

Thesis Title Study on Correlation between Activated Ras Oncogene
and Human Papillomavirus Infection in Cervical Cancer
in Thailand

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ABSTRACT

This project was aimed of investigating whether there was any correlation between point mutation in ras oncogene and human papillomavirus type 16 and 18 infection in cervical cancer.

Three methods were tested for screening of point mutations in K-ras and H-ras genes in cervical biopsies, namely, S1 nuclease cleavage, denaturing gradient gel electrophoresis (DGGE) and PCR primer introduced restriction analysis (PCR-PIRA). The first two methods proved to be unsuitable for our use due to their lacks of consistency, and therefore, PCR-PIRA was selected to be used to analyse the specimens. This method is based on the presence of enzyme recognition site created by bases in primer with base sequence of interest. Only PCR products with normal sequence were recognized and digested by restriction enzyme but not the mutant PCR products. This allowed the detection of a single base change at codon 12 of K-ras gene and codon 61 of H-ras gene by size discrimination between normal and mutant sequences in 8 %

polyacrylamide gel. The digested product was photographed after electrophoresis and then DNA band on negative film was quantitated by scanning method. Samples with high mutant:normal DNA ratio of more than 0.36 were selected for confirmation. Mutant DNA in these samples was enriched by selective amplification of digested PCR product using the same set of primer. Typing of human papillomavirus in each sample was performed by in situ hybridization and PCR-RFLP method.

In total of 49 patients examined for point mutation at codon 12 of K-ras gene, the result showed that samples from six patients, both from cancerous tissues and their normal counterparts, were positive. All samples were found HPV negative except one cancerous sample. A point mutation detected in one pair of specimens was confirmed by direct PCR sequencing method and determined to be a base change from GGT to GTT. Among the same group of patients examined for H-ras mutation at codon 61, only one specimen indicated a base change from A to T at the second nucleotide. This specimen was found HPV negative.

Data summarized from this group of samples showed no association between K-ras or H-ras gene mutations and infection by HPV type 16 or 18. In addition, the mutations observed in normal cervical tissues indicated that alterations at the molecular level of ras genes occurred before histological change.