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论著

艾洛替尼耐药的卵巢癌SKOV3细胞系的建立及其耐药特性

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摘要 目的 通过体外建立艾洛替尼(Erl)耐药的人浆液性卵巢癌细胞系SKOV3/Erl，并探讨其耐药机制，为卵巢癌靶向性治疗的耐药性研究提供细胞模型及依据。方法 采用逐步递增联合大剂量冲击法诱导细胞，取对数生长期的SKOV3细胞，从Erl 1 μmol·L^{-1}开始处理，以Erl 2，4，8，16和 25 μmol·L^{-1}反复换液传代，最终维持浓度为10 μmol·L^{-1}，共诱导10个月。取SKOV3和SKOV3/Erl细胞，加入不同浓度艾洛替尼、紫杉醇、米托蒽醌、表柔比星、拓扑替康、长春新碱和甲氨蝶呤等药物，作用72 h，磺酰罗丹明B染色法检测耐药倍数。流式细胞术检测SKOV3细胞和SKOV3/Erl细胞的细胞周期。Western印迹法检测Erl刺激对敏感和耐药细胞中表皮生长因子受体(EGFR)相关信号通路产生的变化；流式细胞术检测SKOV3/Erl细胞表面ATP结合盒(ABC)转运蛋白中P糖蛋白(Pgp)、多药耐药相关蛋白1(MRP1)、乳腺癌耐药蛋白(BCRP)的表达以及Toll样受体4(TLR4)的表达；检测脂多糖(LPS)刺激对紫杉醇耐受的SKOV3/Erl细胞的敏感度。结果 Erl对SKOV3细胞的IC_{50}为(9.54±1.04)μmol·L^{-1}，而对SKOV3/Erl细胞的IC_{50}为(21.63±1.05)μmol·L^{-1}，耐药倍数约为2.26，诱导成功的SKOV3/Erl对紫杉醇、长春新碱、米托蒽醌和甲氨蝶呤的耐药倍数均在3倍以上。与SKOV3细胞相比，SKOV3/Erl 细胞G_0/G_1期比例上升，S期比例下降，G_2/M期几乎无变化。Erl刺激后，与SKOV3细胞相比，SKOV3/Erl细胞中p-HER1，p-ERK与p-AKT水平上调。SKOV3/Erl细胞膜上Pgp，BCRP和MRP1表达有微弱上调，而TLR4显著上调；脂多糖可强烈刺激SKOV3/Erl细胞增殖，并且对紫杉醇杀伤起到保护作用，而亲本细胞则相对不敏感。结论 成功建立了对Erl耐受的人卵巢癌耐药细胞SKOV3/Erl。其表现出的多药耐药机制可能与TLR4蛋白表达上调有关。

关键词 艾洛替尼 卵巢癌 耐药 酪氨酸激酶抑制剂

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Establishment of human ovarian cancer cell line SKOV3 resistant against erlotinib and its resistant characterization

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Abstract

OBJECTIVE To construct a drug-resistant human serous ovarian cancer cell model induced by erlotinb and explore its possible mechanism of resistance. **METHODS** The cell line SKOV3 was cultured by gradually increasing the concentration of erlotinib from 1, 2, 4, 8, 16 to 25 μmol·L^{-1} until 10 μmol·L^{-1} *in vitro* for 10 months to generate its resistance cell line SKOV3/Erl. In the induction process, the medium was treated with erlotinib 2, 4, 6 and 8 μmol·L^{-1} and changed step by step, accompanied by the passage of cells. The resistance index in SKOV3/Erl was tested by sulforhodamine B (SRB), after being treated with a series of concentrations of erlotinib, paclitaxel, mitoxantrone, epirubicin, topotecan, vincristine and methotrexate. Cell cycle of SKOV3 cells and SKOV3/Erl cells was investigated by flow cytometry. The changes of signal transduction protein in sensitive and drug resistant cells treated with erolotinib were detected by Western blotting. SKOV3 and SKOV3/Erl cells

were stained with antibodies conjugated with fluorescent dyes to determine the expression levels of cell surface P-glycoprotein(Pgp) , breast cancer drug resistance protein (BCRP) and multidrug resistance-related protein 1 (MRP1) and Toll-like receptor 4 (TLR4). To evaluate the drug-resistance function of TLR4, the viability of cells was assayed after stimulation by LPS. **RESULTS** IC_{50} Value of erlotinib to SKOV3 was (9.54±1.04)μmol • L^{-1} while that of erlotinib to SKOV3 was (21.63±1.05)μmol • L^{-1}, with a resistant index of 2.26. The resistant indices of paclitaxel, vincristine, mitoxantrone and methotrexate all exceeded 3. Compared with SKOV3 cells, S-phase of SKOV3/Erl cells was reduced, and G_0/G_1 phase increased while the percentage of G_2/M phase showed no significant change. The phosphorylated HER1 signal was upregulated in SKOV3/Erl cells. p-ERK and p-AKT levels in SKOV3/Erl were also higher than in SKOV3 cells. Major ABC transporter Pgp, BCRP and MRP1