Effect of BRM Shijiesu on Breast Cancer Cell in Culture

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Abstract **Purpose** To investigate the antitumor effects and mechanism of BRM-SJS on breast cancer **Methods** BCaP-37 breast cancer cells culture were studied by culture technique, while fluorescence microscopy, flow cytometry were used for apoptosis test. **Results** BRM-SJS(10μM,for 24-72h)was able to inhibit the growth of BCaP-37 in vitro and the inhibitive response was gradually enhanced; under the observation of fluorescence microscopy, the suspended cells of BCaP-37 were increased by BRM-SJS(10μM, for 12-72h), among them apoptosis cells came to 60%~70%, the flow cytometry analysis showed the rate of apoptosis was 61.4% by BRM-SJS(10μM,for 72h). **Conclusions** These results indicated that BRM-SJS has significant inhibitory effects on breast cancer, it mechanisms may be related to induction of BCaP-37 breast cancer cells apoptosis.

Key Words BRM-SJS; antitumor activity; apoptosis; breast cancer; flow cytometry

Cancer is the local expression of disease in the whole body, which is affected by many factors intersecting action and many genes partaken many procedures accumulated in the body. Breast cancer is a common tumor, the relevant researchers study it thoroughly in the world [1-6]. BRM-SJS is a compound preparations of Chinese traditional medicine, it has antitumor effects in some cancer patients[7]. Since kerry brought up apoptosis theory in 1972, peoples have been realized tumor treatment related to apoptosis gradually.

With the molecular biology development recently peoples find that the antitumor drugs may induce apoptosis, especially many convention preparations used in clinic[8-11]. For further expose antitumor effect of BRM-SJS, we study inhibitive action and induce apoptosis of BRM-SJS on human breast carcinoma cell lines (BCaP-37).

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MATERIALS AND METHODS

Drug and tumor strain

BRM-SJS and human breast cancer cell strain were provided by Beijing Clinical Tumour Gene Research Center, capsule agent, batch number 2001-5, RPMI1640 medium was purchased from Sigma Co.

Influence of BRM-SJS on BCaP-37 cell proliferation in vitro

The index growing period cells were collected, then the cells with trifold times added BRM-SJS (0.5, 1, 2.5, 5, 10μM) respectively, meanwhile set up DMSO for control, under the invert microscope observe the change in different time of action of BCaP-37 cells by BRM-SJS.

The Influence of BRM-SJS in BCaP-37 by fluorescence microscope observation

The index growing period of BCaP-37, 3×10^6 c in to 50ml culture cells flask, after 24h, with trifold times added ① BRM-SJS (2.5μM, 5μM, 10μM), ②control group with equivalent to concentration of DMSO, ③RPMI1640 medium control. After BRM-SJS for 6h, 12h, 48h, and 72h, under the invert microscope the cells were observed, then the cells and suspended liquid were harvested and centrifuged at 1000 rpm, 10 minute then the supernatant was discarded. After repeated washing

cells with PBS, the BCaP–37 cells at final concentration of 1×10^5 c.ml^{-1} were added 10μg.ml^{-1} Rnase, PI and Hoechst 33342 respectively, stained 15 minute, then the small room on the glass slide was round by double –faced glue were coated with added 30μl the suspended cells respectively. The morphology of cells were observed under a invert microscope.

Effect of cell cycle status of BCaP –37 with BRM–SJS by Flow cytometric analysis[12,13]

The index growing period cells were collected, BCaP–37 cells (1×10^6 c/flask) were incubated, untreated or BRM–SJS treated at different concentrations (2.5μM, 5μM, 10μM) were added for 24h, 48h, or 72h respectively. Then the cells were harvested and washed twice with PBS and centrifugated at 1000rpm for 5 min, the pellets cells were stored 700mL.L^{-1} cold ethanol at 4℃ for 24h, then centrifugation 1000rpm, for 5 min, and the supernatant was discarded to remove ethanol completely. The pellets were resuspended in 0.5mL PBS, then added 50μg Rnase at 37℃ incubated for 30 min, then added up propidium iodide (PI) 50μg avoid light for 30 minute, then added PBS to 1mL volume. The fluorescence of 10^4 cells were analyzed for each sample, standard with human lymphocyte. Cells with DNA content below G_1 phase were regarded as apoptotic cells. The percentage of cell in the apoptotic sub– G_1 and G_2 phase and S phase were analyzed by flow cytometry.

RESULTS

The Influence of BCaP–37 cells proliferation by BRM–SJS

Observation under the invert microscope, the normal BCaP–37 cells were monolayer, attachment, shows polygon, the ratio of nuclei/plasma was larger, there was no difference between the control group and with BRM–SJS 0.5–1μM. Under BRM–SJS 10μM for 12h there were a few suspended cells, for 24h the suspended cells increased gradually, for 72h, most of the cells changed suspended cells (Fig 1).

The pathological examination in high dose group showed cell nuclei condensation, heavy stained, cells of partly formed crescentic nucleuses, which demonstrated typical morphological changes of apoptosis, but the negative group displayed degeneration and necrosis (Fig 1, 2).

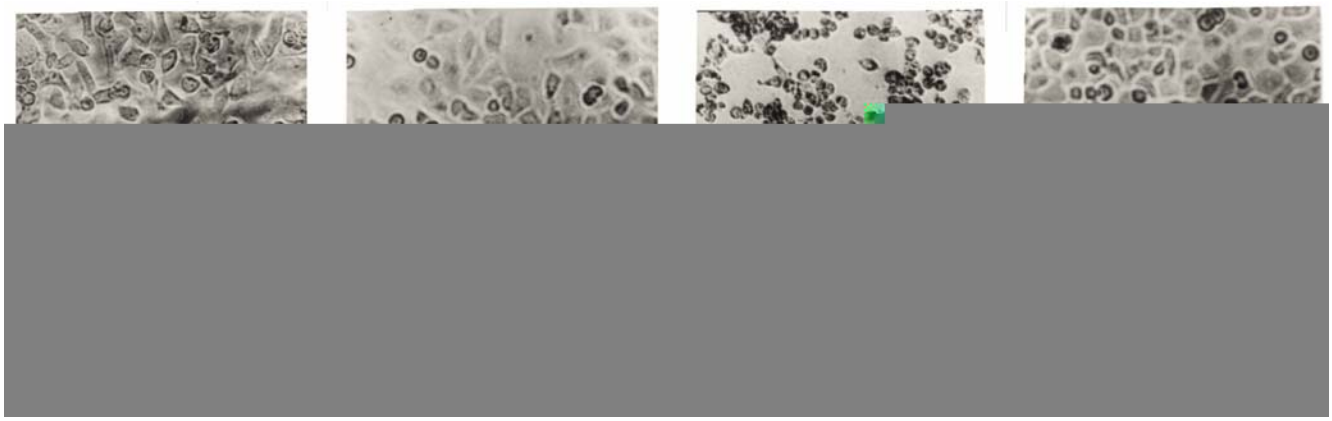
The fluorescent assay of BCaP –37 cells by BRM–SJS

The change of DNA in apoptotic BCaP–37 cells induced by BRM –SJS was determined by using fluorescence microscope after Hoechst 33342 and PI double staining, the vital bisbenzimidazole dye Hoechst 33342 and the DNA intercalating agent proidium iodide (PI) enabled separating and quantitying normal, apoptotic and necrosis cells. The experiment was divided into ① BRM –SJS group (2.5μM–10μM, 6–72h), ②Control group

Hoechst 33342 is taken up by living cells and on binding specifically to DNA(A–T), the necrosis cells fluoresced red due to PI. This result showed that the smaller cells with high blue fluorescence were apoptotic cells, the larger cells with low blue fluorescence were normal. The apoptotic cell membranes were intact and smooth but the nuclei exhibited condensation distribution of heter chromatin. The suspendend cells were rare by BRM –SJS (2.5μM –10μM, 2h –12h) in flask, and mostly stained tangerine (Fig 2). The effect was correlated

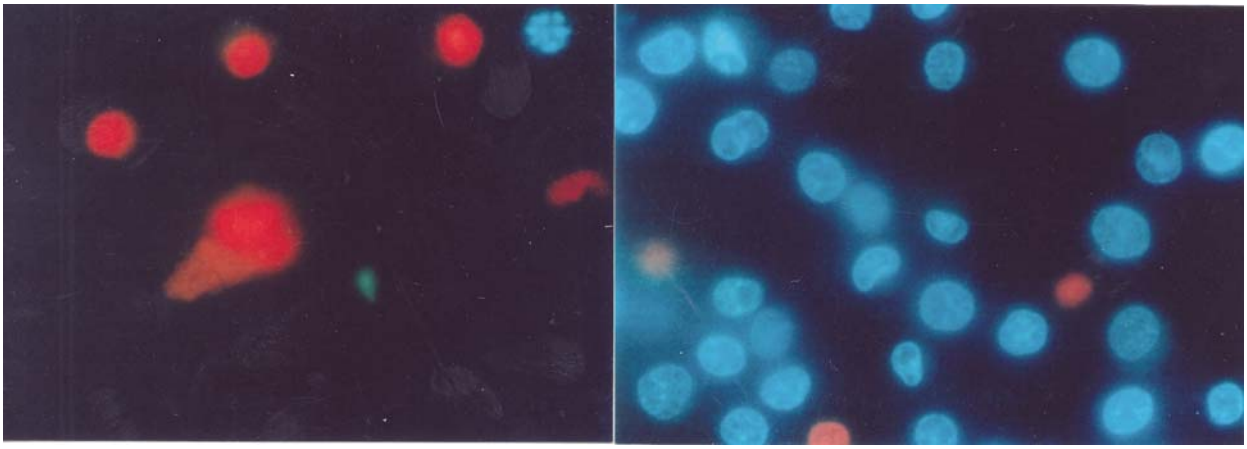
Table 1 fluorescent assay of BCaP–37 cells treated with BRM–SJS

Cell morphology (BCaP–37)	BRM–SJS (2.5、5、10μM)	
	for 2、6、12h	for 24、48、72h
Suspensive cells	a few suspensive cells formed only, most cells of whom are necrosis, few cells are apoptosis.	a lot of suspensive cells formed, the number was associated with drug con–centration and continued time. The main cells consisted two kind, one was necrosis, the other was apoptosis
Attachment cells	most of the cells were normal alive, few cells were necrosis.	These were inversely proportion with n increase by drug concentration and acting time extension.



a. 24h(BRM) b.24h(DMSO) c.72h(BRM) d.72h(DMSO)

Fig. Obervations on morphological change of BCaP−37 in different time with 10μM BRM by invert microscope ×200



a. Suspended cells of BCap−37 with BRM 10μM for 12h b. Attachment cells of BCaP−37 under the same conditions

Fig.2 PI and Hoechst 33342 stained in the meantime observation by fluorescence microscope ×200

with exposure time and concentration of BRM−SJS, the result showed that when BCaP−37 tumor cells were exposed at different concentration of BRM−SJS (2.5μM, 5μM, 10μM) for 24h, 48h or 72h respectively, the apoptosis rate of BCaP−37 cells gradually increased (Table 1, Fig. 3).

The detection of Cell cycle distribution of BCaP−37 by BRM−SJS

After treatment with different concentration of BRM−SJS (2.5μM, 5μM, 10μM) for 24h to 72h, all of DNA of BCaP−37 cells presented characteristic apoptosis Ladder, the flow cytometry analysis showed the rate of apoptosis was 61.4% by BRM−SJS (10μM, for 72h) (Table 2).

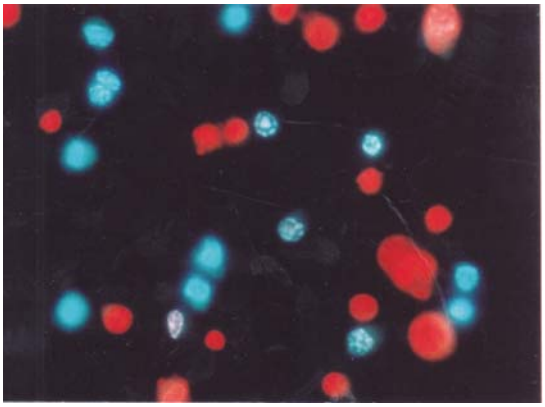


Fig.3 BCaP−37 cells with BRM 10μM for 72h, about to half cells of BCaP−37 exhibit typical appoptosic characteristic ×200

Table 2. Analysis of cell-cycle of BCaP-37 cells apoptosis

Groups	Time (h)	Concentration of BRM-SJS (μm)	Cell Percentage(%)			
			G1	G2	S	Apoptosis
Blank Control			73.5	7.7	19.0	0
BRM-SJS	24	2.5	80.8	7.0	12.2	0
BRM-SJS	24	5.0	68.8	8.7	22.7	0
BRM-SJS	24	10.0	63.2	7.7	29.1	14.0
BRM-SJS	48	2.5	78.2	10.9	20.9	10.6
BRM-SJS	48	5.0	68.7	4.7	6.6	23.8
BRM-SJS	48	0.0	64.6	0.9	34.5	50.2
BRM-SJS	72	2.5	68.5	8.5	22.9	9.1
BRM-SJS	72	5.0	61.1	6.3	32.5	29.9
BRM-SJS	72	10.0	69.4	12.1	18.5	61.4

DISCUSSION

Breast cancer is the most common cancer in the female population worldwide, it is one of the major threats to women health not only in China but in USA and most developed countries. Since the last decade, induction of apoptosis on tumor cells have been formed popular studying, at present a lot of detecting methods were used for apoptosis, but the evidence is directly by using characterized morphologically methods for determination apoptosis. Our studies exhibited that BRM-SJS may induce apoptosis of BCaP -37 cells effectively as well as BRM-SJS displayed time-dependent and dose- dependent manner, the result may be due to an increasing uptake of BRM-SJS by tumor cells. The above outcome suggested that under a range of effective dose, developing its induction apoptosis of tumor cells by BRM-SJS may be beneficial for improve the quality of life in patients and eprolonging the patient live-time.

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