Effect of CD147 monoclonal antibody on paclitaxel resistance in HCE1 multicellular spheroids

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Abstract: Objective To investigate the effect of CD147 monoclonal antibody (mAb) on the natural resistance to paclitaxel (TAX) in the human cervical cancer line (HCE1) multicellular spheroid (HCE1/MCS) model and if CD147 mAb can reverse the HCE1/MCS resistance to TAX. **Methods** HCE1/MCS was obtained by liquid overlay and rotating technique. HCE1/MCS morphological changes were observed before or after the interference of CD147 mAb. The effects of TAX on HCE1/MCS (including inhibition ratio, IC50 and index of multicellular resistance) before or after CD147 mAb treatment were determined by the method of WST-1 and the inhibition ratio curve was mapped. Cell cycle and apoptosis were detected by flow cytometer (FCM). The expression of CD147 and P-gp of both HCE1/MC and HCE1/MCS was detected by immunocytochemistry. **Results** HCE1/MCS was established successfully. CD147 mAb could inhibit HCE1/MCS from forming spheroids. CD147 mAb could enhance the sensitivity of HCE1/MCS to TAX. IC50 in different concentrations of CD147 mAb (5,10,20 μg/mL) HCE1/MCS group were (40.31±3.73), (32.43±1.56), and (30.69±1.01) μg/mL. CD147 mAb resulted in G_1/G_0 arrest in HCE1/MCS. CD147 mAb of low concentrations (0－10 μg/mL) caused a dose-dependent inhibition of HCE1/MCS ($P<0.05$). Combined with TAX, CD147 mAb could also induce HCE1/MCS cell cycle arrest in both G_1/S and G_2/M stage. The expression of CD147 and P-gp was consistent in HCE1/MCS groups. **Conclusion** CD147 plays an important role in muliticellular resistance of cervical cancer and inhibition of CD147 can synergistically reverse the multicellular drug resistance (MCR) in cervical cancer. The MCR of HCE1/MCS mediated by CD147 is related to P-gp.

Key words: cervical cancer; multicellular spheroids; CD147; P-glycoprotein; paclitaxel

CD147 单克隆抗体对 HCE1 多细胞球体紫杉醇耐药的影响

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[摘要] **目的**:研究 CD147 单克隆抗体在人宫颈鳞癌细胞株 HCE1 多细胞球体模型中对紫杉醇天然耐

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Biography HU Yun, master of medicine, gynecologist, mainly engaged in the basic and clinical research of gynecologic tumors.

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药的影响,探讨 CD147 单克隆抗体是否可以逆转 HCE1 多细胞球体天然耐药及其对 P-糖蛋白(P-gp)的影响。**方法**:运用液体重叠法和旋转法建立 HCE1 多细胞球体模型。以单层细胞作为对照,观察 CD147 单克隆抗体干预前后 HCE1 多细胞球体形态学变化。WST-1 法检测不同浓度的 CD147 单克隆抗体干预前后,紫杉醇对 HCE1 多细胞球体的抑制率,计算半数抑制浓度(half maximal inhibitory concentration, IC50)和多细胞耐药指数(index of multicellular resistance, MCRI),并绘制浓度抑制率曲线。用对照组,CD147 单抗,紫杉醇及紫杉醇+CD147 单抗分别干预单层细胞及多细胞球体,流式细胞学检测细胞周期及凋亡率。用免疫组织化学法分别检测药物干预前后单层细胞及多细胞球体中 CD147 和 P-gp 的表达。**结果**:成功建立了 HCE1 多细胞球体紫杉醇天然耐药模型。CD147 单克隆抗体能使 HCE1 多细胞球体解聚。CD147 单克隆抗体能增强 HCE1 多细胞球体对紫杉醇的敏感性。5,10,20 μg/mL CD147 单克隆抗体组 IC50 分别为(40.31±3.73),(32.43±1.56),(30.69±1.01) μg/mL,5 和 10 μg/mL CD147 单抗组呈剂量依赖性($P<0.05$)而 10 和 20 μg/mL CD147 单抗组无明显剂量依赖性($P>0.05$),其凋亡率接近单层细胞($P>0.05$)。CD147 单克隆抗体引起多细胞球体 G_1/G_0 期阻滞,和紫杉醇联用,分别在 G_1/G_0 和 G_2/M 2 个调控点共同阻滞 HCE1 细胞生长。HCE1 多细胞球体各组中 CD147 和 p-gp 表达呈一致性。**结论**:成功建立宫颈鳞癌 HCE1 多细胞球体紫杉醇天然耐药模型;CD147 的表达与宫颈鳞癌 HCE1 多细胞球体对紫杉醇的耐药呈正相关,阻断 CD147 可部分逆转 HCE1 多细胞球体对紫杉醇的天然耐药;CD147 介导的 HCE1 多细胞球体的紫杉醇天然耐药可能与 P-gp 有关。

[关键词] 宫颈癌; 多细胞球体; CD147; P-糖蛋白; 紫杉醇

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Drug resistance and tumor metastasis are the 2 main causes of treatment failure and mortality in cancer patients. These 2 properties of malignant tumors have been studied extensively and there are evidences suggesting functional linkage between the 2 phenotypes[1-2]. CD147 is the major stimulator of matrix metalloproteinases (MMPs). Up-regulation of CD147 has been reported in many multidrug resistance (MDR) cancers[3]. MDR is often associated with overexpression of P-glycoprotein (P-gp), a transmembrane, adenosine triphosphate (ATP)-dependent transporter encoded by the *MDR1* gene. P-gp substrate drug can greatly upregulate the expression of CD147 and MMPs in MDR cancer cells, enhancing the tumor metastatic capability. Inhibition of *CD147* gene expression via RNAi could increase chemosensitivity to paclitaxel (TAX) in the human ovarian cancer cell line[4]. However, these researches were monolayer cell cultured in vitro. It is still unclear whether it has the similar response in vivo. In the meantime, the multicellular spheroid (MCS) was used as tumor model for the in vitro study of solid tumor because it is most similar to avascular tumor regions of practical solid tumors in vivo that are characterized by limited accessibility of cell subpopulations. This study was to investigate the effect of CD147 monoclonal antibody (mAb) on the natural resistance to TAX in the human cervical cancer line (HCE1) multicellular spheroids (HCE1/MCS) model and if CD147 mAb can reverse the HCE1/MCS resistance to TAX and its effects on the expression of P-gp.

1 MATERIALS AND METHODS

1.1 Materials

HCE1, a poorly differentiated squamous carcinoma line, were supplied by Cancer Research Institute of Central South University (Changsha, China). CD147 mAb, which blocks the activity of CD147, was supplied by Cell Engineering Research Center of the Fourth Military Medical University (Xi'an, China). The primary monoclonal mouse anti-human antibody and sencondary rabbit anti-mouse antibody against P-gp were both purchased from Chemicon (USA). The primary polyclonal rabbit anti-human antibody against CD147 and the ABC kit staining systems were both purchased from Wuhan Boster Bio-engineering Limited Company (China). WST-1 dye [3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Beyotime Biotechnology limited Company (Shanghai, China). Propidium iodide (PI) was purchased from Dingguo Biotechnology Co., Ltd. (Beijing, China).

1.2 Methods

1.2.1 Cell culture

Liquid overlay and rotating technique was used to established HCE1/ MCS model. A total of 5×10^5 trypsinized cells was resuspended in 6 mL complete medium (containing 10% calf serum, 100 U/L benzylpenicillin, 100 U/L streptomycin). The suspension was added into 1% sepharose paved Petri dishes so that the cells cohered to one another rather than adhered to the dishes. Then we put it into 37℃ shaking table in slow speed (54 r/min). After 3 days rotating culture, it went on with static culture. Three days later, the cell suspension was centrifuged standingly for 10-15 min and the supernatant was replaced with new culture medium. The cell suspension was then pipetted into 1% sepharose paved Petri dishes to culture and the culture medium was replaced every other day.

1.2.2 Morphology observation

We observed the changes of HCE1/monolayer cells (HCE1/MC) and HCE1/MCS morphology after they exposed to CD147 mAb. HCE1 in logarithmic phase were harvested by trypsinization. HCE1/MC (5×10^5 cells) were pipetted into 2 dishes directly. HCE1/MCS (5×10^5 cells) were pipetted into 2 dishes paved with 1% sepharose, and then these 2 dishes were put into 37℃ shanking table for rotating culture. One of the dishes was added with 10 μg/mL CD147 mAb, the other with complete medium. After 3 days culture, we observed the morphology changes of HCE1/MC and HCE1/MCS, and then take photographs.

1.2.3 Drug sensitivity assay

Using WST-1 to detect the inhibition of proliferation on both HCE1/MC and HCE1/MCS before and after different concentrations of CD147 mAb blocking CD147. There were 4 groups: the control group, 5, 10, and 20 μg/mL CD147 mAb group. HCE1/MC were plated in 96-well plates at a density of 10^4 cells/well and further incubated for 24 h. The medium was then removed and replaced with fresh medium containing TAX respectively, with varying plasma peak concentrations (PPC)[5] (0.125, 0.25, 0.5, 1.0 and 2.0 PPC) for another 24 h. HCE1/MCS were harvested at a density of 20 spheroids/100 μL. Then the spheroids were pipetted into 1% sepharose paved 96-well paltes and cultured with medium containing TAX respectively, with 0.125, 0.25, 0.5, 1.0, and 2.0 PPC[5] for another 24 h. The medium was then removed and replaced with fresh medium containing TAX. After that, both HCE1/MC and HCE1/MCS in 96-well plates were stained with 20 μL sterile WST-1 dye at 37 ℃ for 4 h. Spectrometric absorbance at 490 nm was measured with a microplate reader. Each group contained 3 wells and was repeated 3 times. The half maximal inhibitory concentration (IC50) value was determined by the dose of drug that caused 50% inhibition of cell viability.

1.2.4 Cell cycle and apoptosis

Flow cytometry studies were performed to determine the effect of CD147 mAb-TAX combinations on the cell cycle distribution and cell apoptosis. HCE1/MC and HCE1/MCS were randomly divided into 4 groups: the control group, 10 μg/mL CD147 mAb group, 20 μg/mL TAX group, 10 μg/mL CD147 mAb+20 μg/mL TAX group. After 24 h culture, cells of the 4 groups were harvested at a density of 10^6/mL by trypsinization. One milliliter of cell suspension (containing 10^6 cells) was taken out and centrifuged for 10 min at 1 000 r/min on a 4℃ table top centrifuge. The supernatant was abandoned and the cells were resuspended with 0.3 mL PBS. And then the cells were immobilized with 0.7 mL 4℃ absolute ethyl alcohol. The remaining procedure was performed using the PI kit. Each assay was carried out in triplicate and repeated 3 times.

1.2.5 Immunocytochemistry

Immunohistochemistry was performed using the SABC kit and an antigen retrieval method. HCE1/MC and HCE1/MCS were randomly divided into 4 groups: the control group, 10 μg/mL CD147 mAb group, 20 μg/mL TAX group, 10 μg/mL CD147 mAb+20 μg/mL TAX group. The mean percentage of positive cells was determined in at least ten areas at ×100 magnification and assigned to one of the following categories: <5% marked 0; 5%－25% marked 1; 25%－50% marked 2; 50%－75% marked 3; and >75% marked 4. The immunostaining intensity of CD147 and P-gp was scored as 1+ (weak), 2+ (moderate) or 3+ (intense). The relative intensities of CD147 and P-gp staining defined as 3+ were identical as the high expression.

1.3 Statistical analysis

All data were presented as mean±standard deviation ($\bar{x}\pm s$). Statistical analyses were conducted by SPSS16.0 software. ANOVA and Dunnett's multiple comparison tests were used to compare mean values. Chi-square tests were used to compare proportions. $P<0.05$ was defined as statistical significance.

2 RESULTS

2.1 Establishment of HCE1/MCS model

The growth of HCE1/MC was in a good condition. After 24 h of roller culture, cells began to aggregated into MCS. With the prolongation of culture time, HCE1/MCS became more and more compact and larger in size (Fig. 1).

Connections between HCE1/MC of the CD147 mAb group were loose and could not grow into a film; connections between HCE1/MC of the control group were close, and cells grew into a film (Fig. 2); HCE1/MCS of the CD147 mAb group could not form multicellular spheroid, or formed loose structure; while HCE1/MCS of the control group were stable structured (Fig. 3).

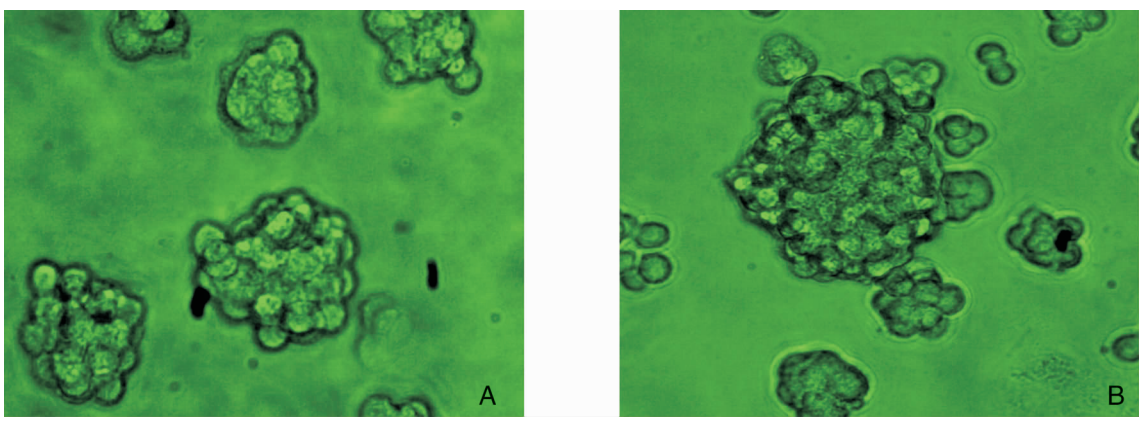

Fig. 1 Morphological features of HCE1/MCS under light microscopy (×200). A: HCE1/MC were in a good condition after 24 h of roller culture; B: Cells began to aggregated into multicellular spheroids.

Fig. 2 Morphological features of HCE1/MC after 72 h treatment (×100). A: Control group; B: CD147 mAb group.

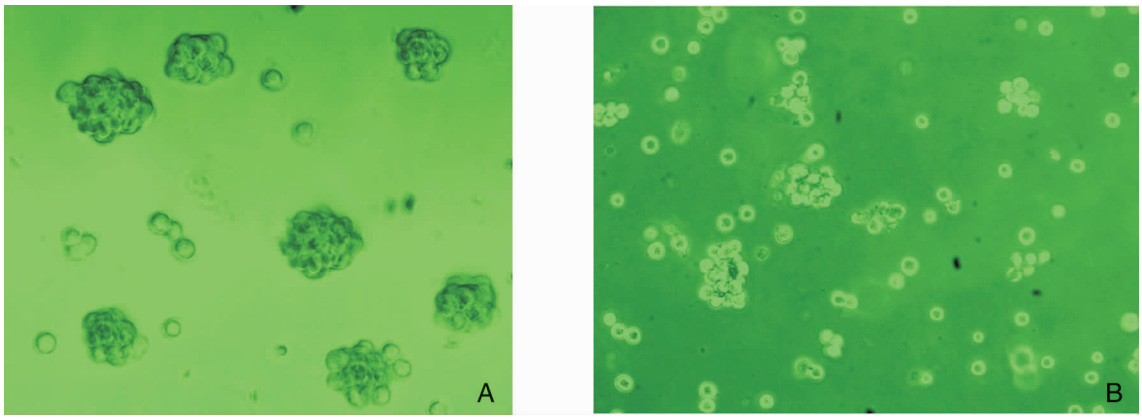

Fig. 3 Morphological features of HCE1/MCS after 72 h treatment (×100). A: Control group; B: CD147 mAb group.

2.2 Proliferation inhibition detected by WST-1

Fig. 4 showed the concentration-inhibition curves of HCE1/MC and HCE1/MCS after different concentrations of TAX treatment. TAX can dose-dependently inhibit the proliferation of both HCE1/MC and HCE1/MCS significantly. And the effect was more significant on HCE1/MC.

The inhibition rates of TAX treated HCE1/MC were not changed significantly after treatment with different concentrations of CD147 mAb (Fig. 5).

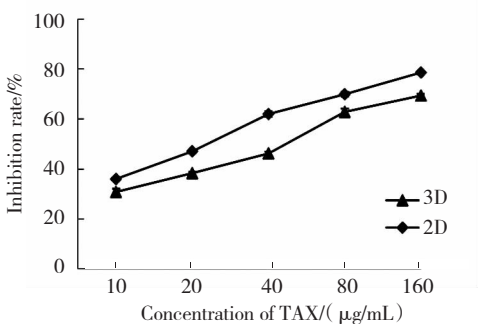

Fig. 4 Concentration-inhibition curves of HCE1/MC and HCE1/MCS. 2D: HCE1/MC; 3D: HCE1/MCS.

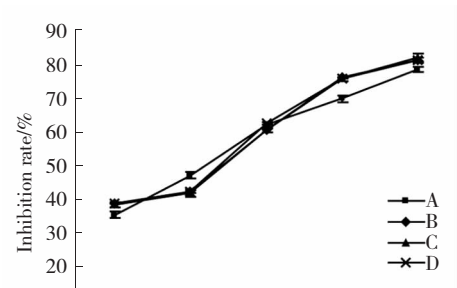

Fig. 5 Concentration-inhibition curves of TAX-treated HCE1/MC after treatment with different concentrations of CD147 mAb. A: Control; B: 5 μg/mL CD147 mAb; C: 10 μg/mL CD147 mAb; D: 20 μg/mL CD147 mAb.

Fig. 6 showed the concentration-inhibition curves of HCE1/MCS after different concentrations of TAX and CD147 mAb.

The IC50 was defined as the concentration of TAX that caused 50% inhibition of cell proliferation. The IC50 values of the HCE1/MC control group and the HCE1/MCS control group were (28.27±1.45) and (64.7±1.67) μg/mL, respectively. The difference between them had statistical significance ($P<0.05$). MCRI of the control group was 2.29. After the activity of CD147 in HCE1/MCS was inhibited by CD147 mAb of different concentrations (5, 10, 20 μg/mL), TAX could easily induce the apoptosis of HCE1/MCS. The values of IC50 were (40.31±3.73), (32.43±1.56), and (30.69±1.01) μg/mL, respectively; and the MCRI were 1.48, 1.20, and 1.18, respectively. CD147 mAb of low concentrations (0—10 μg/mL) caused a dose-dependent inhibition of HCE1/MCS ($P<0.05$). CD147 mAb had no effect on HCE1/MC. Compared with the HCE1/MC control group, the IC50 values of HCE1/MC CD147 mAb groups was not changed obviously ($P>0.05$, Fig. 7).

Fig. 6 Concentration-inhibition curves of HCE1/MCS after treatment with different concentrations of

CD147 mAb had no effect on the apoptosis rate of HCE1/MC ($P>0.05$, Tab. 1).

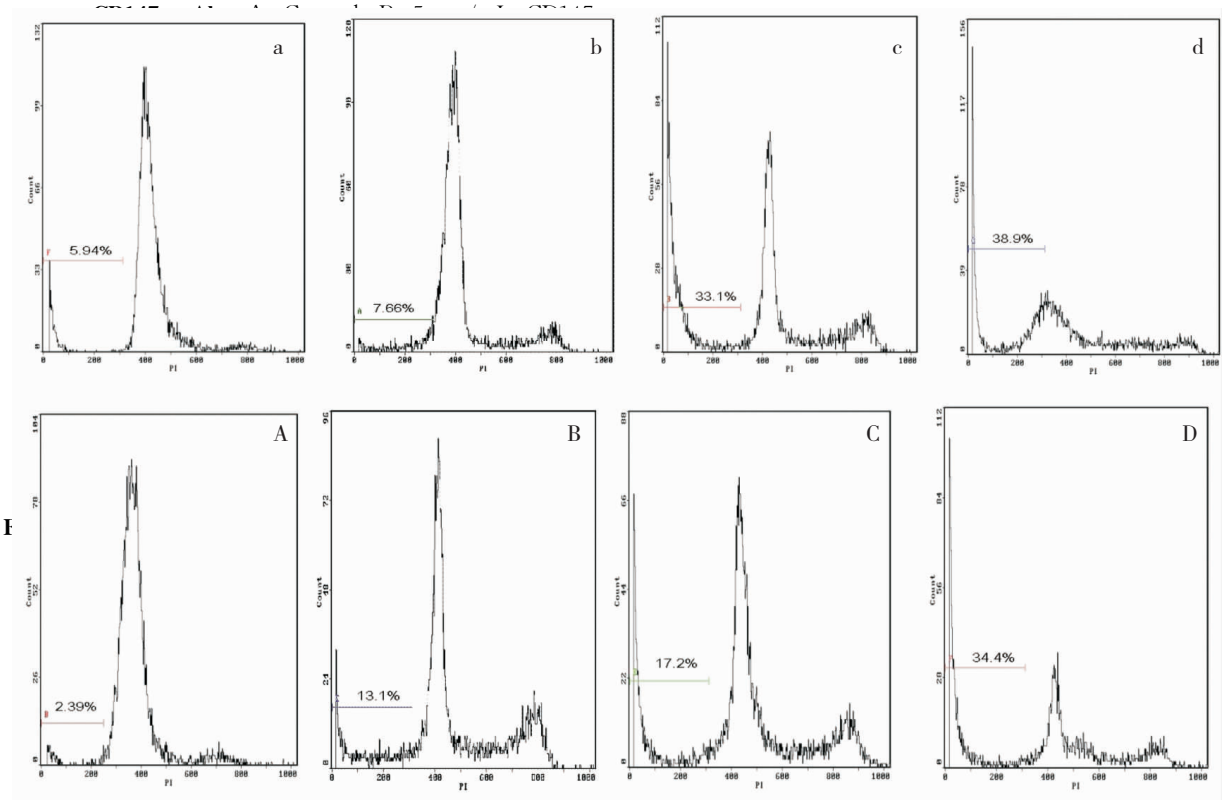

Fig. 8 Apoptosis rates of HCE1/MC and HCE1/MCS in different groups by flow cytometry. a: HCE1/MC control group; b: HCE1/MC CD147 mAb group; c: HCE1/MC TAX group; d: HCE1/MC TAX+CD147 mAb group; A: HCE1/MCS control group; B: HCE1/MCS CD147 mAb group; C: HCE1/MCS TAX group; D: HCE1/MCS TAX+CD147 mAb group.

2.3 Apoptosis and cell cycle of HCE1/MC and HCE1/MCS

2.3.1 Apoptosis of HCE1/MC and HCE1/MCS

Flow cytometric analysis of cell death using PI staining permitted simple measurement of apoptosis (Fig. 8). The apoptotsis rate of the HCE1/MCS control group was (2.39±0.42)%; HCE1/MC were adherent to the bottom of flask, the apoptosis rate of HCE1/MC increased to (6.02±0.20)%. After the treatment of CD147 mAb, the apoptosis rate of HCE1/MCS was (13.77±0.59)%, which was significantly higher than that of the HCE1/MCS control group ($P<0.05$, Tab. 1). After the treatment of TAX, the proliferation of HCE1/MC and HCE1/MCS was inhibited. And compared with the HCE1/MCS TAX group, the apoptosis rate of HCE1/MC was much higher ($P<0.05$, Tab. 1). CD147 mAb could enhance the sensitivity of HCE1/MCS to TAX. After the treatment of TAX+CD147 mAb, the apoptosis rate was (37.67±1.09)%. The difference of apoptosis rate between TAX+CD147 mAb HCE1/MCS group and TAX+CD147 mAb HCE1/MC group had no statistical significance ($P>0.05$, Tab. 1).

Tab. 1 Apoptosis rates of HCE1/MC and HCE1/MCS in the 4 gro

Groups	Control	CD147 mAb	TAX
2D	6.02±0.20	7.57±0.19	33.97±1.97
3D	2.39±0.42	13.77±0.59	17.40±0.53
P	<0.05	<0.05	<0.05

2D: HCE1/MC; 3D: HCE1/MCS.

2.3.2 Cell cycle of HCE1/MC and HCE1/MCS

Flow cytometric analysis was performed to determine the effect of different groups on the cell cycle distribution. Compared with HCE1/MC control group, HCE1/MCS control group predominantly accumulated in G_0/G_1 phase ($P<0.05$, Fig. 9). After the treatment of CD147 mAb, the number of HCE1/MCS in G_0/G_1 phase increased obviously ($P<0.05$), in S

phase decreased ($P<0.05$), and in G_2/M phase had no changes. After the treatment of CD147 mAb, the number of HCE1/MC had no changes in each phase ($P>0.05$, Fig. 10). After the treatment of TAX, HCE1/MC and HCE1/MCS in the G_2/M phase were both accumulated and compared with the control groups, the difference had statistical significance ($P<0.05$). After the treatment of TAX+CD147 mAb, the number of HCE1/MCS in G_1/G_0 phase was evidently increased, which was significantly higher than the HCE1/MCS TAX group ($P<0.05$). Compared with HCE1/MCS TAX group, the number of HCE1/MCS in G_2/M phase had no change ($P>0.05$).

2.4 Expression of CD147 and P-gp

Immunohistochemistry was performed to detect the expression of CD147 and P-gp in HCE1/MCS and HCE1/MC. Positive staining of CD147 and P-gp appeared as buffy grains in cell membrane and intracytoplasm.

The high expression rates of CD147 in the HCE1/MC control group, the HCE1/MCS control group, the HCE1/MCS TAX group, the HCE1/MCS CD147 mAb group, and the HCE1/MCS TAX+CD147 mAb group were 53.33%, 73.33%, 93.33%, 3.33%, and 10.00%, respectively (Tab. 2). Compared with the HCE1/MC control group, increased expression of CD147 was observed in the HCE1/MCS control group ($P<0.05$); treating with TAX resulted in a remarkable overexpression of CD147 in HCE1/MCS. Compared with the HCE1/MCS control group, the difference had statistical significance ($P<0.05$, Fig. 11).

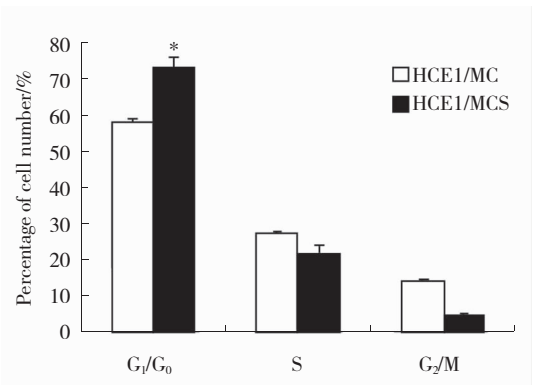

Fig. 9 Cell cycles of HCE1/MC and HCE1/MCS in the control group. Compared with the HCE1/MC control group, $*P<0.05$.

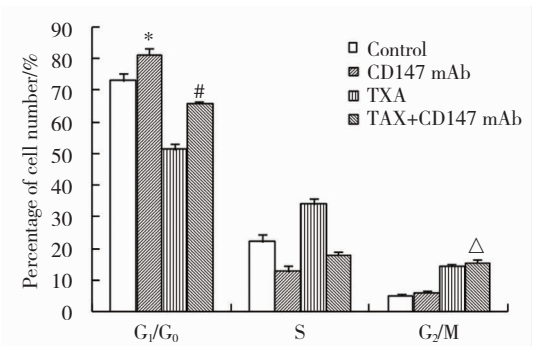

Fig. 10 Cell cycles of HCE1/MCS in different groups. Compared with the HCE1/MCS control group, $*P<0.05$; compared with the HCE1/MCS TAX group, $\#P<0.05$; compared with the HCE1/MCS TAX group, $\triangle P>0.05$.

Tab. 2 Expression of CD147 in HCE1/MC and HCE1/MCS by immunocytochemistry ($n=30$)

Groups		Low expression			High expression	High expression rate/%
		−	+	++	+++	
Control	2D	2	8	4	16	53.33
	3D	0	2	6	22	73.33*
TAX	2D	3	4	5	18	60.00
	3D	0	1	1	28	93.33#
CD147 mAb	2D	29	1	0	0	0
	3D	26	3	0	1	3.33
TAX+CD147 mAb	2D	28	1	0	1	3.33
	3D	22	3	2	3	10.00

2D: HCE1/MC; 3D: HCE1/MCS. Compared with the HCE1/MC control group, $*P<0.05$; compared with the HCE1/MCS control group, $\#P<0.05$.

The high expression rates of P-gp in the HCE1/MC control group, the HCE1/MCS control group, the HCE1/MCS TAX group, the HCE1/MCS CD147 mAb group, and the HCE1/

MCS TAX＋CD147 mAb group were 0, 46.67%, 73.33%, 13.33%, and 23.33%, respectively (Fig. 12, Tab. 3). Compared with the HCE1/MC control group, increased expression of P-gp was observed in the HCE1/MCS control group ($P<0.05$); treating with TAX resulted in a remarkable overexpression of P-gp in HCE1/MCS. Compared with the HCE1/MCS control group, the difference had statistical significance ($P<0.05$). CD147 mAb could inhibit the expression of P-gp in HCE1/MCS ($P<0.05$, Fig. 12, Tab. 3).

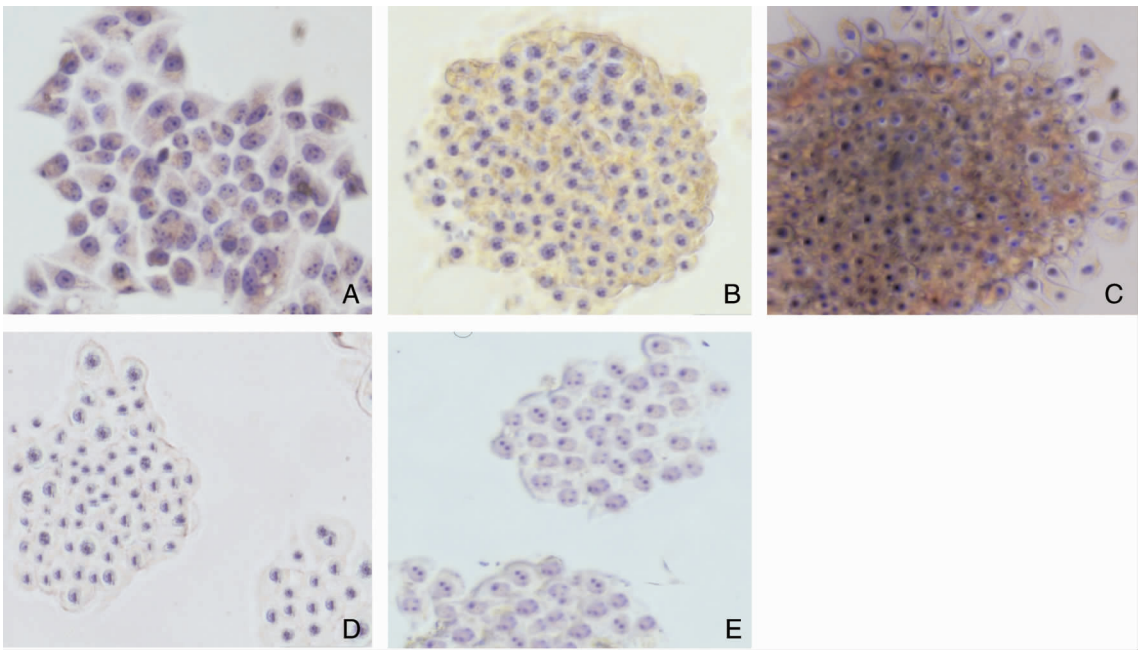

Fig. 11 Expression of CD147 in HCE1/MC and HCE1/MCS by immunocytochemistry (×200). A: HCE1/MC control; B: HCE1/MCS control; C: HCE1/MCS TAX; D: HCE1/MCS CD147 mAb; E: HCE1/MCS TAX+CD147 mAb.

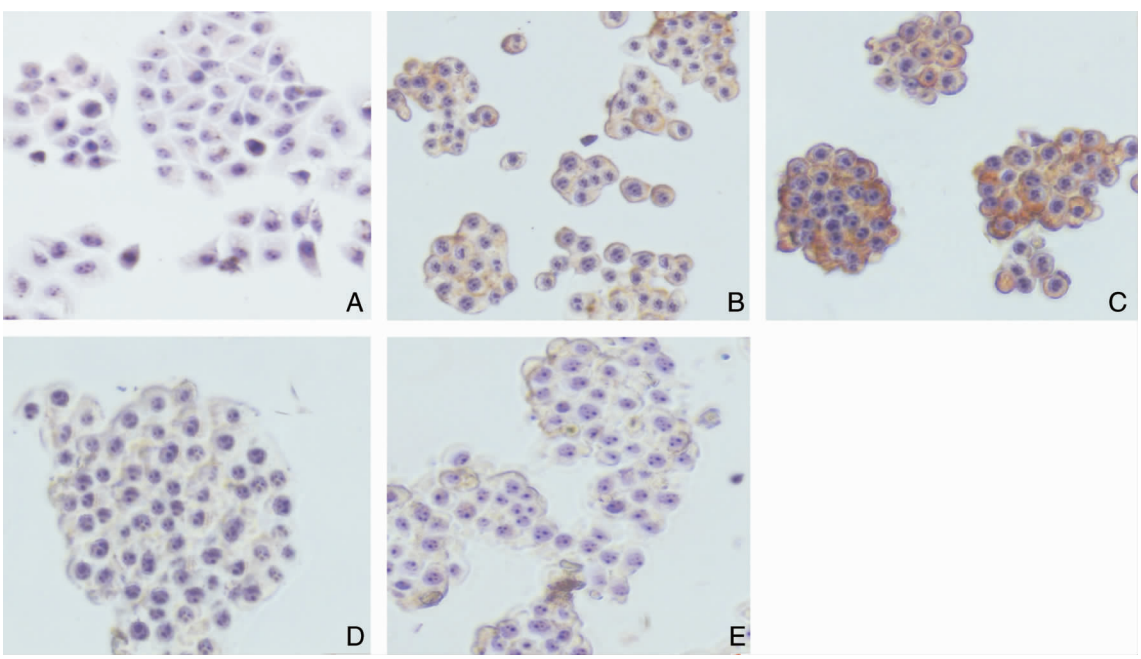

Fig. 12 Expression of P-gp in HCE1/MC and HCE1/MCS by immunocytochemistry (×200). A: HCE1/MC control; B: HCE1/MCS control; C: HCE1/MCS TAX; D: HCE1/MCS CD147 mAb; E: HCE1/MCS TAX+CD147 mAb.

Tab. 3 Expression of P-gp in HCE1/MC and HCE1/MCS by immunocytochemistry ($n=30$)

Groups		Low expression			High expression	High expression rate/%
		−	+	++	+++	
Control	2D	25	3	2	0	0
	3D	8	1	5	14	46.67*
TAX	2D	25	3	1	1	3.33
	3D	5	3	0	22	73.33#
CD147 mAb	2D	28	1	1	0	0
	3D	25	2	6	4	13.33△
TAX+ CD147 mAb	2D	30	28	2	0	0
	3D	12	5	6	7	23.33

2D: HCE1/MC; 3D: HCE1/MCS. Compared with the HCE1/MC control group, * $P<0.05$; compared with the HCE1/MCS control group, # $P<0.05$; compared with the HCE1/MCS control group, △ $P<0.05$.

3 DISCUSSION

Tumor MCS are 3 dimension cultured cell cluster in vitro. Because of the increased interacts between cell-cell and cell-matrix, MCS are resistant to many anticancer drugs. Study[6] has shown that the drug-resistance of MCS may be related to the drug-resistant phenotypic alternation induced by adhesive molecules. TAX is one of the most active agents in the treatment of cervical cancer. TAX acts as a mitotic spindle poison by blocking eukaryotic cells in the G_2/M mitotic phase of the cell cycle. TAX promotes microtubule assembly and stabilization by preventing depolymerization, leading to the inhibition of cell proliferation and induction of cell death. This study showed that the IC50 of TAX in HCE1/MCS was higher than that in HCE1/MC. The results of flow cytometry showed after the exposure to TAX, the apoptosis cell death in HCE1/MCS was significantly lower than that in HCE1/MC; HCE1/MCS in the G_1/S phase increased and in G_2/M phase decreased. The results of immunocytochemistry showed that after 3-dimension culture, the expression of P-gp in HCE1/MCS was increased. After the exposure to TAX, the expression of P-gp was much higher. All of these results reflected that HCE1/MCS were resistant to TAX naturally, the MCRI was 2.29. Compared with the model of cisplatin-resistance, the MCRI was lower[7]. It reflects that HCE1/MCS are more sensitive to TAX. There might be some reasons for the TAX resistance in HCE1/MCS. Firstly, the 3-dimension structure of HCE1/MCS results in reduced drug permeability. The concentration of drugs in the internal cells is lower than that in the external cells, which could not reach the effective concentration. Secondly, the flow cytometry results indicated that the majority of HCE1/MCS were in G_1/G_0 phase. A large number of cells are quiescent, so TAX which is cell-cycle dependent loses its drug-target and supplies abundant time for HCE1/MCS to repair the DNA damage caused by cytotoxic drugs. Finally, TAX is known as the P-gp substrate drug. It is believed to function as an ATP-dependent drug efflux pump to reduce intracellular drug accumulation in resistant cells. The expression of P-gp in HCE1/MCS was higher than that in HCE1/MC, and the expression was increased after its exposure to TAX. This result is similar to the report of Xing, et al[8].

CD147, as an adhesion molecule, is a cell surface glycoprotein that belongs to the immunoglobulin superfamily. CD147 mediates cell-cell interaction and it has been reported to bind to a variety of cell types including endothelial cells and fibroblasts[9]. Previous study[10] demonstrated that CD147 which concentrated expresses on the surfaces of most tumor cells, promoted the invasion of tumor cells by stimulating stromal cells to produce several MMPs. MMPs play very important roles in several aspects of tumor progression, including growth, invasion, metastasis, and angiogenesis. Yang, et al.[10] reported that the expression of CD147 was increased in MDR carcinoma cell lines, MCF-7/AdrR, KBV-1, and A2780Dx5, as compared to their parental counterparts. Inhibition of CD147 may have an anti-tumor effect through enhancing the susceptibility of cancer cells to apoptosis[11]. Our study showed that after the exposure to CD147 mAb,

HCE1/MCS could not form multicellular spheroid. The cell cycle was also changed after exposure to CD147 mAb: the proportion of HCE1/MCS in G_1/G_0 phase increased, the proportion of HCE1/MCS in G_2/S phase reduced, but it had no change in G_2/M phase. This result reflects that CD147 mAb might have anti-adhesive effects. It could inhibit HCE1/MCS from forming spheroids and resulted in HCE1/MCS G_0/G_1 arrest. The anti-cancer agent TAX stabilizes microtubules leading to G_2/M cell cycle arrest and apoptotic cell death. Combining TAX with CD147 mAb induced HCE1/MCS cell cycle arrest in both G_1/S and G_2/M checkpoints. So it inhibited the uncontrolled growth of HCE1/MCS, which resulted in activation of apoptosis and reversing natural resistance of TAX. CD147 mAb is a blocking antibody. The proliferation of HCE1/MCS is inhibited by CD147 mAb from blocking the survival signal based on cell-matrix and cell-cell adherence[12]. After the activity of CD147 was blocked, cell-cell adhesion decreased and survival signals could not be transited between tumor cells. HCE1/MCS were induced to anoikis and their sensitivities to drugs were enhanced. Meanwhile, we found that CD147 mAb had no obvious effect on HCE1/MC. A possible reason for this phenomenon is that cells cultured in "hanging drops" assembled a fibronectin matrix to support aggregate compaction to escape the anoikis[13].

P-gp is an ATP driven transport protein with a very wide range of substrate compounds. It was considered as one of the most important role in multidrug resistance. Data have shown that the expression of CD147 and P-gp was associated with each other in monolayer cells such as MCF7/Adr cells[14]. But all of these studies were cultured monolayer. They ignored the effects of tumor microenvironment on drug resistance. It is still unclear that whether the 3-dimension cultured multicellular spheroids have the same characteristics. Our research showed that the expression of CD147 in HCE1/MCS was higher than that in HCE1/MC. After exposed to TAX, the expressions of CD147 and P-gp in HCE1/MCS were increased. But TAX had no effect on the expression of CD147 in HCE1/MC. After CD147 was blocked by CD147 mAb, the expression of P-gp in HCE1/MCS also decreased. The results reflects that as a model of drug resistance, HCE1/MCS acquired the TAX-resistance phenotype. Inhibiting the activity of CD147 could reduce the expression of P-gp indirectly and it could also reverse MCR in HCE1/MCS actively. The mechanism of the interactions between CD147 and P-gp is still unclear. It may be caused either by direct interaction or by binding to an intermediary protein. Both P-gp and CD147 are ubiquitinated. Researchers have found that ubiquitination plays an important role in the degradation of P-gp and CD147[15]. Some scholars speculated that the interactions might be concerned with mitogen-activated protein kinases pathway, hyaluronidase pathway or integin patheway[16-18].

In summary, HCE1/MCS is naturally resistant to TAX. It is a good model for the study of TAX resistance in vitro. CD147 mAb can inhibit the proliferation of HCE1/MCS and induce apoptosis. CD147 mAb can reverse the natural resistance to TAX partly, which may be associated with P-gp.

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