CHAPTER I

INTRODUCTION

Background and Rationale

One particularly harmful form of DNA damage is the DNA double-strand breaks (DSBs), which are a type of DNA lesion that both DNA strands of the double helix are damaged. DSBs can be induced by intrinsic factors, such as collapse of replication forks, free radicals and special mechanisms in cell (e.g. V(D)J recombination) (1-4). Moreover, DSBs can be induced by extrinsic factors for example, exposure to ionizing radiation, or environmental mutagens. In normal cells, DSBs occur spontaneously at background levels, which are termed endogenous DNA double-strand breaks (EDSBs) (5). EDSBs are particularly dangerous lesions if they occur during the replication of the genome and during the segregation of duplicated chromosomes into daughter cells. Proper genome duplication is hampered by EDSBs. EDSBs repair pathways are composed of at least three major pathways, namely homologous recombination (HR), DNA-PK- dependent non-homologous recombination (NHEJ) and ATM-dependent NHEJ (6). HR repairs precisely using complementary sister chromatid as template. In contrast, NHEJ joins two DNA ends directly but often prone to error, and small sequence deletions. However, ATM-dependent NHEJ is more precise than DNA-PK-dependent NHEJ by use ATM act joint with checkpoint kinase 2 (CHK2) and BRCA1 to controlling the fidelity of DNA end-joining (7, 8).

The fidelity of EDSB repair is important to the fate of the cell. The failure to repair EDSBs or their inaccurate repair can lead to chromosomal instability (CIN) that contributes to carcinogenesis. CIN phenotype is identified by the gross chromosomal rearrangement. Common chromosomal aberrations include the loss or gain of whole chromosomes or chromosomal fragments, and the amplification of chromosomal segments (9). Moreover, global hypomethylaion, which imply aberration level of methylation in the whole genome, has also a causal role in tumor formation by promoting CIN. Evidence for this notion came from the frequently observed genomic hypomethylation in tumor cells, and from a previous study suggesting that defects in

DNA methylation might contribute to the CIN of some colorectal tumor cell lines (10). Therefore, the previous study aimed to explore the association between EDSBs and DNA methylation in each cell cycle. We found that EDSBs are generally hypermethylated in most cell cycles especially G0 phase (11). Besides, hypermethylation of EDSBs is not associated with DNA replication and the level of methylated EDSB should not be positively influenced by the production of EDSBs during DNA replication. Consequently, we hypothesized that hypermethylated EDSBs may occur from a lower rate of repair in methylated EDSB.

To prove this hypothesis, we aim to identify which pathways prefer to repair hypermethylated EDSBs. In the first step, we applied siRNA technique to eliminate the key proteins in HR, DNA-PK-dependent NHEJ and ATM-dependent NHEJ. Rad51 is a target protein in HR pathway. Ku86, DNA-PKcs and ATM were candidate proteins in DNA-PK and ATM-dependent NHEJ. Then, we used three techniques from the previous study such as L1-EDSB-LMPCR to quantitate level of EDSBs and COBRA-L1 and COBRA-L1-EDSB to analyze methylation level of genomic DNA and EDSBs respectively (11).

Objectives

To identify which DNA repair pathways are involved with the methylation level of EDSBs in G0 and S phase.

Research Questions

1. Which NHEJ pathway repair methylated EDSB in Go phase?
2. Does methylated EDSB repair depend on HR pathway in S phase?

Hypotheses

1. Methylated EDSB repair depends on ATM-dependent NHEJ pathway in G0 phase.
2. Methylated EDSB repair does not depend on HR pathway in S phase.

Key Words

Endogenous DNA double-stand breaks, chromosomal instability, global hypomethylation, homologous recombination, non-homologous end joining, DNA methylation

Expected Benefit

The results of this study will help us to understand the mechanism of tumorigenesis.

Conceptual framework

1. Which NHEJ pathway repair methylated EDSB in Go phase?
2. Does methylated EDSB repair depend on HR pathway in S phase?

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To identify which DNA repair pathways are involved with the methylation status of EDSBs in G0 and S phase.

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- Inhibit the targeted proteins of NHEJ and HR pathway using RNA interference (siRNA).
- Synchronize cells at G0, G1/S and S phase.
- Measure the quantity of EDSBs using L1-EDSB-LMPCR.
- Detect the level of methylated EDSBs using COBRA-L1-EDSB and the level of genome methylation using COBRA-L1 in transfected cells during G0, G1/S and S phase.