CHAPTER I

INTRODUCTION

Background and Rationale

The epigenetic modifications, especially modification of DNA methylation patterns, that do not influence the genetic potential of genes, play an important role in tumor formation and progression by the establishment and maintenance of the program of gene expression. Tumor cells are characterized by a paradoxical alteration of DNA methylation pattern: global DNA hypomethylation (loss of methylation) and local hypermethylation of certain genes. Hypermethylation and inactivation of tumor suppressor genes are well documented in tumors. The role of global genome hypomethylation in carcinogenesis is less studied. New data provide evidence for independence of DNA hypo- and hypermethylation processes in tumor cells. These processes alter expression of genes that have different functions in malignant transformation. Recent studies have demonstrated that global decrease in the level of DNA methylation is related to hypomethylation of repeated sequences, increase in genetic instability, hypomethylation and activation of certain genes that favor tumor growth, and increase in their metastatic and invasive potential. It is evident that the targets for aberrant hypomethylation in tumors are represented by DNA sequences that are methylated in normal tissues. The following groups can be attributed to these DNA sequences: 1) unique DNA sequences encoding for genes whose suppression of expression in normal cells is achieved through methylation 2) repeated sequences that are methylated in all studied genome loci[1, 2].

It was demonstrated in one of the first studies of DNA hypomethylation in animal tumors that a significant decrease in 5-methylcytosine content (by 30-40%) occurs in moderately and frequently repeated DNA sequences. Endogenous retrotransposons are attributed to these DNA sequences. These elements of the human genome are methylated in all studied genomic loci. Many transposons have strong promoters; methylation of the latter correlates with suppression of transcription activity and apparently prevents their further expansion throughout the genome[3]. Hypomethylation LINE-1, long interspersed nuclear element 1 that is highly repeated interspersed human retrotransposon account for around 17% of human genome, in tumor cells in

comparison to normal tissues was demonstrated for many tumors[4, 5]. In some cases, hypomethylation of LINE-1 was accompanied by transcription. It suggest that the decrease in the level of methylation of these genomic elements may activate their transcription and provides them competence for transposition and recombination. In form of oncogene process, exogenous expression of LINE-1 in transformed cells is accompanied by LINE-1 transpositions that are connected with genome deletions and inversions[6].

Previously by applying combined bisulfite restriction enzyme assay (COBRA) technique at LINE-1s(COBRA LINE-1), we proved that global hypomethylation is one of the most common epigenetic events in cancer[7]. While hypomethylation of whole genome LINE-1 have already demonstrate, hypomethylation pattern of each LINE-1 in specific location has never studied before. The aim of this study is to molecularly describe how methylation at each LINE-1s loss during the multistep carcinogenesis. Since LINE-1s are interspersed repetitive sequences, it is interesting to evaluate and compare the pattern of demethylation among LINE-1 loci. New method, COBRA unique to LINE-1, is develop for detect methylation status of each LINE-1 in specific location within genome during carcinogenesis, with more detail. 17 COBRA unique to LINE-1 PCR perform with HNSCC, comparing HNSCC cell line, HNSCC microdissect specimen, normal oral epithelial and in leukaemic cancer, leukaemic cell line compare with normal white blood cell. Associations between hypomethylation LINE-1 promoter status and LINE-1's host gene expression are also study. This study will be the first to demonstrate that each LINE-1s have different potential to be hypomethylated and may imply distinctive potential of each LINE-1s in promoting cancer development. Furthermore, loss of LINE-1 methylation may be the regulator of LINE-1's host gene expression.

Objective

1. To establish technique for quantitative detect hypomethylation level among LINE-1 loci.
2. To evaluate and compare the pattern of hypomethylation among LINE-1 loci during multistep carcinogenesis.
3. To test normal tissue differentiation by COBRA unique to LINE-1 technique.

4. To study the affect of specific intronic LINE-1 loss of methylation to it's host gene regulation.

Question

Primary Question

- Does COBRA unique to LINE-1 technique can quantitate hypomethylation level among LINE-1 loci within genome?

Secondary Question

- What are patterns of hypomethylation among LINE-1 loci during the multistep carcinogenesis?
- Are the COBRA unique to LINE-1 technique detect different character of normal tissue specific?
- Does LINE-1 loss of methylation status affect to LINE-1's host gene regulation?

Hypothesis

- COBRA unique to LINE-1 technique can quantitate hypomethylation level among LINE-1 loci within genome.
- Hypomethylation level of each LINE-1 loci by COBRA unique to LINE-1 technique increase during multistep cancer progression, in accordance with COBRA LINE-1.
- COBRA unique to LINE-1 technique show normal tissue differentiation same as COBRA LINE-1 technique.
- LINE-1 promoter loss of methylation status have impact to LINE-1's host gene regulation.

Conceptual framework

In many cancer, COBRA LINE-1 technique reveal whole genome LINE-1 hypomethylation. However, non of the single LINE-1 loci loss of methylation status is never studied before.

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- Does COBRA unique to LINE-1 technique can quantitate hypomethylation level among LINE-1 loci within genome?

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Qualify COBRA unique to LINE-1 technique with COBRA LINE-1 technique association study and COBRA unique to LINE-1 PCR sequencing.

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Pearson correlation study of COBRA unique to LINE-1 technique with COBRA LINE-1 technique to confirm potential of new tumor marker method.

COBRA unique to LINE-1 is the new effective standard method in identify hypomethylation level of each LINE-1 specific location similar to COBRA LINE-1

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- What are patterns of hypomethylation among LINE-1 loci during the multistep carcinogenesis?
- Are the COBRA unique to LINE-1 technique detect different character of normal tissue specific?

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Evaluate and compare the pattern of hypomethylation among LINE-1 loci during multistep carcinogenesis and among normal tissue differentiation by COBRA unique to LINE-1 technique.

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Association of COBRA unique to LINE-1 hypomethylation level with multistep carcinogenesis and normal tissue differentiation were determine by using ANOVA analysis and Independent T-test analysis, respectively.

Hypomethylation level of each LINE-1 loci by COBRA unique to LINE-1 technique are different among each HNSCC cell line.

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- Does LINE-1 loss of methylation status affect to LINE-1's host gene regulation?

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Statistical study, pearson correlation, association of COBRA unique to LINE-1 hypomethylation level with RT-PCR of LINE-1's host gene expression from each

Operation definition

Hypermethylation : gain of methylation than normal tissue methylation status

Hypomethylation : loss of methylation than normal tissue methylation status

(Normal tissue is normal tissue collect from normal healthy donor)

Global hypomethylation: overall decreases methylation status of the entire genome

Expected benefit

The success of this study will be first to completely explore each specific LINE-1 methylation status of 2 types of cancers and normal tissues, HNSCC and leukemia. And, COBRA unique to LINE-1 will be useful for other future genetic studies and convenience tumor marker.

Research methodology

1. Cancer cell line, microdissected HNSCC specimens, normal oral epithelial, normal white blood cell collection.
2. All specimen are extract DNA and extract RNA only HNSCC cell line group.
3. Sodium bisulfite treatment and desalted with DNA clean-up system.
4. Primer design for 17 CORBA unique to LINE-1 and for RT-PCR of LINE-1 host gene.
5. Polymerase chain reaction (PCR).
6. Restriction enzymes combine digestion, *Taq I* & *Tas I*.
7. Gel eletropholesis and quantitation with Molecular Dynamics phosphoimager.
8. Clone COBRA unique to LINE-1 PCR and sequencing.
9. Data collection and statistical analysis.