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实验方法

酪氨酸激酶抑制剂伊马替尼耐药K562细胞系的建立及其耐药特征

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摘要 目的 建立酪氨酸激酶抑制剂伊马替尼(STI-571)耐药K562细胞系并研究其耐药特征。方法采用含递增浓度伊马替尼的培养基培养K562细胞，诱导耐伊马替尼K562细胞系(K562R)。通过细胞生长曲线、细胞形态和细胞凋亡分析确定耐药细胞是否形成；采用MTT法观察耐药细胞的耐药谱；通过半定量PCR和Western印迹法检测相关基因及蛋白的表达，并通过基因测序分析K562R细胞B细胞受体-c-Abelson(*BCR-ABL*)激酶区基因序列。结果 成功地将伊马替尼高敏感的K562细胞诱导成K562R，伊马替尼抑制K562和K562R细胞存活的IC_{50}值分别为0.01±0.00和(2.35±0.01) μmol·L^{-1}，耐药倍数为235.0倍。K562R具有一定的交叉耐药性，对高三尖杉酯碱、长春新碱和对柔红霉素的耐药倍数分别为13.2，63.2和11.8倍。K562R可对抗伊马替尼诱导的细胞凋亡，伊马替尼1.0 μmol·L^{-1}培养24 h K562和K562R细胞凋亡率分别为72.1%和18.2%。耐药形成机制研究表明，与K562细胞相比，K562R细胞*BCR-ABL*基因、多药耐药基因(*MDR*)和*p*-糖蛋白基因(*p-GP*)的表达均明显增加。此外，K562R细胞*BCR-ABL*基因的第696位发生A→C点突变，该位点突变导致激酶区第231位氨基酸由赖氨酸取代原有的天冬酰胺。结论 成功建立了耐伊马替尼细胞系K562R。K562R细胞具有交叉耐药性和对抗伊马替尼诱导的细胞凋亡等特征。K562R细胞*BCR-ABL*基因序列发生点突变，*MDR*和*p-GP*等基因表达亦明显升高。

关键词 伊马替尼 酪氨酸激酶抑制剂 K562细胞 抗药性，肿瘤

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Establishment of human K562 cell line resistant to tyrosine kinase inhibitor imatinib and its resistance characters

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Abstract

OBJECTIVE To screen imatinib(STI-571) resistant human K562 cell line and to study the imatinib resistance mechanisms. METHODS Human K562 cells were induced by culture in the medium with gradually increasing imatinib concentrations to establish the imatinib-resistant K562 (K562R) cells. Biological and pharmacological characters of the cells were examined by MTT method, and cell phenotype and apoptosis were determined. The expression of B cell receptor-c-Abelson (*BCR-ABL*), multi-drug resistance *(MDR)* and *p*-glycoprotein(*p-GP*) genes was examined by semi-QPCR and Western blotting. RESULTS Compared to K562 cells (IC_{50}=0.01 μmol·L^{-1}), K562R cells exhibited 235.0 fold resistance to imatinib (IC_{50}=2.35 μmol·L^{-1}). Besides, K562R also became resistant to homoharringtonine (13.2 folds), vincristine (63.2 folds) and daunorubicin (11.8 folds). These resistance abilities consisted with the expression of *BCR-ABL*, *MDR* and *p-GP* genes. PCR results showed that the expression of *BCR-ABL*, *MDR* and *p-GP* genes increased in K562R cells compared with K562 cells. The DNA sequencing analysis showed that a A→C point-mutant occurred in the *BCR-ABL* gene sequence of K562R cells, and asparagine was submitted by lysine in the 231st site of kinase-domain. CONCLUSION K562R cells are successfully constructed. The expression of *BCR-ABL*, *MDR* and *p-GP* genes is up-regulated in K562R cells. There is a point-mutant in *BCR-ABL* gene of K562R cells.

Key words imatinib tyrosine kinase inhibitor K562 cells drug resistance neoplasm

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