CHAPTER V

Discussion

This thesis aimed to study how DNA methylation is associated with EDSB repair pathway in G0 and S phase since the previous study found that EDSBs are generally hypermethylated in all cell cycles and hypermethylated EDSBs are replication independent (11). Our results suggest that ATM-dependent NHEJ may prefer to repair methylated EDSBs. Consequently, the mechanism of methylated EDSB repair may have higher fidelity than unmethylated EDSBs. In addition, methylated EDSB repair does not depend on HR pathway and this result support our idea that hypermethylated EDSBs are replication independent.

The fidelity of methylated EDSB repair involves with ATM-dependent NHEJ.

We interested in methylated EDSB repair depend on NHEJ pathway in G0 phase since this pathway is the main DSB repair pathway in mammalian cells and major repair in this phase. NHEJ pathway depends on DNA-PK complex, which is composed of DNA-PKcs, Ku70 and Ku86 (7, 8, 17). We found that down-regulation of Ku86 protein did not affect the methylation level of EDSBs. The previous reports showed that in G0 phase has a back up NHEJ (B-NHEJ), which has gain function when Ku86 are inhibited. This pathway utilizes ligase protein (87), which is different from Ku86 pathway. Moreover, B-NHEJ does not normally exist since the ability of a cell to repair DNA breaks is less precise than in the DNA-PK-dependent NHEJ process. This is because the structure of Ku70 bound Ku86 molecule, which is heterodimer, can functionally protect the DNA ends and leads to the more precise and faster repair mechanism. In the absence of Ku86, not only the DNA-PK-dependent NHEJ was absent, this can also lead to increasing of the B-NHEJ activity. Then, it indicated that Ku86 down-regulation cannot be explained whether DNA-PK-dependent pathway effect to repair the methylated EDSBs. In the other hand, lacking of the DNA-PKcs expression, which is the catalytic subunit of the DNA-PK complex, leads to difference in the result of the Ku86 defected

cells. In DNA-PKcs defected cell, B-NHEJ can not function because cells have Ku86, which inhibited B-NHEJ. (86-88).

Methylated EDSBs are retained in heterochromatin, concealed from the early DSB repair response, and repaired by ATM dependent NHEJ pathway. On the contrary, unmethylated EDSBs are not retained and may less depend on ATM. Interestingly, in contrast to general NHEJ and B-NHEJ pathways (84, 89), ATM-dependent repair pathway has been proposed to be more precise (7). The mechanism initiate by ATM acts jointly with checkpoint kinase 2 (CDK2) and BRCA1 in controlling the fidelity of DNA end-joining by precise NHEJ (7). BRCA1 has been shown to inhibit the nuclease activity of Mre11, which possibly inhibit for precise end-joining (8). Consequently, the rate of spontaneous mutations may be limited. This, therefore, could be a reason why more spontaneous mutations arise in hypomethylated genomes. This study supports the idea that global hypomethylation leads to the genomic instability, which is one of the most common molecular events in the multistep carcinogenesis.

Hypermethylated EDSBs did not depend on HR pathway in S phase.

We found that no difference in the HR-DNA repair process in methylated EDSBs. Though HR is ATM-dependent process in which the ATM molecule is considered a signaling transducer protein, this process is similar to the ATM dependent NHEJ which selectively repairs the methylated EDSBs (90). However in this study, no different repair mechanism between methylated and unmethylated EDSBs observed in Rad51-inhibition, may be due to the property of Rad51 that is not transducer, but DNA binding protein. In stead of using ATM, there has been reported previously in the absence of ATM molecule, DNA-PKcs can alternatively be used in the HR process. The interaction and co-expression of DNA-PKcs and ATM can be found in every stages of cell cycle (8). Their function is depended on its downstream proteins in each phase of cell cycle. Though ATM is the main transducer in HR repair process, interactions between ATM and other molecule are needed for completing HR repair process. For example BRCA2 molecule is recruited for regulation of Rad51 expression through the cell cycle check point (CDK2). CDK2 regulates formation of Rad51 filament during S phase in which DNA

replication is proceeded and sister chromatid is synthesized (91). In this process, based on hypothesis of precise-NHEJ, each end of DNA strands is opened and easily repaired by ATM which selectively repairs only in the heterochromatin related methylated EDSBs.

In conclusion, unmethylated and methylated EDSBs preferentially undergo different repair pathways. This study supports the notion that the increase of spontaneous mutation rate in genomic hypomethylation may be related to how differently the methylated and unmethylated EDSBs are processed.