CHAPTER V

DISCUSSION

Hypomethylation of LINE-1 in several cancers have been analyzed previously with methylation-sensitive southern blotting method[65-69]. This method required a large amount of DNA and a high quality of DNA and mixed type of tissue from fresh specimen. This study aimed to develop a new PCR technique, combined bisulfite restriction analysis LINE-1 (COBRA LINE-1), to evaluate methylation status of LINE-1 retrotransposon in various types of malignancies from microdissected paraffin embedded tissues. Alteration of DNA methylation associated with difference in age, sex and cellular differentiation thus three factors may influence result of the COBRA LINE-1.Regarding sex and age, women and youths have more hypermethylated DNA because of X-inactivation in women and loss of methylation in aging respectively[73]. However, COBRA could not distinguished LINE-1 methylation in difference sexes ($p>0.1$) and ages ($p>0.5$) according to the test of sex and ages in leukocytes from peripheral blood. These results suggested that the COBRA may not be sensitive enough to detect the alteration of LINE-1 methylation attributed to sex and age difference. Thus, these two factors would not be accounted for interfere results of COBRA LINE-1 when studying the role of global methylation in cancer development.

Additional backgrounds of LINE-1 hypomethylation in normal tissues should be known for evaluation of hypomethylation in cancer. The results show different level of hypomethylation in normal tissue types such as in uroepitheium and gastric epithelium higher than renal epithelium.The differences in the amounts and distribution of DNA methylation among difference tissues may cause by is tissue specific DNA methylation. Dramatic changes in overall methylation of DNA occur in difference period of embryogenesis development[74]. The result supports the evidence that methylation change continuously in adult tissues following the cellular differentiation.

The different level of LINE-1 hypomethylation between normal and cancer tissues were significant in 8 from 11 cancer types including carcinomas of urinary bladder, head and neck, liver, lung, prostate gland, stomach, colon, breast, and esophagus. The distribution of global hypomethylation level of each tumor type corresponded well to the distinnctive cellular differentiation. The finding are consistent with the previous notion that global hypomethylation is a common epigenetic in cancers.

COBRA LINE-1 protocol could consistently detect 3-6% average of increased level of hypomethylation in most cancers. Owing to difference of genomic hypomethylation may actually represent an alteration, which is equivalent to change of one or two whole chromosomes. This vital of change in the global methylation may be critical to cells as the comparable change of double doses of partial activated X chromosome by X-autosome translocation, observed as a type of skewed X-inactivation in female, leads to cell lethality. Furthermore, methylations of certain repetitive sequences can dramatically alter expressions of neighboring genes[75] and undermethylation of DNA might favor mitotic recombination leading to loss of heterozygosity ,as well as promoting karyotypically detectable rearrangements[76].

Hypomethylation of LINE-1 promoter is known to lead to increased expression of two ORFs in LINE-1 element and probably to retrotransposition. Active retrotransposition of LINE-1 can lead to inactivation of tumor suppressor genes or to activation of oncogenes when LINE-1 was inserted and enhanced expression of oncogene[77]. Therefore, it is expected that hypomethylation of LINE-1 is involved in instability of the genome.

The roles of LINE-1 hypomethylation in the multistep carcinogenesis could be illustrated in colonic carcinoma. This showed significant LINE-1 hypomethylation in the late stage comparing to dyspatic polyps ($p<0.01$) and normal epithelium ($p<0.01$). Through, no significant in dyspatic polyps comparing to normal epithelium. This result suggest that global hypomethylation is a progressive process developing during tumor progression. From to previous study compare carcinogenesis among normal epithelium

which, LINE-1 hypomethylation discovered in early event in carcinogenesis[78-79]. Thus, conclusion from this study is not completely comparing to previous study.

Sera from the gastric cancer patients consistently demonstrated greater hypomethylation levels as compared to the matched controls, with a statistical significance (p <0.05). Nevertheless, the level of LINE-1 hypomethylation in free circulating DNA increased modestly and significantly overlapped. This application of the COBRA LINE-1 as a potential tool to screen cancer is, therefore, limited.

CONCLUSION

In conclusion, This study suggest that hypomethylation is a relative common phenomenon, distinctive among difference cellular differentiation during development process. This mechanism proposes of phenotypic variations in mammals and also leads to increased hypomethylation in several cancers. Moreover variation of hypomethylation in some cancers should contribute to biological and clinical consequence. The difference of methylation implies the possibility to apply COBRA LINE-1 in determining cancer severity. Proposed COBRA LINE-1 method is the first techniques to combine the attractive features rapidly of use, highly quantitative result and compatible with paraffin-embedded samples.