CHAPTER I

INTRODUCTION

Background and Rationale

Methylation of cytosine bases in CpG dinucleotides with in DNA aids in the organization of the mammalian genome. DNA methylation is involved in inactivating one of two X chromosome in women, establishes tissue-specific patterns of gene expression, effects on cellular development and differentiation, and represses proviral and retrotransposon sequences in the genome[22]. Patterns of DNA methylation are often altered in tumor cells. Two distinct changes have been observed, termed DNA "hypermethylation" and "hypomethylation". In tumor DNA hypermethylation usually occurs in a focal fashion affecting the CpG rich promoter regions of tumor suppressor genes, causing or supporting their transcription inactivation. In contrast, genome-wide losses of DNA methylation (global hypomethylation) have been regarded as a common epigenetic event in malignantcies[38], which contribute to carcinogenesis may possibly mediated through facilitating chromosome instability and regulating the expression of proto-oncogenes. Hypomethylated of tumor cells may become reactivated retrotransposon. The movement of retrotransposon may affect genome instability.

Retrotransposons are multicopy genetic elements found in all eukaryotes, and nearly one-half of the human genome consists of these repetitive sequences which contain the bulk of methylated CpG in the genome[24]. In mammalian genomes retrotransposons are commonly maintained in the silent state and are consequently heavily methylated. The long interspersed nuclear element 1 (LINE-1 or L1) ,a highly repeated interspersed human retrotransposon account for around 17% of our DNA[50].There are few reports, qualitative determination of hypomethylated LINE-1 in several epithelial cancers which, evaluated in non-microdissected tissues of cancers by southern blotting and/or distinquish PCR amplicons by methylation sensitive enzyme[65-69].

Limited information is available on the global methylation status of normal tissues and other cancer types beyond colonic carcinoma. Thus, in this study applied the

combined bisulfite restriction analysis (COBRA) PCR [70] to evaluate the methylation status of LINE-1 repetitive sequences in the microdissected materials from various normal, neoplastic human tissues and sera. COBRA LINE-1 technique has no failure rate of PCR from paraffin embedded tissue in which usually yield little amount of degrade DNA. The underlining reasons of this improvement should be due to the shorter of the amplicon size and the significantly larger in number of LINE-1, approximately 3-4,000 full-length copies per cell, as the template. The success of this study will be first to completely explore LINE-1 methylation status of all types of cancers and normal tissues. Second, COBRA LINE-1 will be useful for other future genetic studies and a convenience tumor marker.

Objective

To study methylation status of LINE-1 in cancers and normal tissues.

Question

Primary Question

- How are patterns of LINE-1 hypomethylation in normal and cancer tissues?

Secondary Question

- Are the levels of LINE-1 hypomethylation in different types of cancer the same?
- Are the levels of LINE-1 hypomethylation in normal tissue from different organs and different type of tissues?
- Are the levels of LINE-1 hypomethylation in leukocyte from different age and gender?
- How are patterns of LINE-1 hypomethylation in multistep carcinogenesis in colonic carcinoma?
- How are patterns of LINE-1 hypomethylation in serum of gastric cancer patient compared normal sera?

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Hypothesis

- Hypomethylation level of LINE-1 in cancers is greater than in normal tissues.
- Different level of hypomethylation of LINE-1 is presented among different organs and different type of tissues.
- Women and young persons have more methylated DNA than the men and the elderly.
- Levels of hypomethylation of LINE-1 in late stage of colonic carcinoma are greater than in dysplatic polyps and normal epithelium.

Conceptual framework

1. Global genomic hypomethylation has been observed very often in repetitive sequence of DNA in some cancer types.
2. Global genomic methylation is altered during embryogenesis.

1. Find out the correlation of hypomethylation of 5' UTR LINE-1 in normal and cancer tissues by COBRA LINE-1.
2. Find out the correlation of global methylation among different tissue, age and sex.
3. Prove the application of COBRA LINE-1 as tumor marker by comparing serum of normal and cancer patients.

ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

Operation definition

Hypermethylation: more methylation than in normal tissue

Hypomethylation: less methylation than in normal tissue

(Normal tissue is nonmalignant and nonaging)

Global hypomethylation: overall decreases methylation status of the entire genome

Expected benefit

The success of this study will be first to completely explore LINE-1 methylation status of all types of cancers and normal tissues. COBRA LINE-1 will be useful for other future genetic studies and convenience tumor marker.

Research methodology

1. Paraffin embedded tissue, blood and serum collection.
2. Microdissection materials from various normal and neoplastic tissues.
3. DNA extraction from paraffin embedded tissue, white blood cell and serum.
4. Sodium bisulfite treatment and desalted with DNA clean-up system.
5. Primer design for CORBA LINE-1.
6. Polymerase chain reaction (PCR).
7. Restriction enzymes digestion.
8. Gel eletropholesis and quantitation with Molecular Dynamics phosphoimager.
9. Data collection and statistical analysis.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย