Bacterial Hotspots and Cancer Gene Therapy

In Escherichia coli, a base substitution hotspot occurs in the second C of CC(A/T)GG sequences (where C = cytosine, A = adenine, T = thymine, and G = guanine) (1). It is the consequence of the E. coli dcm (DNA cytosine methylase) methylase, which modifies this cytosine to 5-methylcytosine (2). Then, spontaneous deamination of 5-methylcytosine to thymine introduces a G:C → A:T transition. Because of its bacterial origin, this mutational hotspot will also be found in any plasmid DNA used in gene therapy. Hence, plasmid preparations may contain a small percentage of mutated copies that will not be detected by DNA sequencing if the transition occurred late in the amplification step of plasmid. These mutated plasmids, even if they are quantitatively negligible, could have dramatic biologic consequences if they generate recombinant proteins with a dominant gain of aberrant functions (3). For instance, we have found seven CC(A/T)GG motifs in the p53-coding sequence. The corresponding G:C → A:T transitions will give rise to either proline → leucine substitutions (codons 58, 278, 301, and 359) or nonsense mutations (codons 104 and 354). It is interesting that proline → leucine substitutions at codons 278 and 301 have already been detected in human tumors (4–6). However, hotspots in E. coli will not necessarily overlap hotspots in eukaryotes. It is noteworthy that, in humans, 5-methylcytosine is mainly found at the dinucleotide CG and not in the CC(A/T)GG sequence. Thus, the absence of G:C → A:T transitions at codons 58, 104, 354, and 359 in human tumors does not necessarily mean that they are biologically neutral but may suggest that these codons are not hotspots in humans. The possible biologic consequences of these mutations are, therefore, presently unknown. For all of these reasons, we suggest that the potential hazard of methylated CC(A/T)GG motifs should be evaluated for every plasmid encoding a therapeutic protein for which dominant mutant forms have been described.

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REFERENCES

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