Identification of Mutations at DNA topoisomerase I responsible for camptothecin resistance

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摘要.

A camptothecin-resistant cell line that exhibits more than 600-fold resistance to camptothecin, designated CPTR-2000, was established from mutagen-treated A2780 ovarian cancer cells. CPTR-2000 cells also exhibit 3-fold resistance to a DNA minor groove-binding ligand Ho33342, a different class of mammalian DNA topoisomerase I inhibitors. However, CPTR-2000 cells exhibit no cross-resistance toward drugs such as Adriamycin, amsacrine, vinblastine, and 4'-dimethyl-epipodophyllotoxin. The mRNA, protein levels, and enzyme-specific activity of DNA topoisomerase I are relatively the same in parental and CPTR-2000 cells. However, unlike the DNA topoisomerase I activity of parental cells, which can be inhibited by camptothecin, that of CPTR-2000 cells cannot. In addition, parental cells after camptothecin treatment results in a decrease in the level of DNA topoisomerase I, whereas CPTR-2000 cells are insensitive to camptothecin treatment. These results suggested that the mechanism of camptothecin resistance is most likely due to a DNA topoisomerase I structural mutation. This notion is supported by DNA sequencing results confirming that DNA topoisomerase I of CPTR-2000 is mutated at amino acid residues Gly717 to Val and Thr729 to Ile. We also used the yeast system to examine the mutation(s) responsible for camptothecin resistance. Our results show that each single amino acid change results in partial resitance, and the double mutation gives a synergetic effect on camptothecin resistance. Because both mutation sites are near the catalytic active center, this observation raises the possibility that camptothecin may act at the vicinity of the catalytic active site of the enzyme-camptothecin-DNA complex.

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