CHAPTER VI
CONCLUSION

In the present study, the HDRA was modified from the previous technique which used MTT for detection cell viability. We evaluated the cancer cell viability by estimation the percentage of viable cancer cells in the CCA tissues based on their morphology under microscope. This method was time consuming and a trained scientist was needed. However, the modified HDRA has been successfully set up for screening the response of 28 CCA tissues to 5-FU. The mean of cell viability at day 4 was 83.3±14.2% of those observed at day 0. The relationship between the %I.I. and 5-FU concentrations were observed in a dose-dependent manner. Base on a dose response curve, 200μg/ml 5-FU with the 50% cut-off I.I. was selected as a cut-off point to differentiate between the poorly and well response groups.

Quantitative expression of genes encode for enzymes involved in 5-FU metabolic pathway included TS, DPD, TP and OPRT mRNA were successfully measured in all of 35 specimens by real-time PCR using specific Taqman probes. Among TS, DPD, TP and OPRT, the expressions of DPD and TP in CCA tissues were markedly different between individual patients. Whereas, only moderately different in the expression levels of TS and OPRT mRNA were observed. The variations in these genes expression may cause by the genetic polymorphism which lead to up-or down-regulation of gene and protein expression. In addition, the genetic polymorphism of these genes may also affect the pharmacokinetic profile of 5-FU among individual patients.

The relationships of these four gene expressions in CCA tissues were also examined. We found a positive correlation between the levels of OPRT and TS, OPRT and DPD. TP and DPD. Whereas, no correlation was observed between any other pairs. These findings suggest that de novo synthesis of DNA in CCA is coregulated by both OPRT and TS. Since DPD is involved in the pyrimidine catabolism while TP is a salvage phosphorylating enzyme. Our results suggest that

these salvage pathways may work together to cover the requirement for de novo DNA synthesis.

To determine the role of TS, DPD, TP and OPRT mRNA expression in the response of CCA tissues to 5-FU. We found that TP expression had a tendency to be higher among poorly response group as identified by the HDRA. High TP concentrations in conjunction with the normally low dRib-1-P drive the reaction in the opposite direction (Ackland, 1999). These data support our findings that TP expression is a negative predictor of the 5-FU sensitivity test in CCA. An OPRT mRNA expression was found to be higher in well response group, indicating that OPRT contributes to 5-FU sensitivity.

We were also able to analyze the relationships of the combinations of these mRNA expressions to the 5-FU sensitivity. We found that the OPRT/TP ratios were statistically significant higher in well response group than in poorly response group. Our results suggest that OPRT/TP mRNA expression ratio is a promising indicator of the chemosensitivity of CCA to 5-FU using modified HDRA established in this study. However, a study using a larger numbers of patients will be necessary in order to increase the reliability of the data from this newly established method. Whether the OPRT/TP ratio could be used as predictors of 5-FU chemotherapy in CCA patients needs to be further confirmed *in vivo*.