Thesis Title	Investigation of *Ras* Gene Amplification in Cervical Cancer.
Name	Daraporn Pittayakhachonwut
Degree	Master of Science (Biochemistry)

Thesis Supervisory Committee

Mathurose Ponglikitmongkol, Ph.D.

Prapon Wilairat, Ph.D

Mana Rochanawutanon, M.D.

Date of graduation 15 May B.E. 2540 (1997)

Abstract

Cervical cancer is the most common cancer among Thai women. High risk group of human papillomavirus (HPVs 16 and 18), one of the important etiological factors, was shown to be closely associated with the pathogenesis of cervical cancer. *In vitro* study showed that HPV 16 could cooperate with activated ras oncogene in transformation of cultured cells. However, our previous results showed no correlation between point mutation at codons 12 and 61 of ras genes and HPV infection. In this study, we searched for gene amplification, another activated mechanism frequently found in ras genes, in HPV infected cervical carcinomas. Meanwhile, microsatellite instability was also investigated. Gene amplification was studied in 36 specimens of cervical cancer using differential PCR and densitometric method. Another 9 pairs of cancer and their normal counterparts were determined for microsatellite instability. Analysis of gene amplification by two different cut-off values, using mixtures of

normal tissue and plasmid DNA or normal tissues as standards, exhibited slightly different number of samples with gene amplification. Thirteen (36%) and four (11%) out of thirty-six cases showed *H-ras* and *K-ras* gene amplifications respectively by using the first cut-off value. Seventeen (47%) and ten (28%) out of thirty-six cases showed *H-ras* and *K-ras* gene amplification respectively by using the other cut-off value. Four cases out of thirty six showed *H-ras* gene alterations (both point mutation and amplification) by both cut-off values while none was found for *K-ras*. *Ras* gene alterations (both point mutation and amplification) were found at high frequency (82%) in cervical carcinomas as well as HPV infection (61%).

Microsatellite instability chromosome 2 was observed in four of nine samples and only one case showed microsatellite instability on both chromosomes 2 and 10 using D2S123 and D10S197 markers respectively. No microsatellite instability was observed on chromosome 3 using D3S1277 marker. Results from the study in cell lines containing different types of HPV infection revealed that they all had normal ras genes since no point mutation at codons 12 and 61 and gene amplification were detected.

Our results suggested that HPV (types 16 and 18) infection and ras gene activation (both point mutation and gene amplification) involved in the carcinogenesis of cervical cancer although there was no relationship between HPVs and abnormal ras genes. Microsatellite instability found on chromosomes 2 and 10 might result from accumulations of genetic alterations at the late stage of cervical cancer and might not be the cause of cervical cancer.