

# Reversal in multidrug resistance by magnetic nanoparticle of $\text{Fe}_3\text{O}_4$ loaded with adriamycin and tetrandrine in K562/A02 leukemic cells

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**Abstract:** Drug resistance is a primary hindrance for efficiency of chemotherapy. To investigate whether  $\text{Fe}_3\text{O}_4$ -magnetic nanoparticles ( $\text{Fe}_3\text{O}_4$ -MNPs) loaded with adriamycin (ADM) and tetrandrine (Tet) would play a synergetic reverse role in multidrug resistant cell, we prepared the drug-loaded nanoparticles by mechanical absorption polymerization to act with K562 and one of its resistant cell line K562/A02. The survival of cells which were cultured with these conjugates for 48 h was observed by MTT assay. Using cells under the same condition described before, we took use of fluorescence microscope to measure fluorescence intensity of intracellular ADM at an excitation wavelength of 488 nm. P-glycoprotein (P-gp) was analyzed with flow cytometer. The expression of *mdr1* mRNA was measured by RT-PCR. The results showed that the growth inhibition efficacy of both the two cells increased with augmenting concentrations of  $\text{Fe}_3\text{O}_4$ -MNPs which were loaded with drugs. No linear correlation was found between fluorescence intensity of intracellular adriamycin and augmenting concentration of  $\text{Fe}_3\text{O}_4$ -MNPs. Tet could downregulate the level of *mdr-1* gene and decrease the expression of P-gp. Furthermore, Tet polymerized with  $\text{Fe}_3\text{O}_4$ -MNPs reinforced this downregulation, causing a 100-fold more decrease in *mdr1* mRNA level, but did not reduce total P-gp content. Our results suggest that  $\text{Fe}_3\text{O}_4$ -MNPs loaded with ADM or Tet can enhance the effective accumulation of the drugs in K562/A02. We propose that  $\text{Fe}_3\text{O}_4$ -MNPs loaded with ADM and Tet probably have synergetic effect on reversal in multidrug resistance.

**Keywords:** magnetic nanoparticles, tetrandrine, adriamycin, multidrug resistance reversal, leukemia K562/A02

## Introduction

The multidrug resistance (MDR) phenotype, induced by the over expression of ATP-binding cassette (ABC) transporters, a family of transporter proteins, is responsible for chemotherapy failure in patients with hematological malignancies. This phenotype is generally due to the expression of the *MDR1* gene encoding a 170 kDa ABC transporter P-glycoprotein (P-gp) in the cell membrane of cells pumping chemotherapeutic drugs out of cells. Once MDR emerges, chemotherapy is not effective when using high doses of drugs enough to overcome the resistance, even toxic effects are caused and the resistance mechanism could be further stimulated (Choi 2005). Modulation of P-gp activity by chemotherapy is limited because of toxicity and poor specificity. To reverse the resistance mechanism and reduce side effects during chemotherapy, a promising approach is to combine a conventional chemotherapy with new strategies such as chemosensitizers to inhibit ABC transporters, nanotechnology for targeted drug delivery (Page and Takimoto 2004).

*In vitro* and *in vivo* studies have demonstrated that tetrandrine (Tet), a kind of herbal constituent, possesses potent and specific activity in reversing P-gp-mediated drug resistance (Fu et al 2004; Zhou et al 2004; Zhu et al 2005). This naturally occurring

compound may be used as a chemosensitizer in the treatment of P-gp-mediated MDR malignancies (Liu et al 2003).

Nanotechnology and nanoscience have developed fast during the last few decades. The most important clinical applications of nanotechnology are probably in pharmaceutical development (Thrall 2004). The *in vitro* cytotoxicity test revealed that Fe<sub>3</sub>O<sub>4</sub>-magnetic nanoparticles (Fe<sub>3</sub>O<sub>4</sub>-MNPs) exhibited excellent biocompatibility (Win et al 2003; Cheng et al 2005), furthermore, no carcinogenic effects have been demonstrated for the magnetite particles (Kosar et al 2004).

To date, few studies have approached the mechanism of drug-loaded nanoparticles affecting an drug-resistant leukemia cell (Laurand et al 2004). In our experiment, we prepared Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with ADM and Tet to interfere MDR of K562/A02 cell. By analyzing several biological parameters, we found that ADM and Tet-loaded Fe<sub>3</sub>O<sub>4</sub>-MNPs would contribute to a synergetic reverse in multidrug resistant cells K562/A02, that may owe to the accumulation of drugs caused by its polymerization with Fe<sub>3</sub>O<sub>4</sub>-MNPs; Besides, Tet-loaded Fe<sub>3</sub>O<sub>4</sub>-MNPs enhances MDR1 downregulation but doesn't reduce the total P-gp content.

## Materials and methods

### Main reagents

Adriamycin (ADM, doxorubicin) stock solution 50 mmol/l (Sigma Aldrich Ltd., St. Louis, MO, USA) was prepared with 0.01 mol/l phosphate buffer saline (PBS) (pH 7.4). Tet was kindly provided by Yintao Pharmaceutical (Jiangxi, China) which would be diluted also with 0.01 mol/l PBS (pH 7.4). All reagents used in this study are analytical grade.

### Cell lines and culture conditions

K562 cells, derived from human leukemic cells of a chronic myeloid leukemia patient in blastic crisis, had been constantly preserved in our laboratory. K562/A02 cell line (a gift from Institute of Hematology, Chinese Academy of Medical Sciences, Tianjin, China) was incubated in the medium containing 1 µg/ml ADM for maintaining the resistant characteristics. We kept K562/A02 in medium without ADM for 2 weeks before using these cells. Both the cells were cultured at 37 °C in a 5% CO<sub>2</sub> incubator with 100% humidity in RPMI 1640 medium (Gibco/BRL, Bethesda, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sijiqing, Hangzhou, China), 100 U/ml penicillin, and 100 µg/ml streptomycin.

### Preparation of drug-loaded nanoparticles

Fe<sub>3</sub>O<sub>4</sub>-MNPs were prepared by the electrochemical deposition under oxidizing conditions (EDOC), where the magnetization

and the size of Fe<sub>3</sub>O<sub>4</sub>-MNPs was found to be  $25.6 \times 10^{-3}$  emu/mg and ca. 30 nm, respectively (Zhang et al 2006). Before applied in the present experiment, the Fe<sub>3</sub>O<sub>4</sub> magnetite nanoparticles were well-distributed in RPMI 1640 medium freshly added with 10% heat-inactivated FBS by using ultrasound treatment in order to obtain Fe<sub>3</sub>O<sub>4</sub>-MNPs colloidal suspension. ADM conjugated with Fe<sub>3</sub>O<sub>4</sub>-MNPs (Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM) or ADM and Tet co-conjugated with Fe<sub>3</sub>O<sub>4</sub>-MNPs (Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM-Tet) were prepared by mechanical absorption polymerization referring to previously reported (Bennis et al 1994; Gao et al 2004). We also investigated the temperature effect and have chosen 37 °C or 4 °C for the polymerization process. Briefly, different concentrations (V/V), Fe<sub>3</sub>O<sub>4</sub>-MNPs (0.05, 0.1, 0.2, 0.4) were respectively added under mechanical stirring to 200 µl of an aqueous medium with certain concentration of ADM (Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM0.05, Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM0.1, Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM0.2, Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM0.4) that in the final nanoparticle and cell suspension was 50 µmol/l (pH 7.4) or with ADM and Tet (Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM-Tet0.05, Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM-Tet0.1, Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM-Tet0.2, Fe<sub>3</sub>O<sub>4</sub>-MNPs-ADM-Tet0.4) that its final concentration was 10 µmol/l (pH 7.4). At different temperatures (37 °C, 4 °C), the overall polymerization process lasted for 24 h. Besides, ADM 50 µmol/l, Fe<sub>3</sub>O<sub>4</sub>-MNPs 0.2 and Tet 10 µmol/l were taken as positive controls. RPMI 1640 medium was regarded as blank control and cells with no intervention were negative control.

### MTT assay

Exponentially growing cells ( $1.5 \times 10^5$ /ml) were suspended in 200 µl of culture medium on 96-well culture plate containing different concentrations Fe<sub>3</sub>O<sub>4</sub>-MNPs and other control groups which had been demonstrated above. The survival of cells which were incubated with these conjugates for 48 h at 37 °C was observed through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Sigma). The growth inhibition efficacy (GIE) of cells was determined as follows:  $(1 - A \text{ of tests cells} / A \text{ of blank control}) \times 100\%$ . The experiments were repeated at least three times.

### Fluorescence microscopy study

As described before, cells ( $1.5 \times 10^5$ /ml) were incubated with ADM polymerized with different concentrations of Fe<sub>3</sub>O<sub>4</sub>-MNPs for 48 h at 37 °C, followed by centrifugation at 1000 r/min for 5 min. The resulting supernatant fractions were then discarded. Cells were washed twice with ice-cold PBS and re-suspended in 200 µl PBS in order to measured fluorescence intensity (FI) of intracellular ADM at a wavelength of 488 nm under fluorescence microscope in triplicate different visual

fields at random. FI was quantitated through Image J software and was made a curve to analyze liner regression.

### Flow cytometry study

To detect P-gp expression, cell suspensions ( $1.0 \times 10^6$ ) after treated for 48 h were washed twice with PBS and centrifugation at 1000 r/min for 5 min (Kan et al 1998; Geromin et al 2004). Then the cells were incubated either with the monoclonal antibody (MoAb) P-gp-PE (20  $\mu$ l) (Becton Dickinson, Mountain View, CA, USA) or the mouse isotype-matched control IgG2a-PE (20  $\mu$ l) (Becton Dickinson) at 4 °C for 30 min. After washed with PBS twice, the cells were resuspended in PBS 400  $\mu$ l and fluorescence distributions were generated by a FACS Calibur flow cytometry (Becton Dickinson). P-gp expression were given as the ration of P-gp MoAb mean fluorescence divided by control MoAb fluorescence.

### Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted from K562/A02 cell line with TRIzol reagent (Gibco/BRL) and quantified by UV absorbance spectroscopy and 1.5% agarose gel. Each lane contained RNA from the cells grown with exposure to ADM (50  $\mu$ mol/l), Fe<sub>3</sub>O<sub>4</sub>-MNPs (0.2), Tet (10  $\mu$ mol/l), Tet (1  $\mu$ mol/l) loaded Fe<sub>3</sub>O<sub>4</sub>-MNPs (0.2), Tet (5  $\mu$ mol/l) loaded Fe<sub>3</sub>O<sub>4</sub>-MNPs (0.2) and Tet (10  $\mu$ mol/l) loaded Fe<sub>3</sub>O<sub>4</sub>-MNPs (0.2), respectively. Negative control was also set for a lane. The reverse transcription reaction was performed using TaKaRa RNA PCR kit (AMV) Ver.3.0 (Dalian, China). The newly synthesized cDNA was amplified by PCR (TaKaRa). Primers involved in were: the *mdr1* primer (forward: 5'-TGG TTT GAT GTG CAC GAT GTT GGG-3'; reverse: 5'-AGA TCA GCA GGA AAG CAG CAC CTA-3'); for the  $\beta$ -actin primer (forward: 5'-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3'; reverse: 5'-CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG-3') (Gan et al 2005). The amplified PCR products were 436 bp and 661 bp for MDR1 and  $\beta$ -actin, respectively. The conditions for PCR were as follows: 94 °C predenaturation for 5 min, 94 °C denaturation for 30 s, 56 °C annealing for 30 s, 72 °C extension for 1 min followed by 72 °C 10 min for 35 cycles. Aliquots of the PCR product were electrophoresed on 1.5% agarose gels and PCR fragments were visualized by UV illumination (UVP GDS7500 Gel, Upland, CA, USA) stained by ethidium bromide (Thörn et al 2005). The fluorescence intensity of  $\beta$ -actin fragments served as the criterion for the MDR1 fragments. Densitometric analysis was performed using the electrophoresis image analysis system Smart View 2000 software (Furi, Shanghai, China).

### Statistical analysis

Data were analyzed using the Statistical Package for Social Science (SPSS Release 11.5; SPSS Inc., Chicago, IL, USA). Differences were evaluated using the one-way ANOVA, Student's t-test or paired t-test. A P value < 0.05 or < 0.01 was considered significant. The results were expressed as the mean  $\pm$  SD of data obtained in triplicate. The liner regression between GIE of the two cells and increasing concentrations Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with ADM or fluorescence intensity of K562/A02 intracellular ADM and increasing concentrations Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with ADM was estimated using simple regression analyze (Microsoft Excel; Microsoft, Redmond, WA, USA).

## Results

### Microscope image of cells with different treatments

ADM was polymerized with Fe<sub>3</sub>O<sub>4</sub>-MNPs at 37 °C or 4 °C for 24 h before that was added in K562 or K562/A02 cultured at 37 °C for 48 h in a 5% CO<sub>2</sub> incubator with 100% humidity in RPMI 1640 medium. It is observed that ADM polymerized with Fe<sub>3</sub>O<sub>4</sub>-MNPs aggregated inside the two kinds of malignant hemopathic cells obviously (Figure 1; Figure 2).

### Cell survival

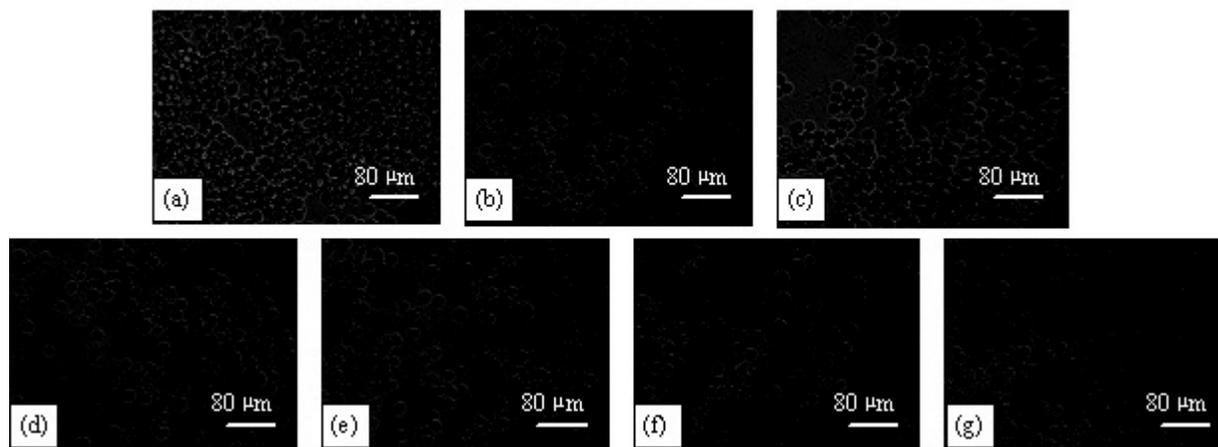
According to MTT assay, the GIE of both the two cell lines increased with augmenting concentrations of Fe<sub>3</sub>O<sub>4</sub>-MNPs which were loaded with drugs: for K562, positive liner regression existed between GIE of the cell line and increasing concentrations Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with ADM ( $r^2 > 0.95$ ,  $P < 0.05$ ) (Figure 3). In contrast, there wasn't good liner regression between those in K562/A02 cell line (Figure 4). Furthermore, the GIE of K562 was significantly higher than that of K562/A02 ( $P < 0.01$ ). It was inferred that better polymerization temperature was 4 °C rather than 37 °C from MTT results ( $P < 0.05$ ).

### Fluorescence intensity of intracellular ADM

At a wavelength of 488nm, ADM was excited to emit a 575 nm wavelength spontaneously which was orange red seen by fluorescence microscope of low power lens ( $\times 600$ ) and high power lens ( $\times 2400$ ), where fluorescence intensity of K562/A02 intracellular ADM could be recorded and analyzed (Figure 5; Figure 6).

### P-glycoprotein (P-gp) expression

Tet or different concentrations of Tet polymerized 0.1 Fe<sub>3</sub>O<sub>4</sub>-MNPs at 4 °C for 48 h were incubated with K562/A02 cells

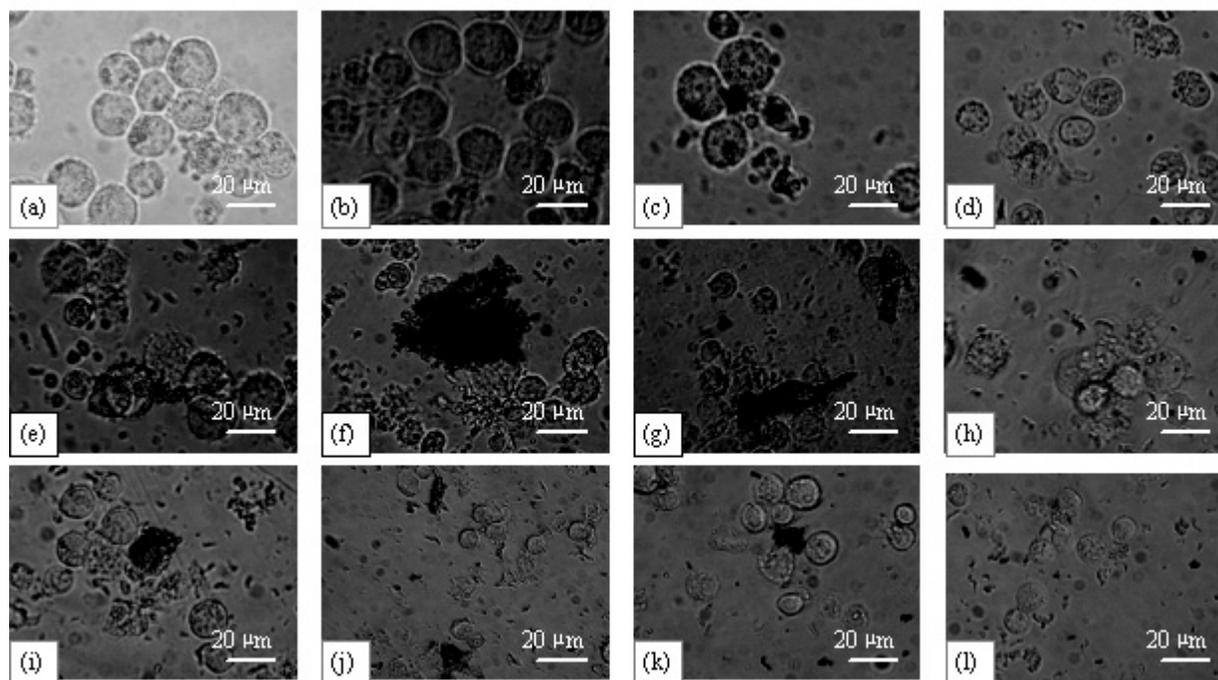


**Figure 1** Optical inverted microscope image of K562 incubating with different concentration of  $\text{Fe}_3\text{O}_4$ -magnetic nanoparticles polymerized with ADM for 48 h (original magnification  $\times 600$ , polymerization condition:  $4^\circ\text{C}$ , 24 h). (a) negative control (K562). (b) K562 + ADM ( $50 \mu\text{mol/l}$ ). (c) K562 +  $\text{Fe}_3\text{O}_4$ -MNPs0.2. (d) K562 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.05. (e) K562 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.1. (f) K562 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.2. (g) K562 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.4.

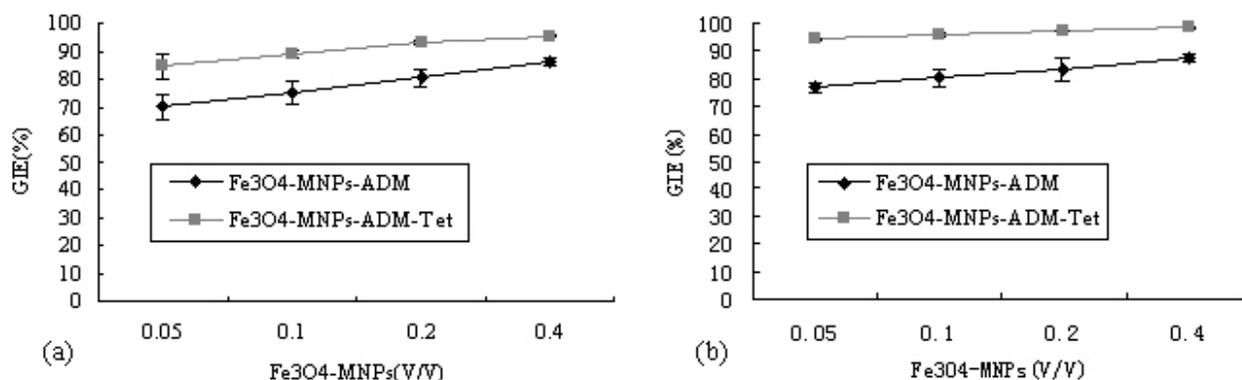
for 48 h while  $\text{Fe}_3\text{O}_4$ -MNPs were incubated with the cells as control (Sun et al 2007). To determine whether Tet or Tet-loaded  $\text{Fe}_3\text{O}_4$ -MNPs could influence P-gp, flow cytometry was performed and it was indicated the total expression content of P-gp was decreased by 24% with Tet, in contrast,  $\text{Fe}_3\text{O}_4$ -MNPs loaded with any concentration of Tet we took in the experiment seemed no quantity degression of P-gp (Figure.7)

## MDR1 mRNA content

Different concentrations of Tet polymerized with  $\text{Fe}_3\text{O}_4$ -MNPs all reinforced down-regulation of MDR1 mRNA content significantly, causing a 100-fold more decrease in MDR1 mRNA level and Tet single used was brought less down-regulation than the same concentration of Tet polymerized with  $\text{Fe}_3\text{O}_4$ -MNPs; nevertheless, ADM or  $\text{Fe}_3\text{O}_4$ -MNPs single used had no obvious effect on MDR1 mRNA (Figure 8).



**Figure 2** Optical inverted microscope image of K562/A02 incubating with different concentration of  $\text{Fe}_3\text{O}_4$ -magnetic nanoparticles polymerized with ADM for 48 h. (original magnification  $\times 2400$ , polymerization condition:  $4^\circ\text{C}$  24 h). (a) negative control (K562/A02). (b) K562/A02+ADM ( $50 \mu\text{mol/l}$ ). (c) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs0.2. (d) K562/A02 + ADM + Tet ( $10 \mu\text{mol/l}$ ). (e) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.05. (f) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.1. (g) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.2. (h) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM0.4. (i) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM-Tet0.05. (j) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM-Tet0.1. (k) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM-Tet0.2. (l) K562/A02 +  $\text{Fe}_3\text{O}_4$ -MNPs-ADM-Tet0.4.

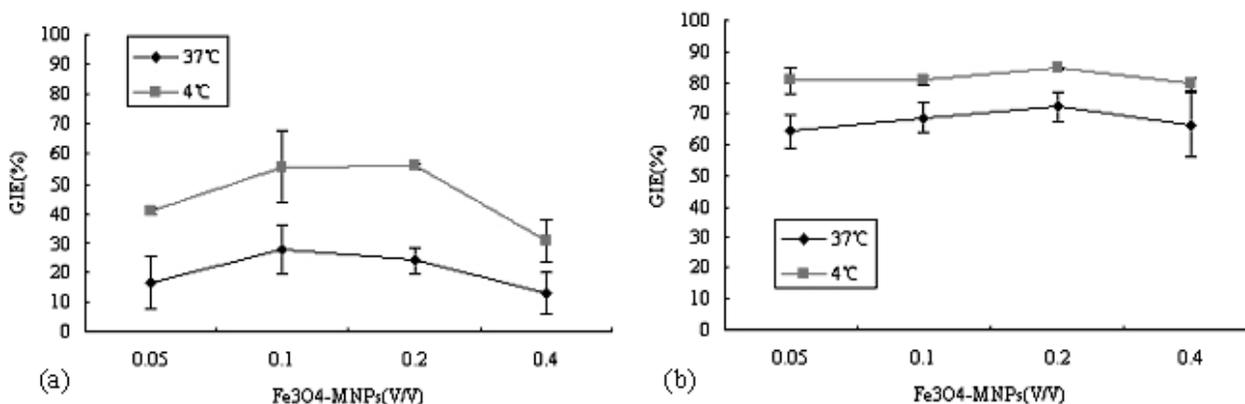


**Figure 3** GIE of K562 cell line by increasing concentrations Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> loaded with ADM or ADM and Tet. (a) After Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> polymerized with ADM at 37 °C for 24 h, the ADM loaded Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> inhibited cells proliferation more with the increasing concentrations Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub>, so did incorporation of ADM and Tet-loaded Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub>,  $R^2 = 0.9996$  and  $0.9805$  respectively,  $P < 0.05$ . Comparatively, GIE of K562 acting with ADM or Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> single used was  $73.67 \pm 3.00\%$  and  $50.56 \pm 6.07\%$  respectively. (b) The similar polymerization at 4 °C for 24 h was done,  $R^2 = 0.9956$  and  $0.9891$  respectively,  $P < 0.05$ . Comparatively, GIE of K562 acting with ADM or Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> single used was  $70.42 \pm 1.88\%$  and  $21.86 \pm 3.80\%$  respectively.

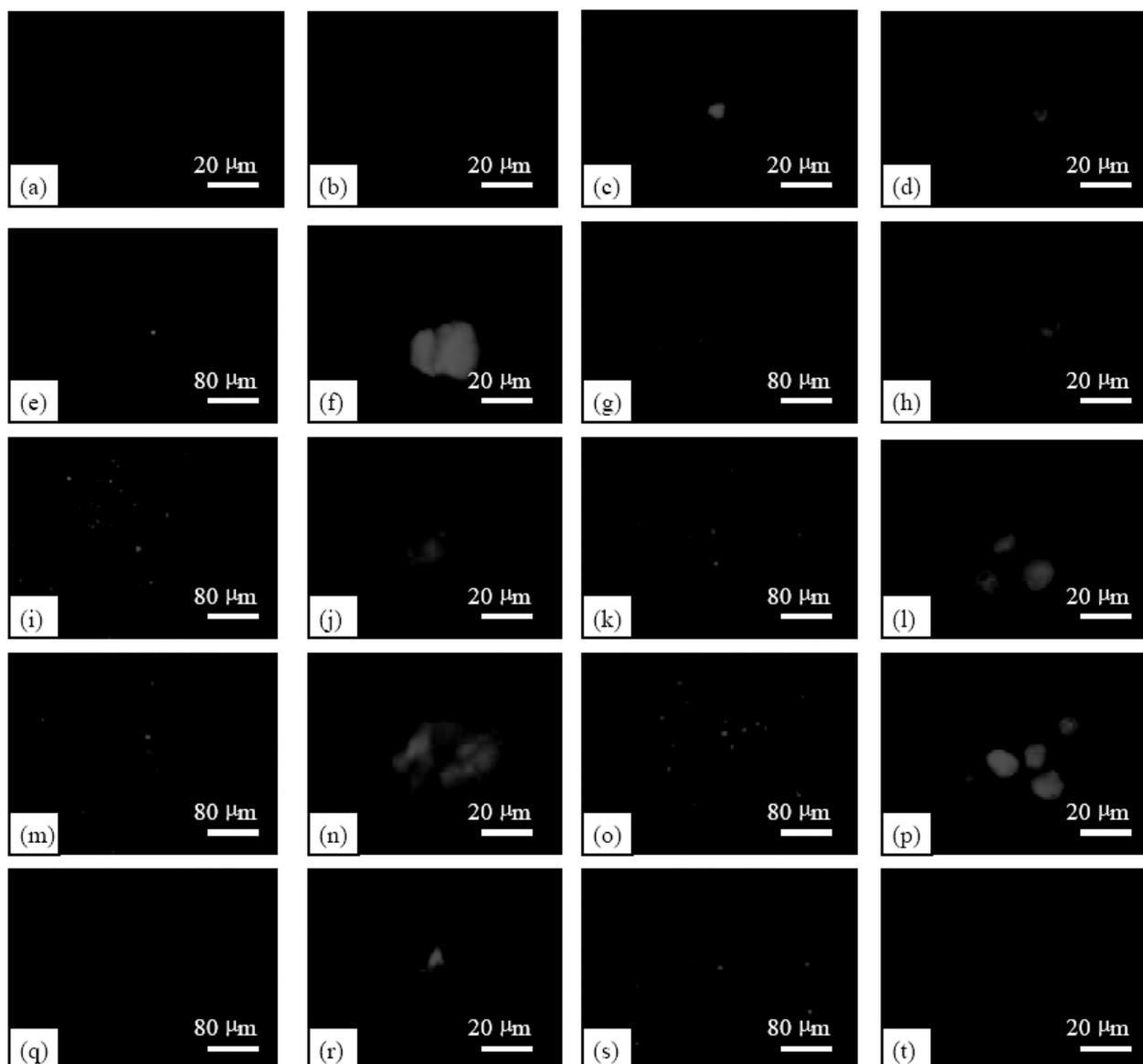
## Discussion

Reversal in MDR is considered to be the most important for the cancer chemotherapy in clinical. The problems of overcoming resistance could be resolved by the use of the some drugs that could overcome the resistance mechanism. These substances would reverse resistance against anticancer drugs to eventually being sensitized for anticancer drugs, so they are called chemosensitizers or MDR reverters. As a result, reversal in MDR is equal to chemosensitizing. Researchers have been seeking for low toxic and high efficient MDR reverters in MDR reversal. Any access which could increase the effective concentration of intracellular chemotherapeutic agent is available. The propensity of macrophages of the reticuloendothelial system for rapid recognition and clearance of particulate matter has provided a rational approach to macrophage targeting

with nanoparticles loaded with drugs (Moghimi et al 2005; Baker 2006). The feature has contribution to treating hematologic malignancies. Due to the unique properties of some biocompatible nanoparticles, they are designed for new approaches to drug-targeting delivery, controlled release and so on (Song et al 2006). As one of the most commonly used magnetic nanoparticles, Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> are gained more easily than other MNP<sub>s</sub> and they may aggregate in water or tissue fluid spontaneously with good biocompatibility and low toxicity (Dresco 1999; Willner and Willner 2002; Thomas and Sayre 2005). Moreover, Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> are found readily to interact with proteins (Chen and Liao 2002). Tet which is regarded to be a potent inhibitor of P-gp has been confirmed to be a kind of block agent of Ca<sup>2+</sup> channels with little cytotoxicity (Liu et al 1990; Mitterdorfer et al 1998; Xu et al 2003). It will be used as a chemosensitizers in



**Figure 4** GIE of K562/A02 cell line by increasing concentrations Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> loaded with ADM or ADM and Tet. (a) After Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> polymerized with ADM, the ADM loaded Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> inhibited cells proliferation the most between Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> concentrations of 0.1–0.2 at 37 °C for 24 h, so was the cell growth under the polymerization at 4 °C for 24 h. (b) There was a similar tendency in cell growth under Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> co-polymerized with ADM and Tet. It's easily observed that GIE under polymerization at 4 °C was significantly higher than that at 37 °C ( $P < 0.05$ ). Comparatively, GIE of K562/A02 acting with ADM, Fe<sub>3</sub>O<sub>4</sub>-MNP<sub>s</sub> single used or ADM and Tet was  $34.69 \pm 8.49\%$ ,  $29.41 \pm 11.21\%$ , and  $64.03 \pm 10.13\%$  respectively at 37 °C,  $51.53 \pm 11.21\%$ ,  $6.78 \pm 11.67\%$  and  $77.8 \pm 12.54\%$ , respectively at 4 °C.

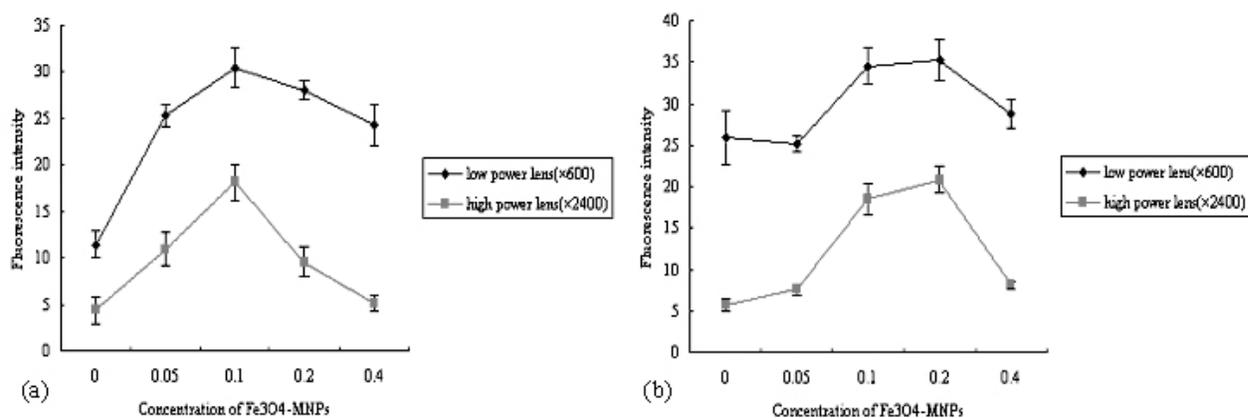


**Figure 5** Fluorescence view of K562/A02 intracellular ADM at a wavelength of 488 nm in scope of low power lens ( $\times 600$ ) (Figure 7e, 7g, 7i, 7k, 7m, 7o, 7q, 7s) and high power lens ( $\times 2400$ ) (Figure 7a~7d, 7f, 7h, 7j, 7l, 7n, 7p, 7r, 7t). The polymerization of  $\text{Fe}_3\text{O}_4$ -MNPs loaded with ADM or ADM and Tet was at  $37^\circ\text{C}$  for 24 h. (a) negative control (K562/A02). (b)  $\text{Fe}_3\text{O}_4$ -MNPs 0.2. (c) ADM. (d) Tet. (e), (f)  $\text{Fe}_3\text{O}_4$ -MNPs 0.05 loaded with ADM. (i), (j)  $\text{Fe}_3\text{O}_4$ -MNPs 0.1 loaded with ADM. (m), (n):  $\text{Fe}_3\text{O}_4$ -MNPs 0.2 loaded with ADM. (q), (r)  $\text{Fe}_3\text{O}_4$ -MNPs 0.4 loaded with ADM. (g), (h)  $\text{Fe}_3\text{O}_4$ -MNPs 0.05 loaded with ADM and Tet. (k), (l)  $\text{Fe}_3\text{O}_4$ -MNPs 0.1 loaded with ADM and Tet. (o), (p):  $\text{Fe}_3\text{O}_4$ -MNPs 0.2 loaded with ADM and Tet. (s), (t)  $\text{Fe}_3\text{O}_4$ -MNPs 0.4 loaded with ADM and Tet.

P-glycoprotein-mediated ADM resistance (Xu et al 1999; Wang et al 2005).

In our experiment, we find that  $\text{Fe}_3\text{O}_4$ -MNPs loaded with ADM can enhance the effective accumulation of ADM in K562/A02 cells, therefore, GIE of K562/A02 and K562 cell lines both increase obviously when cells are incubated with  $\text{Fe}_3\text{O}_4$ -MNPs polymerized with ADM or co-polymerized with ADM and Tet. It is observed that  $\text{Fe}_3\text{O}_4$ -MNPs co-polymerized with ADM and Tet are the most cytotoxic than any other polymerized forms and any other single used drug. Drugs polymerized with  $\text{Fe}_3\text{O}_4$ -MNPs have shown more chemosensitizing activity

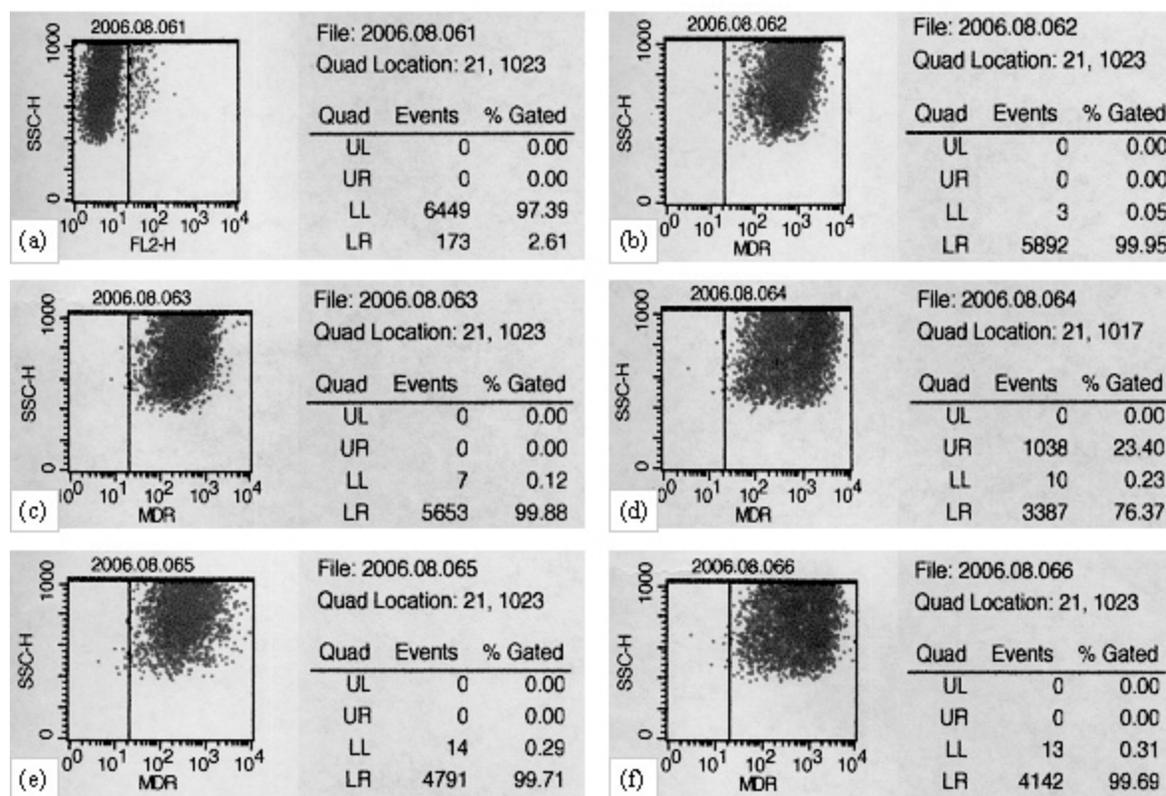
than the drugs alone. It's also observed that the temperature at  $4^\circ\text{C}$  is better for the polymerization and the optimal concentration of  $\text{Fe}_3\text{O}_4$ -MNPs (V/V) for K562 is not the same as that of K562/A02. In K562, there is positive linear correlation between GIE and increasing concentrations  $\text{Fe}_3\text{O}_4$ -MNPs loaded with ADM or ADM and Tet, but no linear correlation is observed for those in K562/A02. The rational behind this may be due to the multidrug resistance of K562/A02 if compared with that of K562. It is known that the anticancer drug can easily enter the intracellular cells of K562 with little drug resistant. However, in K562/A02 cells, the anticancer drug could be readily pumped



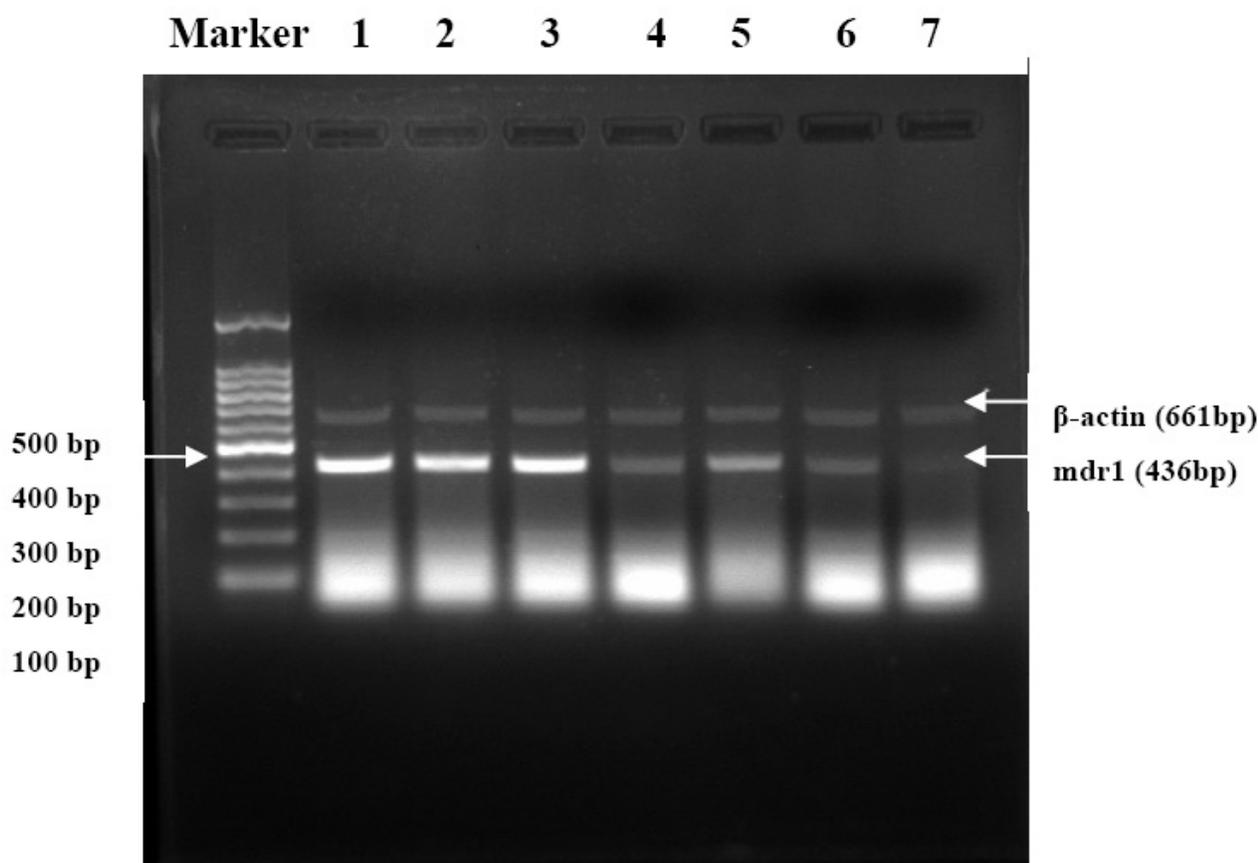
**Figure 6** Fluorescence intensity of K562/A02 intracellular ADM. The polymerization of Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with ADM or ADM and Tet was at 37 °C for 24 h. (a) Fe<sub>3</sub>O<sub>4</sub>-magnetic nanoparticles polymerized with ADM (b) Fe<sub>3</sub>O<sub>4</sub>-magnetic nanoparticles polymerized with ADM and Tet. No linear correlation was found between fluorescence intensity of intracellular adriamycin and augmenting concentration of Fe<sub>3</sub>O<sub>4</sub>-MNPs. The strongest fluorescence intensity was at Fe<sub>3</sub>O<sub>4</sub>-MNPs concentrations of 0.1–0.2. For K562/A02, ADM single or with Tet accumulated less than that loaded Fe<sub>3</sub>O<sub>4</sub>-MNPs.

out or degraded by other drug-resistant mechanisms as soon as it reaches the K562/A02 cells. So the effective intracellular concentration of ADM is influenced by much interference for K562/A02 cells. Thus, that no linear regression exists in the latter situation is easily understood.

K562/A02 cell line has been proved to be resistant to ADM and characterized with P-gp and MDR1 high expression (Yang et al 1995). ADM, a chemotherapeutic agent, has little effect on P-gp and MDR1, while Tet could down-regulate the level of MDR1 gene and decrease the expression of



**Figure 7** P-glycoprotein (P-gp) expression of K562/A02 cell line by 0.1 Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with different concentrations of Tet (the polymerization condition: 4 °C, 48 h). (a) The mouse isotype-matched IgG2a-PE was used as negative control, P-gp was 2.61%. (b)–(f) The monoclonal antibody (MoAb) P-gp-PE was applied in detecting P-gp. (b) The total expression P-gp of K562/A02 was 99.95%. (c) Merely 0.1 Fe<sub>3</sub>O<sub>4</sub>-MNPs were incubated with cells, P-gp was 99.88% with no change. (d) Merely Tet (10 μmol/l l) were incubated with cells, P-gp was significantly decreased to 76.37%. (e) Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with low concentration of Tet (1 μmol/l l), P-gp was 99.71% with no obvious change. (f) Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with high concentration of Tet (10 μmol/l l), P-gp was 99.69% with no obvious change.



**Figure 8** MDR1 mRNA content of K562/A02 cell line by different interferes. (The polymerization condition: 4 °C, 48 h) Lane 1 was total mdr1 mRNA isolated from K562/A02 as negative control. Analyzed density of DNA was 61572. Lane 2 and Lane 3 were from the cells treated with ADM 50  $\mu\text{mol/l}$  and 0.1  $\text{Fe}_3\text{O}_4$ -MNPs respectively. The result showed 55588 and 60514 with no significant change compared with Lane 1. Lane 4 was interfered with Tet 10  $\mu\text{mol/l}$ . The result showed 55588 with significant change compared with Lane 1 ( $P < 0.05$ ). From lane 5 to lane 7 were MDR1 mRNA extracted from K562/A02 incubated with low Tet-, medium Tet- and high Tet-loaded 0.1  $\text{Fe}_3\text{O}_4$ -MNPs mdr1 mRNA was significantly decreased by over 100-fold.

P-gp (Chen et al 2005). Our study has demonstrated that Tet polymerized with  $\text{Fe}_3\text{O}_4$ -MNPs may increase this down-regulation, but can't reduce P-gp on quantity. Quantity or content but not function of P-gp can be detected by flow cytometry. This probably infers that  $\text{Fe}_3\text{O}_4$ -MNPs combining with P-gp may block the pump function of P-gp, which means the pump function of P-gp is cut down but not the quantity of P-gp (Cuvier et al 1992; Henry-Toulme et al 1995). Nanoparticles loaded with anticancer drug could readily approach the cell membrane, leading to drug concentrations at the cell surface higher than those obtained with the same amount of drug diluted in the culture medium, leading in turn to higher intracellular drug concentration (Hu et al 1996; Soma et al 2000). Not only have  $\text{Fe}_3\text{O}_4$ -MNPs the ability to block P-gp function, but also have the potent in aggregation and drug-capsulation (Wong et al 2006a, 2006b). This may be the reason that  $\text{Fe}_3\text{O}_4$ -MNPs which have no cytotoxicity to cells and no reversal in multidrug resistance are able to enhance ADM or Tet effective concentration intracellularly (Colin de

Verdière et al 1994). In K562/A02, ADM polymerized with  $\text{Fe}_3\text{O}_4$ -MNPs is more chemosensitizing than ADM alone, for it bypasses the multidrug resistance. The chemotherapy effectiveness could be further improved with the help of synergetic action of ADM and Tet co-polymerization with  $\text{Fe}_3\text{O}_4$ -MNPs, since 10  $\mu\text{mol/l}$  Tet has both capacity in cytotoxic potency and reversal in multidrug resistant mechanisms. Thus, Tet polymerized with  $\text{Fe}_3\text{O}_4$ -MNPs causes a 100-fold more decrease in MDR1 mRNA level, but did not reduce total P-gp content for the combining of  $\text{Fe}_3\text{O}_4$ -MNPs and P-gp, which could be attributed to the P-gp being blocked by  $\text{Fe}_3\text{O}_4$ -MNPs.

## Conclusions

In summary, a new strategy of mechanical absorption polymerizing is established to make  $\text{Fe}_3\text{O}_4$ -magnetic nanoparticles load with chemotherapeutic agent ADM and(or) chemosensitizer Tet. Our study demonstrates that temperature influenced the effectiveness of the polymerization process.

That 4 °C is better for the polymerization than 37 °C is also observed in this study. Our observations indicate that the polymerizing process can be controlled easily and stably. In addition, it appears that drug(s) polymerized with Fe<sub>3</sub>O<sub>4</sub>-magnetic nanoparticles definitely have curative effect on multidrug resistance by drug accumulating on the basis of certain change of some protein and gene. Tet polymerized with Fe<sub>3</sub>O<sub>4</sub>-MNPs may increase down-regulation of the level of MDR1 mRNA, but Fe<sub>3</sub>O<sub>4</sub>-MNPs can't reduce P-gp on quantity, suggesting that P-gp function is probably blocked off. Totally, Fe<sub>3</sub>O<sub>4</sub>-MNPs loaded with ADM and Tet have synergetic effect on reversal in multidrug resistance, which may be promising for future in vivo studies even in clinic.

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