are electrophoresed on 1.8% agarose gel. Lane 1: 1 kb DNA ladder; Lane 2: water as template; Lane 3: genomic DNA from tissue site A of HLC#576 specimen as the template; Lane 4: genomic DNA from tissue site B of HLC#576 specimen. Lane 5: genomic DNA from HLC#488 specimen as the template.