DNA double strand breaks causing deletion of the p16 tumor suppressor gene in human cancer cells

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Chromosome deletion is a major cause of tumor suppressor gene inactivation in human carcinogenesis. To elucidate the molecular process of chromosome deletion, we determined the structure of breakpoints for deletions of the chromosome 9p21 region containing the p16 tumor suppressor gene in a variety of human cancers. Breakpoints in non-lymphoid tumors were not mapped within 50-bp to each other, and did not show significant sequence homologies with one another. However, the breakpoints were clustered in a 10-kb region covering the p16 gene. Thus, it was indicated that genomic and/or chromatin structure rather than DNA sequence is a critical determinant for the occurrence of DNA double strand breaks (DSBs) in chromosomal DNA. In contrast, breakpoints in lymphoid leukemia were clustered at 5 major sites of <15 bp that contained putative ectopic recombination signal sequences (RSS) for V(D)J recombination, suggesting that illegitimate V(D)J recombination is a major cause of 9p21 deletions. However, clustering in a few among thousands of the ectopic RSS sites in 9p21 also indicated that genomic and/or chromatin structure in addition to DNA sequence is critical for the occurrence of DSBs by the RAG complex. To further elucidate the process of chromosome deletion, we developed a system to probe genomic regions susceptible to DSBs in living cells in vivo. In this system, a restriction enzyme, MspI, was introduced by the Chariot reagent, and inter-regional differences in the susceptibility to DSBs were examined by Southern blot hybridization and linker-mediated capture of DNA fragments digested in vivo. The analysis of the 9p21 region indicated that promoter regions of the p16, p14 and p15 genes were sensitive to MspI digestion in HeLa cells, and breakpoints for p16 deletions in human cancers were significantly over-represented in these three regions (P=6.7x10^-5). Interestingly, the p16 promoter region was less sensitive if it was hypermethylated. These results support our hypothesis that chromatin structure is a critical determinant for the susceptibility of DNA to DSBs by DNA damaging agents. Currently, whole genome mapping of MspI hypersensitive sites is underway to further clarify genomic features underlying DSB susceptibility.

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