CHAPTER V
DISCUSSION

Since global hypomethylation is common in most cancer types, our previous COBRA-L1 analysis was aimed to evaluate if the technique is efficient in cancer diagnosis. However, even if we confirmed that global hypomethylation is a cardinal epigenetic event in cancer, the technique has limited sensitivity and specificity for cancer diagnosis. At first we misunderstood that methylation of repetitive sequences would have followed all or none pattern. We thought that L1 of normal cells would be completely hypermethylated and loss of methylation would be only recognized in cancer. Nonetheless, our previous study[7] proved that the methylation level of L1s in normal cells are not static but there are possibility of de novo methylation and demethylation at L1 in normal tissues by the process of cellular differentiation. Up to 30% in range of L1 methylation can be discovered in normal tissue variation but in general global hypomethylation in cancer revealed as decreased COBRA-L1 at approximately 5%. Therefore, normal tissues from other individuals can be found frequently more hypomethylated than cancer. Consequently, this limits the utilization of COBRA-L1 for cancer diagnosis.

These COBRA-L1 results indicated two possibilities that L1 methylaion altered non-specifically among loci or some L1 loci may change methylation level due to physiologic conditions or carcinogenesis. Here we studied 17 L1 loci and proved they lost L1 methylation during cancer development. We did not observed the correlation between COBRA-L1 and CU-L1 range of variation among normal oral epithelial. This indicated that these selected L1 loci do not alter methylation during oral epithelium differentiation. Therefore L1 methylation changes due to carcinogenesis and tissue differentiation may occur at different loci.

Our information is not sufficient to conclude the characteristic of L1 that specific to global hypomethylation in cancer. We selected to study full length L1s located within intron for simplicity and possibility of gene control. It is interesting to further explore if

classification of L1s for example truncated vs full lenghted may determine the role of L1 methylation for tissue differentiation and carcinogenesis.

When analyzed CU-L1, each L1 locus demonstrated distinctive patterns of methylation levels. Whereas some loci are hypermethylated in normal tissues, others are partially methylated. Moreover, some loci demonstrated different level of methylation between oral epithelial and WBCs. The underlining mechanism of this finding is not known. Nevertheless, EPHA3 and PPP2R2B experiments proved that L1 methylation may upregulate gene expression in dosage dependent manner. Further experiments are needed to prove if L1 methylation level are important in tissue differentiation by the same mechanism.

CU-L1 demonstrated several characteristics of L1 hypomethylation in cancer. Whereas COBRA-L1 detected hypomethylation in cancer approximately 5%, CU-L1 demonstrated various degree of methylation loss. Interestingly, some loci lost methylation up to 100%. Moreover, each locus possesses differential potency for demethylation. Similar to what we discussed in normal tissue, L1 hypomethylation may represent control of gene regulation in dosage dependent manner. Moreover, the complete loss in methylation in some loci also represent selective clonal expansion, a characteristic of multistep carcinogenesis. As a result, such loci would represent genes with similar roles as tumor suppressor genes. In otherword, our result may demonstrated an unprecendented epigenetic mechanism in down regulating tumor suppressor genes. This technique will be a crucial technique of the future to map molecular targets of cancer.

The mechanism, how L1 hypomethylation down regulated EPHA3 and PPP2R2B is not known. It is also as crucial to determine if the mechanism is universal to all L1s or specifically to some genes. List of our proposed mechanisms are L1 promoter as silencer or antisense transcript from L1 promoter interfere with the linked gene RNA processing. Both mechanisms should be explored in the future.

Finally, CU-L1 possesses higher sensitivity and specificity than COBRA-L1. Our results revealed that CU-L1 methylation level of normal tissue have limited range. As a result, more hypomethylation of each L1 locus is cancer characteristic with high

specificity. In addition, frequency of L1 hypomethylation varied among loci. The more CU-L1 tests are added, the higher sensitivity of the test will be. Therefore, CU-L1 will be very promising tumor marker of the future.