# Usefulness of *p53* Gene Mutations in Bile juice for Diagnosis of Biliary Tract Carcinoma: Comparison With K-*ras* Mutations

Y. Yamaguchi, Y. Wang, K. Ohtsubo, H. Watanabe, and N. Sawabu

Biliary tract carcinoma (BTCa) is still difficult to detect at an early stage resulting in a poor prognosis despite recent progress in various imaging modalities, such as ERCP, EUS, CT, and MRI. Cytological examinations of bile have been conducted to diagnose BTCa qualitatively. Although some skillful cytologists have reported relatively high positive rates in BTCa, the accuracy of bile cytology for the diagnosis of BTCa has been limited due to cell injury and degradation induced by various proteases present in bile juice. Thus, the analyses of cancer-related genes in bile for the specific diagnosis of BTCa have been anticipated. Only a few researchers have tried to analyze *p53* or K-*ras* mutations in bile from patients with biliary malignancy, but the detective incidence of *p53* or K-*ras* genetic analyses seems to be limited because of insufficient sensitivity or false positivity. To improve the molecular diagnosis for BTCa, we analyzed *p53* and K-*ras* mutations in DNA extracted from not only the sediment but also the supernatant of bile samples.

For analysis of bile about *p53* and K-*ras* mutations, polymerase chain reaction-single strand conformation polymorphism (PCR - SSCP) and direct sequencing were used for analyses of *p53* mutations in exon 5 through 8. K-*ras* mutations at codon 12 were examined by mutant allele specific amplification, in which the mutation sequence could be determined with high sensitivity. In bile supernatant from patients with BTCa, *p53* and K-*ras* mutations were detected in 50.0% (15/30) and 56.7% (17/30) of cases, respectively. The incidence of *p53* and K-*ras* mutations in the sediment was 33.3% and 43.3%, respectively. On the other hand, in 20 patients with cholelithiasis, *p53* mutations were detected in neither supernatant nor sediment, though K-*ras* mutations were found in the sediment alone in 20%, but not in the supernatant. These data indicated that the supernatant is more favorable for genetic analysis than the sediment. When a combination assay with both genes was used, molecular abnormalities were detected in 80.0% of cases including three in which *p53* alone was positive, In addition, for the cases with BTCa in which bile cytology was examined, either *p53* or K-*ras* mutations were detected in 12 of 15 (80.0%) cases with negative cytology and there were no cases which showed cytology positive and genetic analyses negative as shown in Fig.

In conclusion, the incidence of *p53* and K-*ras* mutations is higher in the supernatant than sediment, and simultaneous analyses of *p53* and K-*ras* in the two bile fractions could enhance the genetic diagnosis of BTCa. Notably, the specificity of *p53* mutations for cancer is very high in bile samples, and the sensitivity is also relatively high.

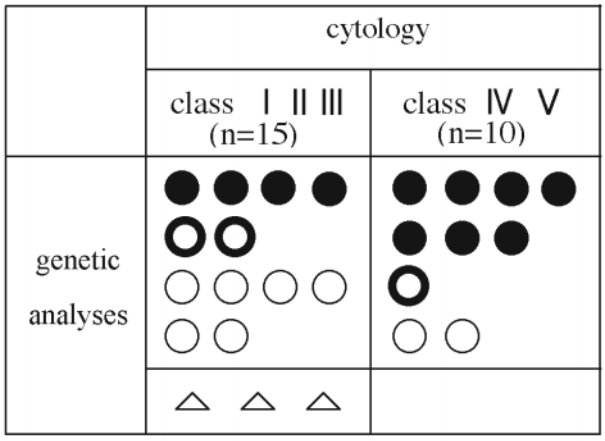


Figure: Comparison between genetic analysis and cytological examination in BTCa. ●, *p53* and K-*ras* mutants; ⭘, *p53* mutant only; ○, K-ras mutant only; △, *p53* and K-*ras* wild- type