CHAPTER V

DISCUSSION

Eukaryotic genome has been widely recognized as an enormously long continuous stretch of DNA. Our study discovered three unprecedented findings which are universal characteristics of genome. First, in normal physiologic circumstance DNA breakages are common and are hypermethylated. Second, level of EDSBs is cell type specific. Finally, the EDSBs are concealed within heterochromatin.

DNA breakages are common.

The discovery of hypermethylated EDSBs in all cell phases are not surprising. Although DSBs are hazardous to cells, leading to a complete loss of function of the broken genes and faulty DNA recombination, DSBs in methylated DNA should be less harmful because methylated genes usually have limited activity (25). In addition, usually in association with DNA methylation (26), the tightly packed structure of heterochromatin (86) may brace the broken chromosome and hide EDSB ends from random recombination. In S phase, EDSBs are still hypermethylated albeit with less significance than G0. This result implies that because DNA replication does not occur simultaneously throughout the genome, heterochromatin may still capture the broken late replicating DNA even during cell proliferation.

Levels of EDSBs are cell type specific.

Nonetheless, it is surprising that the quantity of EDSBs is not directly associated with carcinogenesis but is influenced by cellular physiologic process. Here, we reported that L1-EDSB-LMPCR and γ-H2AX-bound L1s are cell type specific and inversely related. Whereas WBCs possessed more L1-EDSB-LMPCR than did epithelial cells, epithelial had more γ-H2AX-bound L1s than WBCs. Since EDSBs are concealed in heterochromatin, the higher quantity of EDSBs in WBCs may be due to their different chromatin organizations. Moreover, the level of γ-H2AX binding reflects the level of early DSB repair response, which in turn limits the level of remaining DSBs detectable by L1-EDSB-LMPCR at a given time. Nonetheless, it is interesting to explore if there are

specific causes and physiologic consequences for WBCs in maintaining the higher level of methylated EDSBs.

The retention of EDSBs in heterochromatin may also help explain the antineoplastic mechanism of TSA. Histone deacetylase inhibitors have been found to induce cell cycle arrest and apoptosis in several tumors (87). Interestingly, cells chemically or genetically defective in non-homologous end-joining (NHEJ) have been found to respond hypersensitively to TSA in a dose-dependent manner (88). We showed here that TSA increased the amount of γ-H2AX-bound DNA as an early cellular DSB response. Therefore, the TSA- induced cell cycle arrest and apoptosis may be consequent cellular responses to exposed methylated EDSBs.

Connection between genomic hypomethylation and instability

Finally, we may present evidence that differential repair of EDSBs could therefore be a concrete connection between global hypomethylation and genomic instability. This is further supported by the report by Chen and colleagues (6) that murine embryonic stem cells nullizygous for the major DNA methyltransferase (*Dnmt1*) gene exhibited global hypomethylation and significantly elevated rate of mutations with predominant small deletions, similar to NHEJ repair errors. Here, we showed suggestive experiments that methylated EDSBs are able to avoid error-prone NHEJ repair and consequently the rate of spontaneous mutations is limited. The unmethylated EDSBs are preferentially repaired by DNA-PKcs-dependent imprecise NHEJ pathway. In contrast, the methylated EDSBs are retained in heterochromatin, which delays cellular repair responses. Further experiments in our laboratory indicated that methylated EDSBs are repaired by ATM-dependent NHEJ repair pathway (Appendicx). As a result, more spontaneous mutations could arise in hypomethylated genomes (Fig. 26).

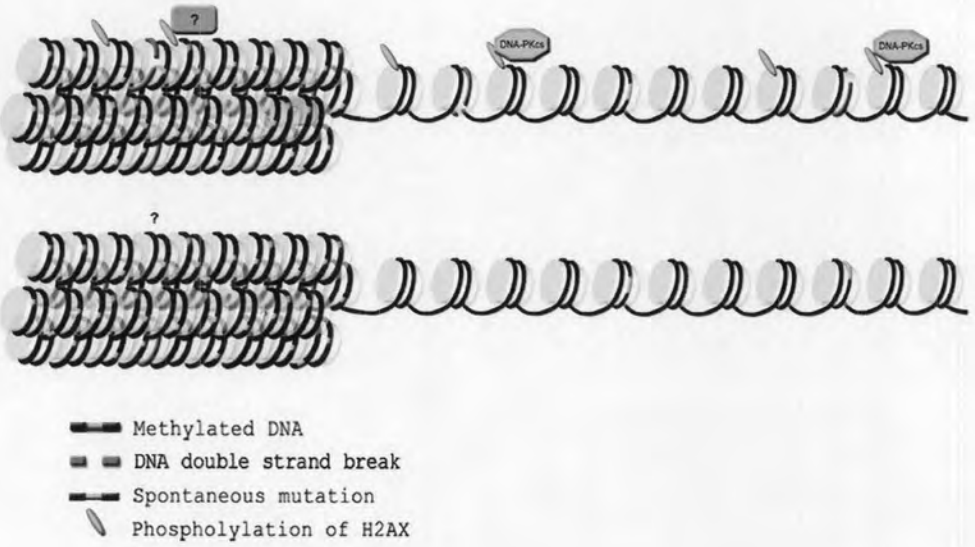

Figure 26 EDSBs are hypermethylated; methylated EDSBs are retained in heterochromatin while unmethylated EDSBs undergo less-precise repair. Diagrammatic representations of the quantity of EDSBs under normal physiologic circumstances show the differences between hyper- and hypo-methylated DNA, which associate with hetero- and eu-chromatin, respectively. (A) While methylated EDSBs are concealed in heterochromatin, the earliest DSB repair responses, γ-H2AX, are more prevalent in hypomethylated DNA. The differential nonhomologous end-joining repair pathways in nonreplicating cells between hyper and hypo-methylated DNA are shown. (B) Consequently, spontaneous mutations are accumulated in relation to the genomic hypomethylation level.