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Antitumor Activity of Erythromycin on Human Neuroblastoma Cell Line (SH-SY5Y)

Yongsheng JIA (贾勇圣), Xiaoyun MA (马晓芸), Xiaoli WEI (魏晓莉), Xin LI (李 昕), Haitao YAN (闫海涛), Xiaoyan LIU (刘晓燕), Jianquan ZHENG (郑建全)[#]
Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China

Summary: Antitumor effects of erythromycin and the related mechanism were investigated in the present study. Neuroblastoma cells (SH-SY5Y) were exposed to erythromycin at different concentrations for different durations. Cell proliferation was measured by cell counting, and cell viability was examined by MTT assay. Cell cycle phase distribution and the cytosolic calcium level were detected by flow cytometry. Mitochondrial membrane potential was measured by the JC-1 probe staining and fluorescent microscopy. The expression of an oncogene (c-Myc) and a tumor suppressor [p21 (WAF1/Cip1)] proteins was analyzed by using Western blotting. Erythromycin could inhibit the proliferation of SH-SY5Y cells in a concentration- and time-dependent manner. The cell cycle was arrested at S phase. Mitochondrial membrane potential collapsed and the cytosolic calcium was overloaded in SH-SY5Y cells when treated with erythromycin. The expression of c-Myc protein was down-regulated, while that of p21 (WAF1/Cip1) protein was up-regulated. It was concluded that erythromycin could restrain the proliferation of SH-SY5Y cells. The antitumor mechanism of erythromycin might involve regulating the expression of c-Myc and p21 (WAF1/Cip1) proteins.
Key words: erythromycin; c-Myc; p21 (WAF1/Cip1); neuroblastoma

Erythromycin is a clinically important broad-spectrum antibiotic that belongs to the macrolide class. Many esters of erythromycin are well established as agents to treat a variety of respiratory and cutaneous infections, particularly in children[1]. Recently, increasing evidence has revealed that erythromycin exerts a variety of effects that are not related to its antibiotic activity, including an effective aid in the management of aleukemic myeloblastic leukemias[2]. Treatment with erythromycin significantly reversed the resistance of MDR WEHI 164 murine fibrosarcoma cells to the chemotherapeutic drugs by saturating the drug-binding sites on the P-glycoprotein[3]. When given by oral administration, it prolonged the survival time of tumor-bearing mice in both allogeneic and syngeneic mouse systems by two- to three-fold as compared with those of vehicle control mice[4]. In some cancers with HERG (ether-à-go-go related gene) protein preferentially expressed, the antitumor effect of erythromycin was considered to be related to inhibiting the HERG channels[5]. However, the molecular and cellular mechanism which underlines the cell proliferation inhibitory effect of erythromycin remains obscure.

Neuroblastoma is the most common extracranial solid cancer in childhood and the most common cancer in infancy[6]. In the present experiment, human neuroblastoma cells (SH-SY5Y) were used to observe the antitumor effects of erythromycin. The mechanism was explored by observing the effects of erythromycin on cell proliferation, viability, cell cycle phase distribution, and mitochondrial membrane potential. Cancer arises from a stepwise accumulation of genetic changes that liberates neoplastic cells from the homeostatic mechanisms that govern normal cell proliferation[7]. Oncogene and tumor-suppressor gene mutations all operate similarly at the physiologic level: they drive the neoplastic process by increasing tumor cell number through the stimulation of cell birth or the inhibition of cell death or cell-cycle arrest[8]. C-Myc gene encodes for a transcription factor that is believed to regulate expression of 15% of all genes[9] through binding on Enhancer Box sequences (E-boxes) and recruiting histone acetyltransferases (HATs). This means that in addition to its role as a classical transcription factor, c-Myc also regulates apoptosis in part through interactions with the p53/Mdm2/Arf signaling pathway. Mutation in p53 is commonly observed in patients with relapsed neuroblastoma, contributing to both biology and therapeutic resistance[10]. The cyclin-dependent kinase inhibitor p21 (WAF1/CIP1) is the major transcriptional target of the tumor suppressor gene, p53; despite this, loss-of-function mutations in p21 do not accumulate in cancer nor do they predispose to cancer incidence. It was earlier reported that p21 (WAF1/CIP1) expression was required for survival of those differentiating neuroblastoma cells[11]. Recent studies showed that endogenous p21 (WAF1/CIP1) protein in neuroblastoma cells was inactive and may be bound either to a protein complex or in a conformation that precluded its binding to cdk2. The dysfunction of

Yongsheng JIA, E-mail: jiays@yahoo.com
[#]Corresponding author, E-mail: zhengjq@bmi.ac.cn

p21 in neuroblastoma cells represented a novel mechanism by which the G_0/G_1 cell cycle checkpoint could be inactivated[12]. Furthermore, Myc could repress p21 transcription, thereby overriding a p21-mediated cell cycle checkpoint[13]. Taking this together, c-Myc and p21 play a pivotal role in the process of neuroblastoma survival, differentiation and chemotherapy. To find out whether they are involved in the antitumor effects of erythromycin, the levels of c-Myc and p21 (WAF1/Cip1) protein expression were determined by western blotting assay. It was found that erythromycin could inhibit growth and proliferation of SH-SY5Y cells. The antitumor effects of erythromycin might involve down-regulation of an oncogene, c-Myc, and up-regulation of a tumor suppressor, p21 (WAF1/Cip1).

1 MATERIALS AND METHODS

1.1 Cell Culture and Treatment

The human neuroblastoma SH-SY5Y cell line (from the Cell Center at Peking Union Medical College) was used in the study. The cells were cultured in RPMI 1640 medium (Sigma, USA) containing 10% fetal calf serum (Hyclone, USA) and incubated at 37°C in a humidified atmosphere with 5% CO_2.

1.2 Cell Growth Curve

The cells were detached by trypsinization, and seeded into 12-well plates (Costar, USA.) at 5000 cells/well in 500 μL of medium and further cultured at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. Different concentrations of erythromycin were added, and cells were incubated for an additional 24 h, 48 h, 72 h or 96 h before quantification of cell growth. At each indicated time point, cells were harvested and counted with a hemocytometer. Experiments were performed in triplicate.

1.3 Cell Viability Assay

The effects of erythromycin on the viability of SH-SY5Y cells were determined by MTT-based colorimetric assay in three separate experiments performed in triplicates. Cells were seeded into a 96-well plate at a density of 2000 cells/well and cultured in 150 μL of medium and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. Different concentrations of erythromycin were added, and cells were incubated for an additional 24, 48, or 72 h before quantification of cell viability. At each indicated time point, the cells were incubated with 100 μg/well MTT solution. Four h later, the medium was replaced with 150 μL dimethyl sulfoxide (DMSO, Sigma, USA) for 10 min. Absorbance (*A*) was recorded at 492 nm using an automatic microwell plate reader (Bio-Rad 3350, USA). Cell viability (%) was calculated as percentage of the untreated cells.

1.4 Measurement of Cell Cycle

Cells were seeded into the 6-well plate at a density of 2×10^4 cells/well and cultured in 2 mL of medium without serum and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. Different concentrations of erythromycin were added, and cells were incubated for an additional 24, 48, or 72 h before measurement of cell cycle. At each indicated time point, cells were collected by trypsinization, washed in PBS and centrifuged at 800 r/min for 5 min. Cells were resuspended at 1×10^6 cells/mL in PBS containing 5% fetal bovine serum and fixed in ice-cold ethanol overnight at 4°C. Fixed cells were centrifuged and washed once with PBS. Each sample was resuspended in propidium iodide (PI) stain buffer (0.1% Triton X-100, 200 μg of DNase-free RNase A, 20 μg of PI) in PBS for 15–30 min at room temperature. After staining, 1×10^4 cells per sample were analyzed using a FACScan flow cytometer (Becton Dickinson, USA).

1.5 Mitochondrial Membrane Potentials Assay

JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide) probe (Beyotime, China) was employed to measure mitochondrial depolarization in SH-SY5Y cells. Briefly, cells cultured in 6-well plates after indicated treatments were incubated with an equal volume of JC-1 staining solution (5 μg/mL) at 37°C for 20 min and rinsed twice with PBS. Mitochondrial membrane potentials were monitored by determining the relative amounts of dual emissions from mitochondrial JC-1 monomers or aggregates using an Olympus fluorescent microscope under Argon-ion 488 nm laser excitation. Mitochondrial depolarization was indicated by an increase in the green/red fluorescence intensity ratio.

1.6 Measurement of Cytoplasmic Free Calcium

Cells were seeded into the 6-well plate at a density of 2×10^4 cells/well and cultured in 2 mL of medium and incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. Erythromycin (500 μmol/L) was added, and cells were incubated for 72 h. Then cells were collected by trypsinization, washed in PBS and centrifuged at 800 r/min for 5 min. Cells were resuspended in 200 μL PBS, 200 μL Fluo-3AM (AM ester, Biotium, USA) was added, and the final work concentration was 5 μmol/L. Cells were incubated for 40 min at 37°C, and then washed in PBS three times. After staining, the fluorescence intensity of 1×10^4 cells per sample was analyzed using the FACScan flow cytometer[14].

1.7 Western Blotting

The levels of c-Myc and p21 (WAF1/Cip1) protein expression were detected by western blotting assay. Briefly, the cell monolayer was washed three times with ice-cold PBS. Then, the cells were collected by scraping and lysed with RIPA buffer containing 50 mmol/L Tris-HCl (pH 7.4), 1% NP-40, 0.25% sodium-deoxycholate, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L Na_3VO_4, 1 mmol/L NaF and protease inhibitor mixture (phenylmethylsulfonyl fluoride 1 mmol/L, aprotinin, leupeptin, pepstatin 1 mg/mL each) at 4°C for 20 min. Cell debris was removed by centrifugation at 14 000 g for 30 min at 4°C, and protein concentration was determined by the Bradford method with BSA as a standard. The protein samples (p21 50 μg, c-Myc 20 μg) were separated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene-fluoride membrane. After being blocked in TBST (25 mmol/L Tris, pH 7.5, 135 mmol/L NaCl, 2.7 mmol/L KCl, 0.05% Tween 20) containing 5% nonfat dry milk the membrane was incubated overnight at 4°C with anti-c-Myc antibody (1:200 dilution; eBioscience, 14-6755-63, USA) and anti-p21 (WAF1/Cip1) antibody (1:200 dilution; eBioscience, 14-6715-63, USA). Thereafter, the membrane was washed three times with TBS for 10 min each and incubated with sheep

anti-rabbit IgG peroxidase conjugate secondary antibody (1:4000 dilution; Zhongshan Company, China, ZDR-5306) for 1 h at room temperature; the membrane was washed four times with TBS for 10 min and developed with the enhanced chemiluminescence method (ECL; Santa Cruz, USA). Membranes probed for c-Myc and p21 (WAF1/Cip1) were reprobed for β-actin (1:5000 dilution, Sigma, USA) to normalize for loading.

1.8 Statistical Analysis

Data were expressed as $\bar{x}\pm s$. Statistical significance of difference was determined by analysis of variance (ANOVA) using GraphPad Prism 5.01 (GraphPad Software, Inc., USA). A value of $P<0.05$ was considered significant.

2 RESULTS

2.1 Erythromycin Inhibited Cell Growth and Proliferation

When SH-SY5Y cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, they proliferated quickly. The cell count was increased from $(3.4\pm0.4)\times10^4$ to $(38.1\pm5.1)\times10^4$ after incubation for 96 h (table 1). However, the growth of cells was repressed remarkably when incubated with erythromycin. After incubation for 96 h, the inhibition rate of cell proliferation was 31.5%, 34.6%, 53.0% and 86.4% at the concentrations of 62.5, 125, 250 and 500 μmol/L, respectively. In the presence of 250 μmol/L erythromycin, the inhibition rate was 5.9%, 25.6%, 37.0%, and 53.0% after incubation for 24, 48, 72 and 96 h, respectively. Two-way ANOVA revealed that the inhibitory effect of erythromycin was enhanced with increased concentrations ($F=59.37$, $P<0.001$) and incubation time ($F=189.0$, $P<0.001$). The interaction between erythromycin concentration and incubation time was significantly different ($F=13.78$, $P<0.001$). The results implied that erythromycin could repress SH-SY5Y cell proliferation in a concentration- and time-dependent manner.

Table 1 Effect of erythromycin on proliferation of SH-SY5Y cells (n=3)

Erythromycin (μmol/L)	Cell count ($\times10^4$ cell/per well) at different incubation durations (h)*			
	24	48	72	96
0	3.4±0.4	7.8±2.0	20.0±8.1	38.1±5.1
62.5	3.3±0.3	7.3±0.6	17.3±3.2	26.1±1.6
125	3.1±0.1	7.2±0.1	15.0±0.1	24.9±1.2
250	3.2±0.3	5.8±0.1	12.6±2.2	17.9±0.1
500	1.4±0.1	2.6±0.4	1.8±0.4	5.2±0.5

*Two-way ANOVA was made. The inhibitory effect of erythromycin on SH-SY5Y cells was enhanced significantly with increased concentrations and prolonged incubation duration (P<0.001).

2.2 Erythromycin Inhibited Cell Viability

The survival rate of SH-SY5Y cells was decreased remarkably when incubated with different concentrations of erythromycin and for different durations (fig. 1). Two-way ANOVA revealed that the inhibitory effects of erythromycin on cell viability were potentiated with increased concentrations ($P<0.001$, n=3) and prolonged incubation ($P<0.001$, n=3). The interaction between erythromycin concentration and incubation was significantly different ($P<0.001$). It was suggested that erythromycin exerted inhibitory effect on viability of SH-SY5Y cells in a concentration- and time-dependent manner.

2.3 Erythromycin Influenced Cell Cycle Distribution

In control group, the percentage of cells in the S phase was (3.66±0.49)%, (5.66±0.11)% and (7.05±1.02)% at the different incubation time points of 24, 48 and 72 h, respectively (fig. 2). When the cells were incubated with 500 μmol/L erythromycin for 24 and 48 h, there was no visible change (The percentage of cells in the S phase was 3.50%±1.56% and 6.12%±0.26%, respectively). However, the percentage of cells in the S phase was increased more than twice as many (14.95%±1.63%, $P<0.01$) when the incubation time was prolonged to 72 h. It was suggested that increasing erythromycin exposure time would result in a cell cycle-arresting effect on SH-SY5Y cells.

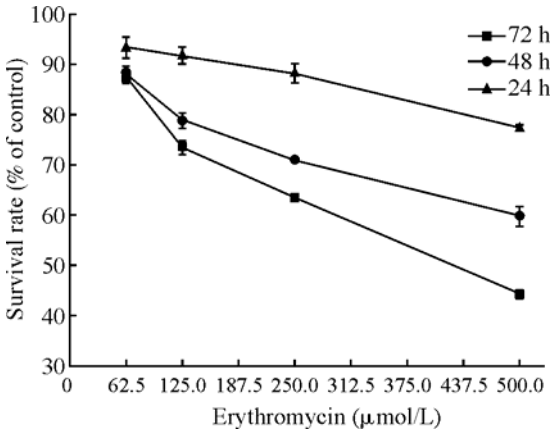

Fig. 1 Effect of erythromycin on SH-SY5Y cell viability. The cell survival rate was determined by MTT assay. All data were presented as $\bar{x}\pm s$ (n=3). Two-way ANOVA revealed that the inhibitory effects of erythromycin on cell viability was potentiated with increased concentrations ($P<0.001$) and prolonged incubation durations ($P<0.001$). The interaction between erythromycin concentration and incubation was significantly different ($P<0.001$).

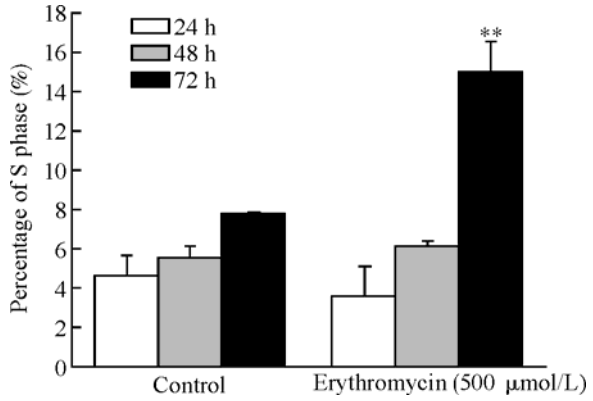

Fig. 2 Effect of erythromycin on S phase distribution of SH-SY5Y cells. The cells were incubated with 500 μmol/L erythromycin for different durations. The distribution of the cell cycle was analyzed using a FACScan flow cytometer. All data were presented as $\bar{x}\pm s$ (n=3).
$^{**}P$<0.01 *vs* control group (*t* tests)

2.4 Erythromycin Influenced Mitochondrial Function

Normal SH-SY5Y cells with JC-1 emitted mitochondrial red fluorescence with a little green fluorescence (fig. 3A). The aggregated JC-1 within normal mitochondria was dispersed to the monomeric form (green fluorescence) after treatment with 500 μmol/L erythromycin for 72 h. This was further clarified in the merged images and erythromycin-induced mitochondrial depolarization was shown as the fluorescent color change from red to yellow (fig. 3B, green merged with red). The increased green/red fluorescence intensity ratio implied that mitochondrial membrane potential was depolarized remarkably[15].

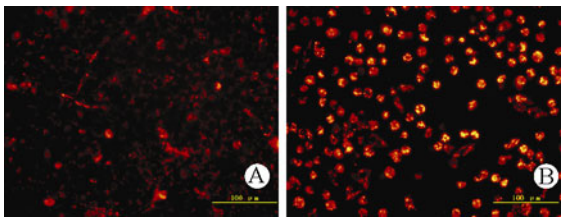

Fig. 3 Effect of erythromycin on mitochondrial membrane potential. SH-SY5Y cells were incubated for 72 h in the absence (A) or presence of 500 μmol/L erythromycin (B). Mitochondrial depolarization was indicated by an increase in the green/red fluorescence intensity ratio.

2.5 Erythromycin Increased the Cytoplasmic Free Calcium

The change of intracellular calcium level was determined by measuring the fluorescence intensity using FACScan flow cytometer. The mean fluorescence intensity in control group was significantly lower (74.82±0.24, fig. 4A) than in erythromycin group (152.62±18.42, fig. 4B) after treatment with 500 μmol/L erythromycin for 72 h. The cytosolic calcium level was 2.04-fold higher in SH-SY5Y cells of erythromycin group than in control group (P<0.01, n=3). The data showed that cytosolic calcium overloading happened in SH-SY5Y cells after treated with erythromycin.

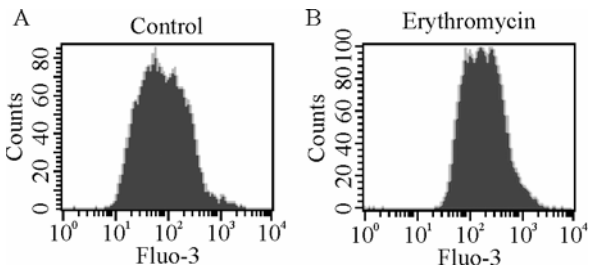

Fig. 4 Effect of erythromycin on SH-SY5Y cell [Ca^{2+}]i after incubation for 72 h without (A) or with (B) 500 μmol/L erythromycin
$^{**}P$<0.05 *vs* control group (n=3, $\bar{x}\pm s$)

2.6 Erythromycin Influenced Expression of c-Myc and p21 (WAF1/Cip1) in SH-SY5Y Cells

The expression of c-Myc and p21 (WAF1/Cip1) was detected by western blotting. When SH-SY5Y cells were treated with 62.5—500 μmol/L erythromycin for 72 h, a concentration-dependent decrease in c-Myc expression was observed. As shown in fig. 5, the expression of c-Myc protein was significantly decreased by treatment with 62.5—250 μmol/L (P<0.01) and 500 μmol/L erythromycin (P<0.001) for 72 h. Erythromycin (500 μmol/L) treatments for 24 and 48 (P<0.01) and 72 h (P<0.001) caused a time-dependent decrease of c-Myc expression (fig. 6). p21 (WAF1/Cip1) protein expression was not observed in normal SH-SY5Y cells and controls. When SH-SY5Y cells were treated with 62.5—500 μmol/L erythromycin for 48 h, a concentration-dependent increase in p21 (WAF1/Cip1) expression was detected. The expression of p21 (WAF1/Cip1) protein was significantly increased by treatment with 125—250 μmol/L erythromycin (P<0.01) and 500 μmol/L erythromycin (P<0.001) for 48 h (fig. 7).

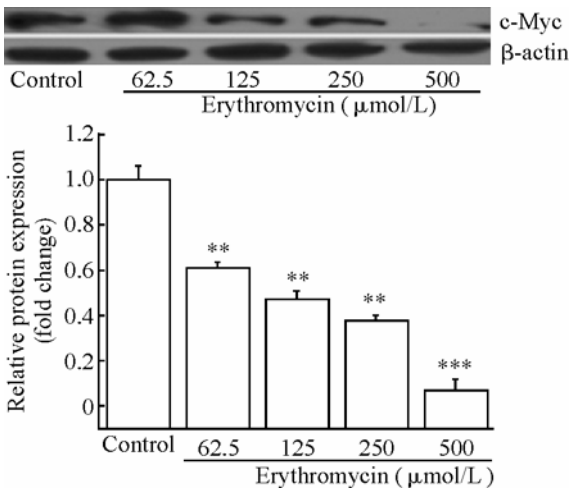

Fig. 5 Western blotting assay of c-Myc protein expression in SH-SY5Y cells after incubation with erythromycin for 72 h. β-actin was employed as a control. Data were expressed as $\bar{x}\pm s$ of 3 independent experiments
$^{**}P$<0.01, $^{***}P$<0.001 *vs* control group

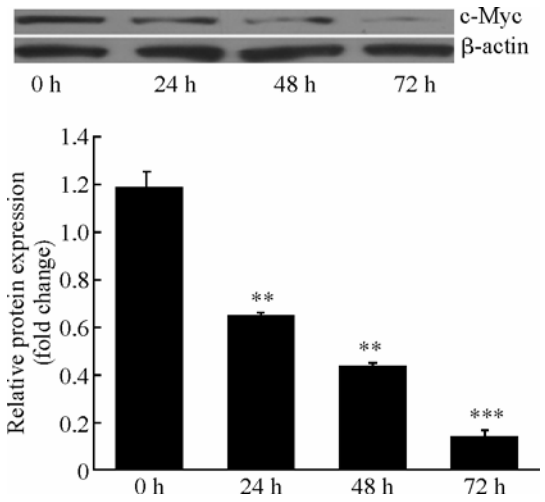

Fig. 6 Western blotting assay of c-Myc protein expression in SH-SY5Y cells after incubation with 500 μmol/L erythromycin. β-actin was employed as a control. Data were expressed as $\bar{x}\pm s$ of 3 independent experiments
$^{**}P<0.01$, $^{***}P<0.001$ *vs* 0 h

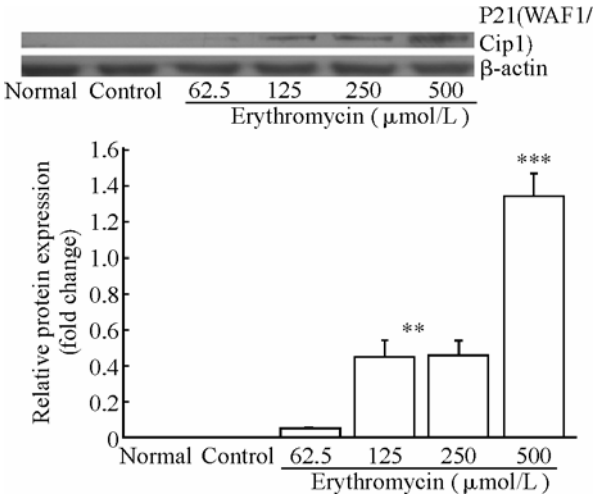

Fig. 7 Western blotting assay of p21 (WAF1/Cip1) protein expression after incubation with different concentrations of erythromycin for 48 h. β-actin was employed as a control. Data were expressed as $\bar{x}\pm s$ of 3 independent experiments.
$^{**}P<0.01$, $^{***}P<0.001$ *vs* control group

3 DISCUSSION

Erythromycin and other macrolides compounds represent an important class of antibiotics that block protein synthesis by interacting with the large ribosomal subunit[16]. However, erythromycin has been shown to exert anti-tumor activity in some tumor cell strains[17]. The results of this study also exhibited the antitumor effect of erythromycin on the neuroblastoma SH-SY5Y cells. Erythromycin could drastically reduce cell proliferation and viability in a dose- and time-dependent manner. In some cancers with HERG protein preferentially expressed, the antitumor effect of erythromycin was considered to be related to the inhibition of HERG potassium channel[5]. In addition, evidence revealed that erythromycin exhibited an indirect antineoplastic activity by enhancing the production of IL-4 which augmented the tumoricidal activity of macrophages[4]. However, a direct effect of erythromycin on the growth of tumor cells has not been fully evaluated. Our results revealed that reduction of cell growth induced by erythromycin was accompanied by collapse of mitochondrial membrane potential. In addition, erythromycin caused an intracellular Ca^{2+} level ($[Ca^{2+}]_i$) increase in SH-SY5Y cells. Ca^{2+} is an important signaling molecule that regulates multiple cellular processes, including apoptosis[18]. In this study, erythromycin-induced cell proliferation inhibition was well in agreement with the role of collapse of mitochondrial membrane potential and cytosolic calcium overloading, which is an early events in apoptosis.

Based on a deep understanding of the molecular basis of cancer, current therapeutic strategies now focus on inhibiting the molecular drivers of cancer[19]. Myc (c-Myc) protein is a transcription factor that regulates the expression of a number of genes involved in cell cycle control and metabolism[20, 21]. Myc gene is also a very strong proto-oncogene and it is very often found to be upregulated in a large percentage of human tumors, including human neuroblastoma SH-SY5Y cells (fig. 5, 6). It was newly reported that c-Myc regulated genes involved in the biogenesis of mitochondria, and regulation of glucose and glutamine metabolism[22, 23]. Myc-mediated altered cancer cell energy metabolism could be translated for the development of new anticancer therapies[24]. These experimental approaches have provided important details on mitochondrial dysfunction followed by down-regulation of c-Myc. Therefore, erythromycin-induced down-regulation of c-Myc might contribute, at least partially, to its antitumor activity, which would trigger a "vicious cycle" of mitochondrial calcium overloading and restrain the proliferation of SH-SY5Y cells. Moreover, c-Myc has a direct role in the control of DNA replication and interacts with the pre-replicative complex and localizes to early sites of DNA synthesis[25], which lends a support to the occurring of accumulation of SH-SY5Y cells in the S phase of the cell cycle following erythromycin administration.

The molecular balance between oncoprotein and tumor suppressor is finely tuned to meet cell specific energetic, metabolic, and signaling demands in cancer cells. This tuning is largely achieved at the level of transcriptional regulation, and is believed to be critical in cell's decision to survive or to die[26]. P21 (WAF1/Cip1) is a cyclin-dependent kinase inhibitor (CKI) that directly inhibits the activity of cyclin E/CDK2 and cyclin D/CDK4 complexes. P21 functions as a regulator of cell cycle progression at S phase. We here provide evidence that there is a concurrent of p21 (WAF1/Cip1) up-regulation and c-Myc down-regulation after erythromycin administration in SH-SY5Y cells. It is probably a new clue to explore the antitumor mechanism of erythromycin. Obviously, much more researches are needed to elucidate how erythromycin regulates the expression of c-Myc and p21 (WAF1/Cip1) proteins. And it is also needed to clarify whether erythromycin-induced down-regulation of c-Myc relates to its blockade on HERG potassium channel. Nevertheless, the present findings might offer some insights into a possible molecular mechanism of erythromycin on modulating the

function between oncoprotein and tumor suppressor.

In conclusion, the results demonstrate that erythromycin can exert an inhibitory control on the growth and proliferation of SH-SY5Y cells, which implies a potential application of erythromycin in human neuroblastoma therapy. Erythromycin-induced mitochondrial membrane potential collapsing, the cytosolic calcium overloading and cell cycle arrest at the S phase might relate to its modulation on the expression of c-Myc and p21 (WAF1/Cip1) proteins.

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REFERENCES

1 Neu HC. The development of macrolides: clarithromycin in perspective. J Antimicrob Chemother, 1991, 27(Suppl A):1-9
2 Reich C. Erythromycin as an aid in the management of aleukemic myeloblastic leukemias. N Y State J Med, 1954,54(15):2208-2209
3 Hofsli E, Nissen-Meyer J. Reversal of drug resistance by erythromycin: erythromycin increases the accumulation of actinomycin D and doxorubicin in multidrug-resistant cells. Int J Cancer, 1989,44(1):149-154
4 Hamada K, Kita E, Sawaki M, *et al*. Antitumor effect of erythromycin in mice. Chemotherapy, 1995,41(1):59-69
5 Volberg WA, Koci BJ, Su W, *et al*. Blockade of human cardiac potassium channel human ether-a-go-go-related gene (HERG) by macrolide antibiotics. J Pharmacol Exp Ther, 2002,302(1):320-327
6 Park J R, Eggert A, Caron H. Neuroblastoma: biology, prognosis, and treatment. Hematol Oncol Clin North Am, 2010,24(1):65-86
7 Hahn WC, Weinberg RA. Modeling the molecular circuitry of cancer. Nat Rev Cancer, 2002,2(5):331-341
8 Gorlov IP, Byun J, Gorlova OY, *et al*. Candidate pathways and genes for prostate cancer: a meta-analysis of gene expression data. BMC Med Genomics, 2009,2:48
9 Gearhart J, Pashos EE, Prasad MK. Pluripotency redux—Advances in stem-cell research. N Engl J Med, 2007,357(15):1469-1472
10 Gustafson WC, Weiss WA. Myc proteins as therapeutic targets. Oncogene, 2010,29(9):1249-1259
11 Poluha W, Poluha DK, Chang B, *et al*. The cyclin-dependent kinase inhibitor p21 (WAF1) is required for survival of differentiating neuroblastoma cells. Mol Cell Biol, 1996,16(4):1335-1341
12 Mckenzie PP, Danks MK, Kriwacki RW, *et al*. P21Waf1/Cip1 dysfunction in neuroblastoma: a novel mechanism of attenuating G0-G1 cell cycle arrest. Cancer Res, 2003,63(13):3840-3844
13 Seoane J, Le HV, Massague J. Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. Nature, 2002,419(6908): 729-734
14 June CH, Moore JS. Measurement of intracellular ions by flow cytometry. Curr Protoc Immunol, 2004, Chapter 5:Unit 5.5
15 Hu Y, Xia XY, Pan LJ, *et al*. Evaluation of sperm mitochondrial membrane potential in varicocele patients using JC-1 fluorescent staining. Zhonghua Nan Ke Xue (Chinese) 2009,15(9):792-795.
16 Lovmar M, Nilsson K, Vimberg V, *et al*. The molecular mechanism of peptide-mediated erythromycin resistance. J Biol Chem, 2006,281(10):6742-6750
17 Chen SZ, Jiang M, Zhen YS. HERG K+ channel expression-related chemosensitivity in cancer cells and its modulation by erythromycin. Cancer Chemother Pharmacol, 2005,56(2):212-220
18 Baumann S, Fas SC, Giaisi M, *et al*. Wogonin preferentially kills malignant lymphocytes and suppresses T-cell tumor growth by inducing PLCgamma1- and Ca^{2+}-dependent apoptosis. Blood, 2008,111(4): 2354-2363
19 Dick JE. Stem cell concepts renew cancer research. Blood, 2008,112(13):4793-4807
20 Dang CV. c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol, 1999,19(1): 1-11
21 Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. Nat Rev Cancer, 2002, 2(10):764-776
22 Li F, Wang Y, Zeller KI, *et al*. Myc stimulates nuclearly encoded mitochondrial genes and mitochondrial biogenesis. Mol Cell Biol, 2005,25(14):6225-6234
23 Gao P, Tchernyshyov I, Chang TC, *et al*. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature, 2009, 458(7239):762-765
24 Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res, 2009,15(21):6479-6483
25 Dominguez-Sola D, Ying CY, Grandori C, *et al*. Non-transcriptional control of DNA replication by c-Myc. Nature, 2007,448(7152):445-451
26 Dai MS, Jin Y, Gallegos JR, *et al*. Balance of Yin and Yang: ubiquitylation-mediated regulation of p53 and c-Myc. Neoplasia, 2006,8(8):630-644

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