人類第一型去氧核糖核酸拓樸異構酵素其耐喜樹鹼之突變研究

Identification of Mutations at Human DNA Topoisomerase I Responsive to Camptothecin Resistance

中文摘要

喜樹鹼(Camptothecin)係作用在真核細跑第一型去氧核糖核酸拓樸異構酵素(Eucaryotic DNA topoisomerase I)上的抗癌藥物，我們首先以突變劑EMS(Ethyl methane sulfonate)處理人類卵巢癌 A2780 細胞株(Human ovarian cancercell line A2780)，然後再逐步增加喜樹鹼的濃度，目前已成功的篩選並分離出能夠耐喜樹鹼的突變株，稱之爲 CPT-2000。我們發現，此突變株並不耐其他的抗癌藥物，如 adriamycin、vinblastine 和 VM-26。然而，細胞在第一型去氧核糖核酸拓樸異構酵素的蛋白質及活性上，原 A2780 細胞株和耐藥的 A2780 細胞株 CPT-2000 並無兩樣。初步的研究結果發現，其耐藥性乃由於人類第一型去氧核糖核酸拓樸異構酵素基因的突變所導致，利用去氧核糖核酸定序法得知是在胺基酸 Gly717 突變成 Val，及 Thr729 突變成 Ile。另外，我們由喪失第一型去氧核糖核酸拓樸異構酵素的酵母菌(Saccharomyces cereoisiae strain:JN2-134)送入含有人類第一型去氧核糖核酸拓樸異構酵素及突變在人類第一型去氧核糖核酸拓樸異構酵素胺基酸 Gly717→Val 和 Thr729→Ile 的質體(YcpGaltopI 及 YcpGaltopI717,729)做耐藥性實驗時，也證明第一型去氧核糖核酸拓樸異構酵素結構上的改變是導致其對喜樹鹼耐藥性的原因。由於雙點突變非常接近酵素的活化中心(Tyr#723)，因此這個結果建議喜樹鹼也許是作用在酵素活化中心附近。

同時，我們也利用桿狀病毒表達蛋白系統(baculovirus expression system)的重組核多角體病毒(nuclear polyhedrosis virus, NPV)來表現並純化人類第一型去氧核糖核酸拓樸異構酵素和突變的人類第一型去氧核糖核酸拓樸異構酵素。我們可以獲得大量的 100kDa 人類第一型去氧核糖核酸拓樸異構酵素，經由 BioRex-70 ion exchangercolumn 純化所得的人類第二型去氧核糖核酸拓樸異構酵素，我們以西方墨點法(Western blot)確認，發現它與 A2780 細胞獲得的人類第一型去氧核糖核酸拓樸異構酵素無異，且其酵素活性也和人類第一型去氧核糖核酸拓樸異構酵素沒有差別。

英文摘要

We have isolated and cloned a camptothecin resistant cell line, designated CPT-2000, and found that CPT-2000 did not cross resist to other antitumor drugs such as adriamycin, vinblastine, and VM-26. Both protein level and enzyme specific activity of DNA topoisomerase I are relatively constant in parental and resistant cells. However, the enzymatic activity of DNA topoisomerase I from parental cells can be

inhibited by camptothecin, but not from CPT-2000 cells. We believed that 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 camptothecin resistant cells is mutated at amino acid residues Gly717 to Val and Thr729 to lie. In addition, yeast JN2-134 bearing YCpGAL1-htop I (G717V, T729I) grew well on galactose plates containing 10 U.g/ml camptothecin, while JN2-134 bearing YCpGAL1-htop I failed to grow on galactose plates in the presence of camptothecin. This observation further suggests that either or both of the two amino acid changes identified in the mutant DNA topoisomerase I is responsible for the resistance to camptothecin. Since both mutation sites are near the catalytic active site, this observation raises the possibility that camptothecin may act at the vicinity of the catalytic active site of the enzyme-camptothecin-DNA complex.
In addition., we also used the baculovirus expression system to express wild type human topo I and Cpt-resistant topol in SF21 insect cells. The human topo I cDNA and Cpt-resistant topo I cDNA were integrated into the Autographa californica multiple nuclear polyhedrosis virus genome by cotransfecting linear BacPAK6/AcMNPV DNA with pVL1393/hTopo I and pVL1393/hTopo 1(G717V,T7291), respectively. We have used the Bio-Rex 70 column to purify the recombinant topoisomerase 1. Reasonable amount of 100-KDa recombinant wild type hTopo I (about 2000 mg from 500 ml SF21 cell culture) can be obtained. The baculovirus expressed recombinant hTopo I -was then characterized by immunoblotting with anti-topo I antibody and DNA relaxing activity. We found that there are no difference in terms of the enzymatic activity and antigenicity between the recombinant hTopo I and native human topoisomerase I isolated from cultured cells.