Cancer Chemother Pharmacol (2005) 56: 212–220
DOI 10.1007/s00280-004-0960-5

ORIGINAL ARTICLE

Shu-zhen Chen · Min Jiang · Yong-su Zhen

HERG K^+ channel expression-related chemosensitivity in cancer cells and its modulation by erythromycin

Received: 22 June 2004 / Accepted: 15 October 2004 / Published online: 6 April 2005

Abstract *Purpose*: Previous studies have found that the HERG K^+ channel is highly expressed in some cancers. In the study reported here, we investigated HERG expression in various cancer cell lines, its correlation with chemosensitivity to vincristine, paclitaxel, and hydroxy-camptothecin, and its biochemical modulation. *Methods*: The MTT assay and clonogenic assay were used to detect the cytotoxicity of anticancer drugs in vitro. HERG expression was analyzed by Western blotting or immunocytochemistry. Gene transfection was used to examine the changes in HERG-related chemosensitivity. Cell cycle phase distribution was detected by flow cytometry and drug combinations were evaluated by the MTT assay. *Results*: HERG expression levels differed widely between various human cancer cell lines and HT-29 cells expressing high levels of HERG were more sensitive than A549 cells expressing low levels of HERG to vincristine, paclitaxel, and hydroxy-camptothecin. In terms of IC_{50}, the chemosensitivities of *herg*-transfected A549 cells to vincristine, paclitaxel and hydroxy-camptothecin were significantly increased. However, for cisplatin and 5-fluorouracil, no significant difference between *herg*-transfected A549 cells and parent A549 cells was detected. Erythromycin, a HERG K^+ channel blocker, suppressed the growth of various cancer cells and the potency was correlated with HERG expression levels. Combinations of erythromycin and vincristine, paclitaxel or hydroxy-camptothecin showed synergy in cytotoxicity to HT-29 cells. Erythromycin also enhanced the G_2/M arrest induced by vincristine in HT-29 cells. There were synergistic effects between erythromycin and vincristine, paclitaxel, and hydroxy-camptothecin, and chemosensitivity was correlated with HERG expression level. *Conclusions*: HERG expression levels and chemosensitivity were positively correlated for vincristine, paclitaxel, and hydroxy-camptothecin. Erythromycin was active as a modulator. These results suggest that HERG may serve as a molecular marker and modulating target for individualized cancer therapy.

Keywords HERG K^+ channel · Chemosensitivity · Erythromycin · Biochemical modulation · Synergistic effects

S. Chen · M. Jiang · Y. Zhen (✉)
Department of Oncology,
Institute of Medicinal Biotechnology,
Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100050, China
E-mail: zhenys@public.bta.net.cn
Tel.: +86-10-83158065
Fax: +86-10-63017302

Introduction

Potassium channels are the most diverse plasma membrane ion channels [1]. The *herg* gene (human ether-a-go-go-related gene) encodes a K^+ channel similar to the rapid delayed rectifier channel current [I(Kr)] in cardiac myocytes and has been identified as the gene involved in chromosome-7-associated long QT syndrome [2, 3]. As a class III antiarrhythmic drug, azimilide modulates the activation of the HERG channel [4]. HERG channels are tetramers, with each subunit consisting of six transmembrane domains (denoted S1–S6), and both N and C termini are located intracellularly [5]. Some studies have demonstrated that HERG is expressed in a variety of cancer cell lines of different histogenesis, but HERG is absent in the healthy cells from which the respective cancer cells are derived [6–8]. In neuroblastoma cells exposed long-term to hypoxia, HERG potassium channels undergo a profound modulation, which leads to stabilization of the resting potential in hypoxic environments [9]. In signaling transduction, an ERG K^+ current is regulated by Src tyrosine kinase in MLS-9 rat microglia cells [10]. Furthermore, HERG channels represent an important molecular device being involved in both integrin-mediated outside-to-in and inside-to-out signaling and in this way in some signaling pathways controlling cell differentiation in the hemo-

poietic system [11]. HERG channels are also involved in the regulation of cell proliferation and apoptosis [8, 12]. However, there are no reports on the correlation between HERG expression and chemosensitivity in cancer cells. The possible roles of HERG in cancer chemotherapy remain unclear.

There is increasing evidence that ion channels, such as Ca^{2+} and K^+ channels, play a key role in modulation of the cell cycle. Calcium channel blockers have been shown to inhibit cell growth and metastasis in various models of neoplasia [13, 14] and to potentiate the antiproliferative activity of some anticancer drugs [15, 16]. Moreover, some K^+ channel blockers have been reported to inhibit the growth of several kinds of cancer cells and to display synergistic effects in combination with anticancer drugs [17–19]. Ion channels have become potential molecular targets for modulation of chemosensitivity in cancer therapy. As reported previously, erythromycin, a macrolide antibiotic, inhibits the *herg*-encoded potassium current in a concentration-dependent manner [20]. Apparently, erythromycin may serve as a K^+ channel blocker. In the present study, we demonstrated that HERG K^+ channel expression is related, at least in part, to the chemosensitivity of cancer cells to vincristine, paclitaxel, and hydroxy-camptothecin (HCPT), and erythromycin acts as a modulator of the effect of chemotherapeutic agents.

Materials and methods

Cell culture and treatments

HT-29 and T84 (human colon carcinoma) cells, MCF-7 and SK-BR-3 (human mammary adenocarcinoma) cells, PG (highly metastatic human lung giant-cell carcinoma) cells, and A549 (human lung adenocarcinoma) cells were maintained in RPMI 1640 medium (GIBCO-BRL, Grand Island, N.Y.) supplemented with 2 mM L-glutamine, 10% (v/v) heat-inactivated newborn bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin, and cultured at 37°C in a humidified atmosphere containing 5% CO_2. Erythromycin (Sigma Chemical Co., St. Louis, Mo.) was dissolved in ethanol. HCPT (Shenzhen Main Luck Pharmaceuticals, China) and vincristine sulfate (Shanghai Hualian Pharmaceuticals, China) were dissolved in phosphate-buffered saline (PBS) as 1 mg/ml stock solutions and stored at −20°C. Paclitaxel (Taxol; Sino-America Squibb Pharma) was diluted with 0.9% NaCl. Cisplatin (Jingzhou Jiutai Pharmaceutical, China) and 5-fluorouracil (Shanghai Xudong Haipu Pharmaceuticals, China) were dissolved in PBS as 1 mg/ml stock solutions and stored at −20°C. Cells were harvested at approximately 80% confluence and subcultured in RPMI 1640 medium. After incubation overnight, cells were treated with the agents tested. Medium containing 0.1% ethanol was used as a control.

DNA constructs and purification

The *herg* plasmid was obtained from Dr. Gea-Ny Tseng (Virginia Commonwealth University, Richmond, Va.). First, *herg* cDNA was subcloned into *Bam*H I/*Hind* III sites of the pCDNA3.1 vector (Invitrogen, Carlsbad, Calif.). This vector contains a CMV promoter and a SV40 promoter, which drives the expression of the inserted cDNA and neomycin-resistance gene, respectively. JM109 cells transformed with pCDNA3.1 plasmid containing *herg* were cultured in Luria-Bertani medium with 60 μg/ml ampicillin. The growing phase bacteria were collected and plasmids were purified using the Wizard PureFection plasmid DNA purification system according to the manufacturer's manual (Promega, Madison, Wis.).

Western blot analysis

Cells were lysed in RIPA buffer containing 50 m*M* Tris, pH 8.0, 1% Nonidet P-40, 150 m*M* NaCl, 1 m*M* EDTA, 2 m*M* Na_3VO_4, 1 m*M* NaF, and the protease inhibitors phenylmethylsulfonyl fluoride (10 m*M*), aprotinin (1 μg/ml), and leupeptin (1 μg/ml). The protein content of the crude membrane was measured using the Bradford protein assay. Aliquots of 60 μg (BCA protein assay kit, 23225; Pierce Biotechnology, USA) of the crude membrane were boiled for 3 min in 4× loading buffer and then separated by 7.5% SDS-PAGE. The proteins were transferred to a nitrocellulose membrane, blocked with 5% nonfat milk in TBS containing 0.05% Tween 20 (TBST), and then incubated with anti-HERG antibody (C-20, sc-15968; Santa Cruz Biotechnology, Santa Cruz, Calif.) or anti-actin antibody (sc-1616; Santa Cruz Biotechnology) overnight at 4°C. After five washes with TBST and two washes with TBS, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody (sc-2922, Santa Cruz Biotechnology). Following another two washes with TBST, labeled proteins were visualized using ECL (sc-2048; Santa Cruz Biotechnology) on high-performance chemiluminescence film [21].

MTT assay

Cells were detached by trypsinization, and seeded into 96-well plates (Costar, Cambridge, Mass.) at 2– 5×10^3/well, depending on the cell line, in 180 μl of medium and incubated for 24 h at 37°C. Various concentrations of paclitaxel, HCPT, or erythromycin were added, and cells were incubated for an additional 24, 48, or 72 h before quantification of cell growth. The inhibitory effect on cell growth was examined using the MTT (3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium) assay according to a described previously method [22].

Clonogenic assay

Cytotoxicity was assayed by determining the ability of A549 and HT-29 cells to form colonies after treatment. The cells, suspended by trypsinization and counted, plated into 24-well plates (Costar, Cambridge, Mass.) at a density of 50 or 100 cells/well and cultured overnight. Different concentrations of vincristine were added and colonies were allowed to grow for 7–10 days at 37°C in an atmosphere containing 5% CO_2. The medium was then removed, and colonies were stained with 1% crystal violet (Sigma Chemical Company, St. Louis, Mo.) in 70% ethanol and then counted. Plating efficiency of the controls was approximately 85%. The cytotoxicity of the tested drugs was evaluated by calculating the percentage of surviving cells relative to the control cultures(untreated cells).

Stable gene transfection

The transfection of the *herg*gene into A549 cells was performed using the lipofectamine 2000 method (GIBCO, Grand Island, N.Y.) according to the manufacturer's manual. After transfection for 24 h, the cells were detached by trypsinization and then regenerated according to 1:8 dilution into fresh growth medium. The transfected cells were then cultured in RPMI 1640 medium containing 600 μg/ml of G418 for 7–10 days and further cultured in medium containing 300 μg/ml G418 for 2 weeks. The established cell lines were maintained under G418-free conditioned for at least 1 week before use to avoid any effects of G418.

Immunocytochemistry

A549 cells and transfected A549 cells were detached by trypsinization and suspended in RPMI 1640 medium at 1×10^5cells/ml, and 3-ml portions therefrom were placed on cover glasses present in 6-well plates (Costar, Cambridge, Mass.). After incubation for 20 h, cells adhering to the cover glass were freed from the medium by rinsing with PBS three times, fixed in ethanol for 10 min at room temperature, and air-dried before further analysis. The fixed cells were treated with 3% H_2O_2 for 15 min, and then blocked by 10% normal rabbit serum. The cover glasses were extensively rinsed with PBS and then incubated with the anti-HERG primary antibody (Santa Cruz Biotechnology; C-20, sc-15968) for 1 h at 37°C. They were rinsed again and incubated with biotinylated polyvalent anti-goat antibody and with the horseradish peroxidase-conjugated streptavidin. After extensive rinsing with PBS, color was developed with DAB (3,3′-diamino-benzidine) chromogen solution. The cover glasses were then counterstained with Mayer's hematoxylin. The stained cells were covered with 50% (v/v) glycerol/PBS and inspected under a microscope.

Determination of cell cycle phase distribution

HT-29 cells (1×10^6) were treated with various drugs for different times. The cells were then resuspended in ice-cold 70% ethanol and stored at −20°C until analysis. The cells were then washed two times with PBS and stained with PBS containing 50 μg/ml propidium iodide and 25 μg/ml RNase A. Flow cytometric analysis was performed with a flow cytometer (Coulter EPICS XL).

Evaluation of drug combinations

Coefficients of drug interaction (CDI) were calculated as follows: $CDI = AB/(A\times B)$, where A and B are the survival values with single agents and AB is the observed values of the two-drug combination [23, 24]. There was considered to be a synergistic effect of a two-drug combination for $CDI < 0.85$, and a significant synergistic effect of a two-drug combination for $CDI < 0.7$ ($P < 0.05$, combination vs antitumor drug alone, Student's *t*-test).

Statistical analysis

The results are presented as means ± standard deviation. The data were analyzed by the non-paired Student *t*-test and $P < 0.05$ was considered statistically significant. IC_{50} values were calculated using STAT statistical software.

Results

HERG expression in various cancer cell lines

Western blot analysis was applied to detect the expression of HERG in a series of cancer cell lines. As shown in Fig. 1, the antibody recognized two bands of HERG proteins, an upper broad band with an apparent molecular mass of about 155 kDa and a lower band with an apparent molecular mass of about 135 kDa. The 155-kDa protein represents the fully glycosylated form of HERG channels, whereas the 135-kDa protein is a core-glycosylated form of HERG channels [21]. Among all of the tested human cancer cell lines, the level of HERG expression in HT-29 cells was the highest, and then decreased in the order of T84, MCF-7, PG and A549 cells.

HERG expression and chemosensitivity to various anticancer drugs

The MTT assay and clonogenic assay were used to determine the cytotoxicity of various anticancer drugs in HT-29 cells expressing high levels of HERG and in A549 cells expressing low levels of HERG. The IC_{50} values of vincristine by the clonogenic assay in HT-29 cells and A549 cells were 1.06 ± 0.09 n*M* and 15.23 ± 0.03 n*M*,

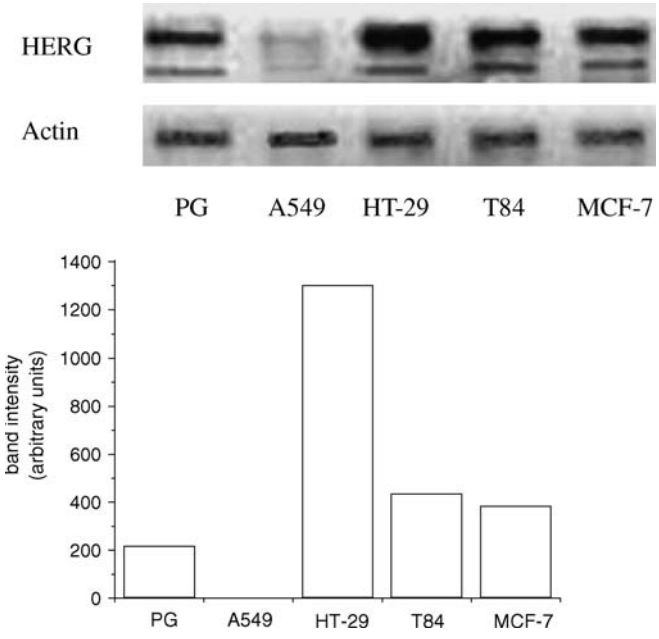


Fig. 1 Western blot analysis of the expression of HERG channel protein in various cancer cell lines. Crude membrane protein (60 μg in 30 μl) was diluted with 4× sample loading buffer and separated by SDS-PAGE in 7.5% acrylamide gel. Anti-HERG and anti-actin antibodies were detected by chemiluminescence as described in Materials and methods

respectively. The IC_{50}values of other drugs by the MTT assay are shown in Table 1. In terms of IC_{50} value, the highly HERG-expressing HT-29 cells were more sensitive than the low HERG-expressing A549 cells to a series of drugs including vincristine, paclitaxel and HCPT. However, for cisplatin and 5-fluorouracil, no difference were found between HT-29 cells and A549 cells.

Cancer cells expressing high levels of HERG are sensitive to erythromycin

The MTT assay was used to evaluate the inhibitory effect of erythromycin on cancer cell proliferation. Erythromycin suppressed the growth of HT-29, T84, MCF-7, PG, and A549 cells in a dose-dependent manner (Fig. 2). HT-29 cells expressing high levels of HERG were the most sensitive to erythromycin among the tested cell lines and A549 cells expressing low levels of HERG were the least sensitive. Among the tested human cancer cell lines, the sensitivity to erythromycin correlated with the level of HERG expression. The IC_{50} of erythromycin in HT-29 cells was 106.61 ± 12.13 μ*M*. Inhibition of proliferation in HT-29 cells was increased with prolongation of the exposure time (Fig. 3), indicating that the effect was time-dependent.

Table 1 Comparison of the chemosensitivity of highly HERG-expressing HT-29 cells and low HERG-expressing A549 cells to various anticancer drugs. Cells were exposed to drug for 72 h. The data presented are the average of three different experiments, each performed in triplicate. The IC_{50} values were determined by the MTT assay

Drug	IC_{50}(μ*M*)		Ratio (A549/HT-29)
	A549	HT-29	
Vincristine	0.24 ± 0.03	0.057 ± 0.002	4.2
Paclitaxel	2.37 ± 1.54	0.04 ± 0.01	59.6
HCPT	32.52 ± 3.94	6.59 ± 3.23	4.9
CDDP	12.64 ± 1.84	14.10 ± 2.67	0.9
5-FU	13.02 ± 1.07	14.36 ± 0.58	0.9

HERG expression in *herg*-transfected A549 cells

Western blot analysis and immunocytochemistry were applied to determine the expression of HERG protein in *herg*-transfected A549 cells. After stable transfection with *herg*, the expression of HERG in A549 cells was significantly increased as compared with that of control (Fig. 4). As reported, SKBR-3 cells express the HERG protein [6, 12], so the cells were chosen as the positively immunostaining control in the experiment. As shown in Fig. 5d,e, SKBR-3 cells and *herg* stably transfected A549 cells were positive to anti-HERG antibody, revealing a prevalent, diffuse cytoplasmic immunostaining, and scantily positive nuclei. On the other hand, the immunohistochemical picture of parent A549 cells and pCDNA3.1 stably transfected A549 cells turned out to be scantily positive for the presence of the HERG protein (Fig. 5a, b) as compared to the PBS control (Fig. 5c).

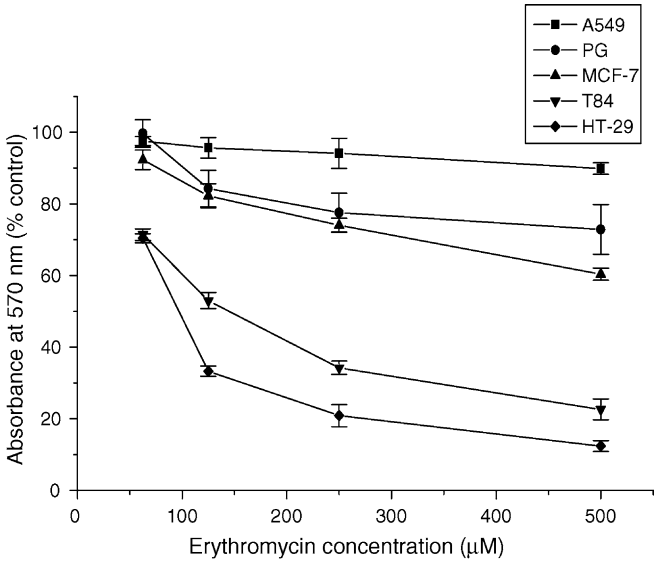


Fig. 2 Effects of erythromycin on the proliferation of A549, PG, MCF-7, T84 and HT-29 cells. Cells were exposed to the indicated concentrations of erythromycin for 48 h. Cell viability was determined by the MTT assay. The data presented are the average of three different experiments, each performed in quadruplicate (*bars* SD)

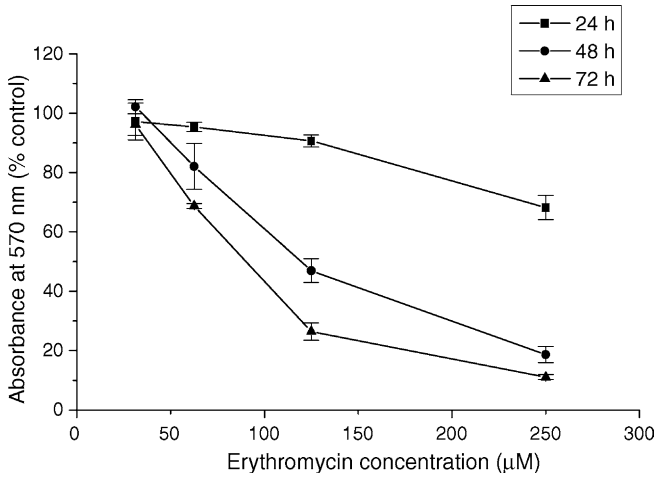


Fig. 3 Time-dependent and dose-dependent effects of erythromycin on the proliferation of HT-29 cells. Cells were exposed to the indicated concentrations of erythromycin for 24, 48, and 72 h. Cell viability was determined by the MTT assay. The data presented are the average of three different experiments, each performed in quadruplicate (*bars* SD)

Chemosensitivity of *herg*-transfected A549 cells

The MTT assay was used to evaluate the cytotoxicity of various anticancer drugs in parent A549 and *herg*-transfected A549 cells. All the IC_{50} values are the mean from three different experiments. In A549, pCDNA3.1-transtected A549, and *herg*-transfected A549 cells, the IC_{50} values of vincristine were 0.24 ± 0.03, 0.35 ± 0.05, and 0.019 ± 0.06 μ*M*, respectively, of paclitaxel were 2.37 ± 1.54, 3.56 ± 1.13, and 0.14 ± 0.03 μ*M*, respectively, and of HCPT were 32.52 ± 3.94, 49.78 ± 18.90, and 5.42 ± 0.93 μ*M*, respectively. Vincristine, paclitaxel and HCPT inhibited the proliferation of A549 and stably pCDNA3.1-transfected or *herg*-transfected A549 cells in a dose-dependent manner. Notably, the chemosensitivity of *herg*-transfected A549 cells was significantly greater than that of parent A549 or pCDNA3.1-transfected A549 cells (Fig. 6a–c).

In A549, pCDNA3.1-transfected A549, and *herg*-transfected A549 cells, the IC_{50}values of cisplatin were 4.07 ± 0.48, 4.16 ± 0.50 and 3.64 ± 0.37 μ*M*, respectively, and of 5-fluorouracil were 9.16 ± 2.17, 14.63 ± 2.15 and 10.83 ± 0.70 μ*M*, respectively. There were no significant differences in the IC_{50}values of cisplatin and 5-fluorouracil between *herg*-transfected A549 cells and parent A549 cells.

Potentiation of drug cytotoxicity by erythromycin in HT-29 cells expressing high levels of HERG

The MTT assay was used to evaluate the potentiation effects of erythromycin in combination with various anticancer drugs in HT-29 cells expressing high levels of HERG. In drug combination experiments, erythromycin was used at subcytotoxic levels. Erythromycin enhanced the cytotoxicity of vincristine, paclitaxel, and HCPT (Fig. 7a-c). However, the potentiation of HCPT by erythromycin was weaker than that of vincristine and paclitaxel. The IC_{50} values of vincristine, vincristine plus erythromycin (25 μ*M*) and vincristine plus erythromycin (50 μ*M*) were 57.12 ± 2.00, 20.50 ± 0.65, and 5.37 ± 0.48 n *M*, respectively. The IC_{50} values of paclitaxel, paclitaxel plus erythromycin (50 μ*M*) and paclitaxel plus erythromycin (100 μ*M*) were 29.59 ± 4.06, 12.69 ± 1.66, and 0.39 ± 0.14 n *M*, respectively. The IC_{50} values of HCPT, HCPT plus erythromycin (50 μ*M*) and HCPT plus erythromycin (100 μ*M*) were 6.59 ± 3.23, 2.59 ± 0.59, and 0.26 ± 0.14 μ*M*, respectively. The CDI values of erythromycin combined with cisplatin/5-fluorouracil were higher than 1, indicating no synergistic interaction between erythromycin and cisplatin/5-fluorouracil in HT-29 cells (data not shown).

Potentiation of vincristine-induced G_2/M arrest by erythromycin in HT-29 cells

As shown in Table 2, 50 μ*M* erythromycin or 5 n*M* vincristine used separately did not change the cell cycle

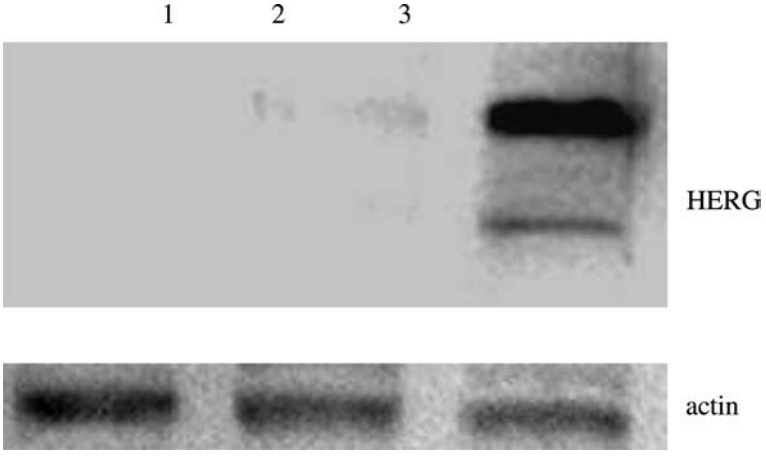


Fig. 4 Western blot analysis of the expression of HERG channel protein in stably pCDNA3.1-transfected or *herg*-transfected A549 cells. Crude membrane protein (60 μg in 40 μl) was diluted with 6× loading buffer and separated by SDS-PAGE in 7.5% acrylamide gel. *Lane 1*parent A549 cells, *lane 2* pCDNA3.1-transfected A549 cells, *lane 3herg*-transfected A549 cells

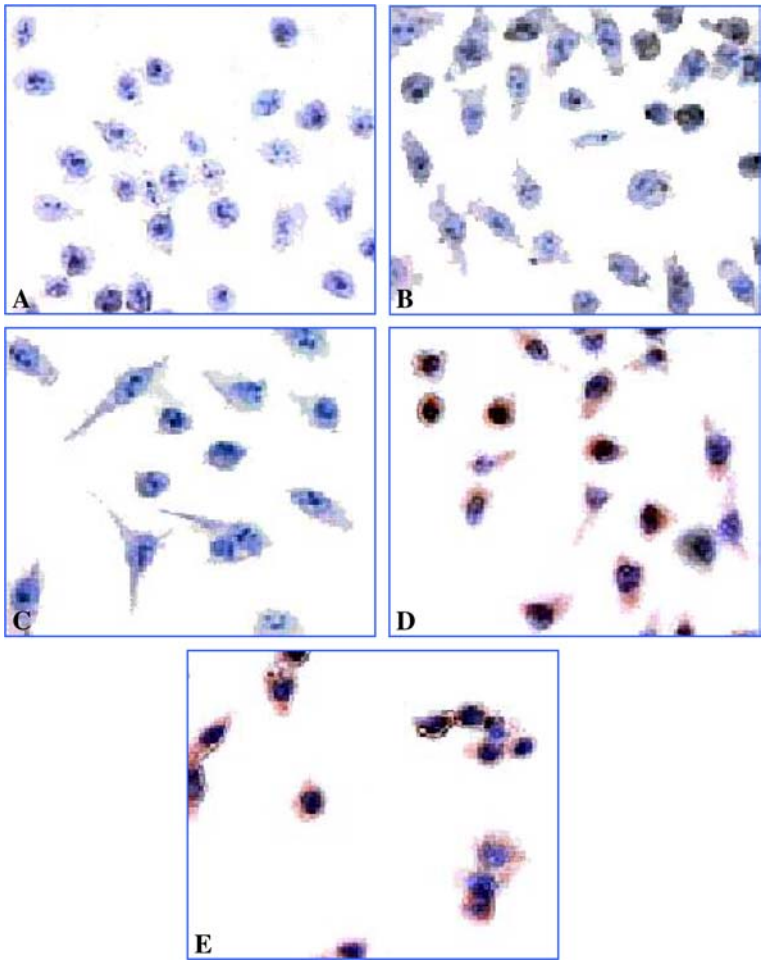


Fig. 5 Immunohistochemical detection of HERG protein. Anti-HERG C-terminus antibody, developed as described in Materials and methods, was used for cells cultured in vitro. A549 cells (**a**) and pCDNA3.1 stably transfected A549 cells (**b**) show scantily positive cytoplasmic immunostaining as compared to PBS control (**c**). A clear cytoplasmic immunostaining was found in SKBR-3 cells (**e**) and *herg* stably transfected A549 cells (**d**)

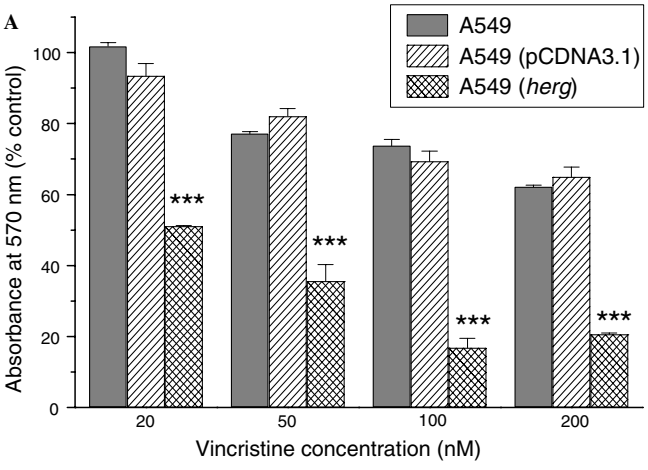
phase distribution. Notably, erythromycin potentiated the G_2/M arrest of vincristine in HT-29 cells, and this was accompanied by a decrease in cells in the G_1phase.

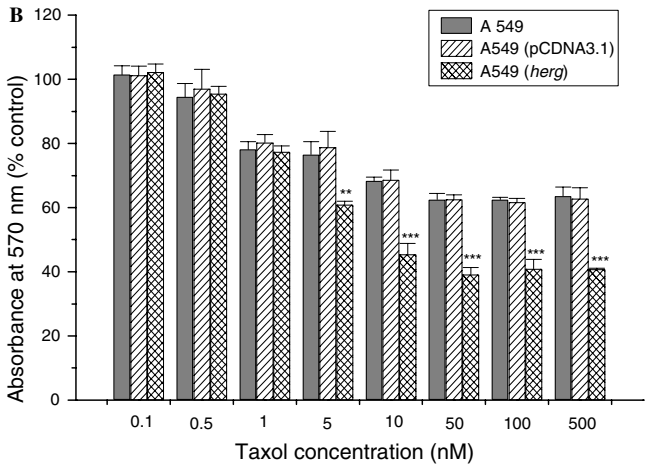
Discussion

Previous studies have shown that *herg* is highly conserved in tumors of different histogenesis [6, 7, 25] and the *herg* gene is expressed in a high percentage of colorectal cancer specimens with respect to normal colonic mucosa [26]. In our experiments, HERG expression levels varied widely between different human cancer cell lines including HT-29, T84, MCF-7, PG and A549 cells. The HERG expression level was highest in colon carcinoma HT-29 cells, and decreased in the order of T84, MCF-7, PG and A549 cells.

The role of K^+ channel activity during the cell cycle progression has become a research topic of considerable interest. Blocking of K^+ channels inhibits the proliferation of many types of cancer cells. It is reported that the blocking of HERG channels dramatically impairs cell growth of HERG-bearing tumor cells. Modulated expression of different K^+ channels is the molecular basis of a novel mechanism regulating neoplastic cell proliferation [27]. There is increasing evidence that the HERG K^+ channel is involved in the process of cancer cell proliferation. However, there have been no reports on the possible relationship between HERG expression and chemosensitivity in cancer cells. In this study cancer cells expressing high levels of HERG were more sensitive than those with low levels of HERG expression to vincristine, paclitaxel and HCPT. Notably, chemosensitivity to the drugs in *herg*-transfected cells was also increased. The level of HERG expression positively correlated with the cytotoxicity of vincristine, paclitaxel and HCPT. However, there was no correlation between chemosensitivity to cisplatin/5-fluorouracil and HERG expression level. To our knowledge, the present results are the first to demonstrate that HERG expression in cancer cells might be related to chemosensitivity to vincristine, paclitaxel, HCPT. We therefore postulate that these chemotherapeutic agents might exert a blocking effect on the HERG channel. Nevertheless, the mechanism of this drug-dependent action remains unclear. Further investigations are needed.

Several physiological ions play key roles in cellular homeostasis. Ions pass the cellular membrane mostly via ion channels and the electrophysiological characteristics of cancer cells are different from those of normal cells





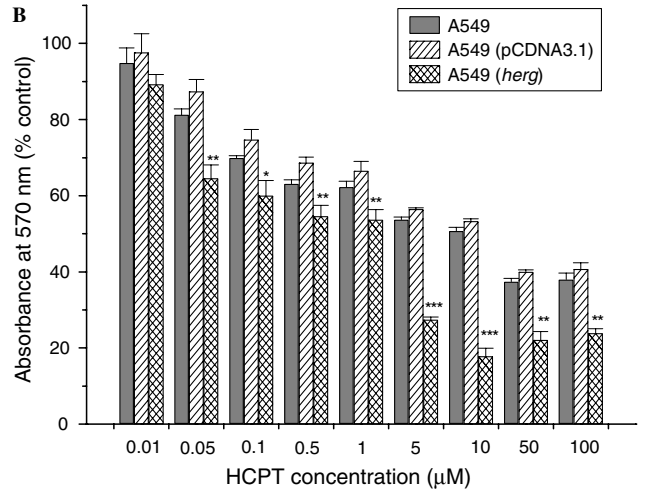
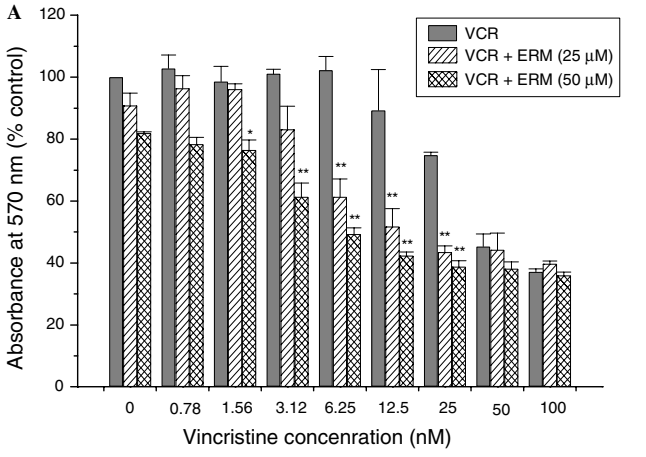
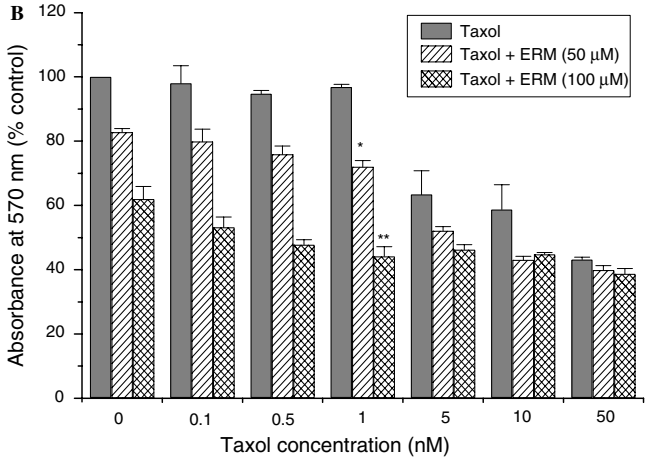


Fig. 6 Cytotoxicity of various anticancer drugs in A549, pCDNA3.1-transfected and *herg*-transfected A549 cells as determined by the MTT assay. Cells were exposed to drug for 72 h. The data presented are the average of three different experiments, each performed in triplicate (*bars* SD) (*A549* parent cell line, *A549 (pCDNA3.1)* cells stably transfected with pCDNA3.1, *A549 (*herg*)* cells stably transfected with *herg*). There are significant differences between A549 and A549 (*herg*) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$), but no significant differences between A549 and A549 (pCDNA3.1) ($P > 0.05$)





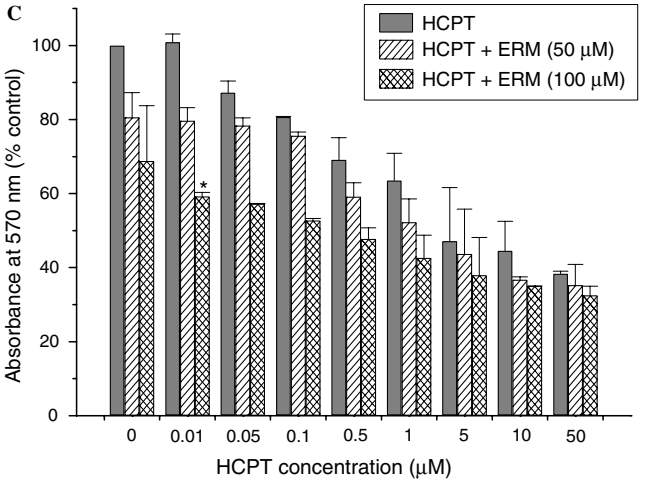


Fig. 7 Potentiation of drug cytotoxicity by erythromycin (*ERM*) in HT-29 cells expressing high levels of HERG. The cells were exposed to erythromycin for 2 h, and then vincristine (*VCR*), paclitaxel or HCPT was added. Cells were cultured for another 72 h, and then cell viability was determined by the MTT assay. The data presented are the average of three different experiments, each performed in triplicate (*bars* SD; **CDI < 0.7, *CDI < 0.85)

Table 2 Cell cycle distribution of HT-29 cells treated with vincristine alone and in combination with erythromycin. After exposure to drug for 24 h, cells were collected and resuspended in ice-cold 70% ethanol and stored at −20°C until flow cytometric analysis

	Erythromycin (μ*M*)	Vincristine (n *M*)	Cell cycle distribution (%)		
			G_0/G_1	S	G_2/M
Control	–	–	50.3	38.6	11.1
Erythromycin	50	–	50.7	36.9	12.4
Vincristine	–	5	49.2	37.5	13.3
	–	10	25.2	11.2	63.6
	–	20	10.4	7.7	81.9
Erythromycin	50	5	17.7	15.8	66.5
+ vincristine	50	10	1.3	4.0	94.7
	50	20	1.2	3.5	95.2

from the same tissues of origin [28, 29]. Calcium channel blockers may play a neutral or even beneficial role in the treatment of tumors when used either alone or in combination with standard chemotherapeutic drugs. The calcium channel blocker verapamil can inhibit the growth of tumors and drug-resistant tumors and enhance the effect of P-glycoprotein-independent anticancer drugs markedly [13, 30]. In one study, the antitumor activity of the calcium channel blocker, nifedipine, was demonstrated in human glioblastoma cells. In combination with the anticancer agent cisplatin, nifedipine inhibited tumor growth by inducing apoptosis [31]. There are reports that the proliferation of cancer cells is often reduced and sometimes blocked by K^+ channel inhibitors [12, 32, 33]. The selective HERG channel blocker, E-4031, reduced the proliferation of CEM, U937, and K562 cells which show higher *herg* transcript levels [26]. The K^+ channel blockers, dequalinium and amiodarone, show marked inhibitory effects on MCF-7 cell proliferation and potentiate the growth-inhibitory effects of tamoxifen in human breast cancer, prostate cancer and colon cancer cell lines [17]. Ion channels have become one of the potential targets for biochemical modulation in cancer chemotherapy. In this study erythromycin, a HERG channel blocker, inhibited the proliferation of some cancer cells in a dose-dependent manner and the potency was correlated with the HERG expression level. Moreover, there were synergistic effects between erythromycin and vincristine, paclitaxel and HCPT on the proliferation of HT-29 cells. Erythromycin also enhanced the arrest of the cell cycle by vincristine in HT-29 cells. The mechanisms of the effects could be related to the HERG channel blocked by erythromycin. Erythromycin displayed a modulating effect on the chemosensitivity of vincristine, paclitaxel, and HCPT in cancer cells, possibly mediated, at least in part, by HERG proteins. HERG channel blockers could be used as modulators in cancer chemotherapy.

In summary, this study demonstrated that HERG expression in cancer cells correlates with chemosensitivity to various anticancer drugs. Erythromycin is an active agent able to potentiate the effect of relevant drugs in cancer cells. The HERG channel may serve as a molecular marker for chemosensitivity and a relevant target for biochemical modulation, and therefore may be potentially useful for individualized cancer chemotherapy.

References

1. Shieh C, Coghlan M, Sullivan JP, Gopalakrishnan M (2000) Potassium channels: molecular defects, diseases, and therapeutic opportunities. Pharmacol Rev 52:557
2. Jiang M, Dun W, Tseng GN (1999) Mechanism for the effects of extracellular acidification on HERG-channel function. Am J Physiol 277:H1283
3. Tseng GN (2001) Basic cardiac electrophysiology I_{kr}: the hERG channel. J Mol Cell Cardiol 33:835
4. Jiang M, Dun W, Fan JS, Tseng GN (1999) Use-dependent 'agonist' effect of azimilide on the HERG channel. J Pharmacol Exp Ther 291:1324
5. Warmke JW, Ganetzky B (1994) A family of potassium channel genes related to eag in Drosophila and mammals. Proc Natl Acad Sci U S A 91:3438
6. Cherubini A, Taddei GL, Crociani O, Paglierani M, Buccoliero AM, Fontana L, Noci I, Borri P, Borrani E, Giachi M, Becchetti A, Rosati B, Wanke E, Olivotto M, Arcangeli A (2000) HERG potassium channels are more frequently expressed in human endometrial cancer as compared to non-cancerous endometrium. Br J Cancer 83:1722
7. Bianchi L, Wible B, Arcangeli A, Taglialatela M, Morra F, Castaldo P, Crociani O, Rosati B, Faravelli L, Olivotto M, Wanke E (1998) *herg* encodes a K^+ current highly conserved in tumors of different histogenesis: a selective advantage for cancer cells? Cancer Res 58:815
8. Pillozzi S, Brizzi MF, Balzi M, Crociani O, Cherubini A, Guasti L, Bartolozzi B, Becchetti A, Wanke E, Bernabei PA, Olivotto M, Pegoraro L, Arcangeli A (2002) HERG potassium channels are constitutively expressed in primary human acute myeloid leukemias and regulate cell proliferation of normal and leukemic hemopoietic progenitors. Leukemia 16:1791
9. Fontana L, D'Amico M, Crociani O, Biagiotti T, Solazzo M, Rosati B, Arcangeli A, Wanke E, Olivotto M (2001) Long-term modulation of HERG channel gating in hypoxia. Biochem Biophys Res Commun 286:857
10. Schlichter LC, Cayabyab FS (2002) Regulation of an ERG K^+ current by Src tyrosine kinase. J Biol Chem 277:13673
11. Hofmann G, Bernabei PA, Crociani O, Cherubini A, Guasti L, Pillozzi S, Lastraioli E, Polvani S, Bartolozzi B, Solazzo V, Gragnani L, Defilippi P, Rosati B, Wanke E, Olivotto M, Arcangeli A (2001) HERG K^+ channel activation during beta(1) integrin-mediated adhesion to fibronectin induces an up-regulation of alpha(v)beta(3) integrin in the preosteoclastic leukemia cell line FLG 29.1. J Biol Chem 276:4923
12. Wang H, Zhang Y, Cao L, Han H, Wang J, Yang B, Nattel S, Wang Z (2002) HERG K^+ channel, a regulator of tumor cell apoptosis and proliferation. Cancer Res 62:4843
13. Mason RP (1999) Effect of calcium channel blockers on cellular apoptosis. Cancer 85:2093
14. Tayor JM, Simpson RU (1992) Inhibition of cancer cell growth by calcium channel antagonists in the athymic mouse. Cancer Res 52:2413
15. Abdul M, Hoosein N (2003) Potentiation of the antiproliferative activity of MKT-077 by loperamide, diltiazem and tamoxifen. Oncol Rep 10:2023
16. Taylor CW, Dalton WS, Mosley K, Dorr RT, Salmon SE (1997) Combination chemotherapy with cyclophosphamide, vincristine, adriamycin, and dexamethasone (CAVD) plus oral quinine and verapamil in patients with advanced breast cancer. Breast Cancer Res Treat 42:7

17. Abdul M, Santo A, Hoosein N (2003) Activity of potassium channel-blockers in breast cancer. Anticancer Res 23:3347
18. Chin LS, Park CC, Zitnay KM, Sinha M, DiPatri AJ Jr, Perillan P, Simard JM (1997) 4-Aminopyridine causes apoptosis and blocks an outward rectifier K^+ channel in malignant astrocytoma cell lines. J Neurosci Res 48:122
19. Choi BY, Kim HY, Lee KH, Cho YH, Kong G (1999) Clofilium, a potassium channel blocker, induces apoptosis of human promyelocytic leukemia (HL-60) cells via Bcl-2-insensitive activation of caspase-3. Cancer Lett 147:85
20. Volberg WA, Koci BJ, Su W, Lin J, Zhou J (2002) Blockade of human cardiac potassium channel human ether-a-go-go-related gene (HERG) by macrolide antibiotics. J Pharmacol Exp Ther 302:320
21. Zhou Z, Gong Q, Ye B, Fan Z, Makielski JC, Robertson GA, January CT (1998) Properties of HERG channels stably expressed in HEK 293 cells studied at physiological temperature. Biophys J 74:230
22. Scudiero DA, Shoemaker RH, Paul KD (1988) Evaluation of a soluble tetrazolium formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res 48:4827
23. Cao SS, Zhen YS (1989) Potentiation of antimetabolite antitumor activity in vivo by dipyridamole and amphotericin B. Cancer Chemother Pharmacol 24:181
24. Zhen YS, Taniki T, Weber G (1992) Azidothymidine and dipyridamole as biochemical response modifiers: synergism with methotrexate and 5-fluorouracil in human colon and pancreatic carcinoma cells. Oncol Res 4:73
25. Smith GA, Tsui HW, Newell EW, Jiang X, Zhu XP, Tsui FW, Schlichter LC (2002) Functional up-regulation of HERG K^+ channels in neoplastic hematopoietic cells. J Biol Chem 277:18528
26. Lastraioli E, Bencini L, Guasti L, Polvani S, Pastorekova S, Olivotto M, Moretti R, Arcangeli A, Mugnai G (2003) A possible novel mechanism of tumor progression: cell surface concomitant expression of HERG K^+ channel and carbonic anhydrase IX in colorectal cancers. Proc Am Assoc Cancer Res 44:39
27. Crociani O, Guasti M, Becchetti A, Wanke E, Olivotto M, Wymore RS, Arcangeli A (2003) Cell cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. J Biol Chem 278:2947
28. Soroceanu L, Manning TJ Jr, Sontheimer H (1999) Modulation of glioma cell migration and invasion using Cl^- and K^+ ion channel blockers. J Neurosci 19:5942
29. Gerard V, Rouzaire-dubois B, Dilda P, Dubois J (1998) Alteration of ionic membrane permeabilities in multidrug-resistant neuroblastoma × glioma hybrid cells. J Exp Biol 201:21
30. Vilpo J, Koski T, Vilpo L (2000) Calcium antagonists potentiate P-glycoprotein-independent anticancer drugs in chronic leukemia cells in vitro. Haematologica 85:806
31. Kondo S, Yin D, Morimura T, Kubo H, Nakatsu S, Takeuchi J (1995) Combination therapy with cisplatin and nifedipine induces apoptosis in cisplatin-sensitive and cisplatin-resistant human glioblastoma cells. Br J Cancer 71:282
32. Rybalchenko V, Prevarskaya N, Van Coppenolle F, Legrand G, Lemonnier L, Le Bourhis X, Skryma R (2001) Verapamil inhibits proliferation of LNCaP human prostate cancer cells influencing channel gating. Mol Pharmacol 59:1376
33. Nilius B, Wohlrab W (1992) Potassium channels and regulation of proliferation in human melanoma cells. J Physiol 445:537