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A STUDY OF MAST CELL-MEDIATED CYTOTOXICITY TO HEPATOMA CELL *IN VITRO*

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The effects of mast cells (MCs) isolated from rat peritoneal cavity on rat hepatoma cell line (CBRH7919) *in vitro* were studied with phase contrast microscope, scanning and transmission electron microscope. The results showed that different degrees of degeneration were presented in all CBRH7919 cells and a few of them exhibited necrosis or disruption when CBRH7919 cells were cocultured with MCs for 24 h. *In situ* hybridization demonstrated that the expression of c-*myc* mRNA in CBRH7919 cells was markedly reduced by MCs. These results suggest that MCs had an antitumor effect.

Key words: Liver neoplasms, Mast cells, Oncogenes.

The studies in recent years have showed that the mast cells could suppress the growth of some tumor cells.[1] Our previous studies demonstrated that mast cells could inhibit the growth of rat primary hepatoma cells.[2] At present, there are not any reports about the effects of mast cells on cultured hepatoma cells. In this paper, the effects of rat peritoneal mast cell on hepatoma cell line (CBRH7919) were observed *in vitro*.

MATERIALS AND METHODS

Cell Culture

Rat hepatoma cell line CBRH7919 (obtained from Institute of Cytobiology, Chinese Academy of Science, Shanghai) was cultured in RPMI-1640 medium containing 10% fetal calf serum, 10 μg/ml penicillin/streptomycin, 10 nM HEPES, and L-glutamine, 37 ℃ in a humidified atmosphere of 5% CO_2 and 95% air.

Isolation and Purification of Rat Peritoneal Mast Cells

Peritoneal cell suspensions were harvested from healthy Sprague-Dawleys rats (300–450 g, purchased from the Experimental Animal Center of Tongji Medical University) as described.[3] Macrophages containing in suspensions were removed by adhering to tissue culture flasks. Mast cells were obtained by density gradient centrifugation with Ficoll-Hypaque (density 1.070) and dense medium of density 1.806 or 1.090 (Ficoll-Hypaque+40% Ficoll) referring to the method described by Yurt.[4] The purity of mast cells was >98%, as determined by Alcian blue staining and transmission electron microscope. The purified mast cells with a viability of >95% as determined by trypan blue exclusion.

Morphological Observation of Mast Cell Cytotoxicity to Hepatoma Cell Line CBRH7919

The indexal hepatoma cell (5×10^2 cells/well) were cultured in 24-well plates with or without cover glasses. 12 h later, the fleshly purified mast cells were added to the wells, and the ratio of mast cells/tumor cells was 40/1. The equal amount of hepatoma cells instead of mast cells was added to as

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control. The total volume of medium was 3.0 milliliter each well. After 24 h, the following observations were carried out.

1. Observation with phase contrast microscope;

2. Observation with scanning electron microscope: The hepatoma cells adhering to the cover glasses were fixed with glutaraldehyde and osmium tetraoxide respectively, dehydrated, dried at the critical-point using carbon dioxide, coated with gold and observed with a Hitachi S-520 scanning electron microscope;

3. Observation under transmission electron microscope: The cells from the wells without cover glasses were centrifugated, fixed, dehydrated, and embedded in Epon 812. Ultrathin sections stained with uranyl acetate and lead citrate were observed under an Opton EM 10C electron microscope.

In Situ Hybridization to Detect the Effect of Mast Cell on the Expression of C-*myc* Gene in Hepatoma Cell

The c-*myc* cDNA probe (1.4 kb, Beijing Zhong Shan Biological Technology CO. Ltd) was labeled with digoxigenin (DIG). The DIG-labeling and detection kit was purchased from Boehringer Mannheim Company. The hepatoma cells were prepared by the methods described above and fixed with cooled acetone. *In situ* hybridization was performed as follows: The fixed cells were treated with 0.2 mol/L HCl for 15 min, washed with 0.25 glycine/PBS for 2 min, treated with 250 μg/ml proteinase K for 15 min at 37 ℃, prehybridized in prehybridization buffer containing 6×SSC, 45% deionized formamide, 5×Denhard's solution, and 100 μg/ml sheared salmon sperm DNA, then hubridized in hybridization solution containing 2.6 μg/ml sheared probe at 42 ℃ for 36 h. After hybridization, the hepatoma cells were washed. Immunoenzyme detection was performed as the methods given in kit. As negative controls (1) The hybridization solution without probe was used. (2) Sheep anti-DIG-AP complex was omitted. One hundred positive cells were randomized to measure their mean optical density (OD) with TJTY-300 Imaging Analysis System. The data were dealt with *t* test.

RESULTS

Morphological Observation of Hepatoma Cell

Under the phase contrast microscope the density of hepatom cells of control groups increased and their cytoplasma were transparent, the nucleoli were distinct. In the test groups, the cnacer cells contained rich reflected granules, parts of them couldn't adhere to flasks. Some small round and light cells bound to the hepatoma cells with unclear nucleoli. By the scanning electron microscopy the shape of hepatoma cells was irregular, and their surface was coarse. One or more mast cells attached to surfaces of the cancer cells, but the hepatoma cells had regular shape and smooth surface in controls. Ultrastructurally the shape and structure of cancer cells maintained intact in the controls, but a widespread degenerative changes emerged in the hepatoma cells of test groups: Mitochondria were swollen, cytoplasma presented electronic transparent matrix and endoplasmic reticula dilated, but plasma membrane and nuclear membrane remained intact. A few of hepatoma cells exhibited karyopyknosis, karyorrhexis and necrosis or disruption.

The Expression of C-*myc*

In situ hybridization demonstrated that the expression of c-*myc* mRNA in hepatoma cells was strong positive in the test groups. In the controls, the major amount of cancer cells showed weak positiveness and their optical density was lower than that of cancer cells in the test group (Table 1).

Table 1. Analysis of optical density measured the intensity of c-myc gene expression

Group	n	OD ($\bar{x}\pm s$)	*P*
Hepatoma cell	104	0.5791±0.1372	
Hepatoma cell+MC	106	0.3294±0.08683	<0.01

DISCUSSION

Our studies indicated that rat peritoneal mast cells showed marked cytotoxicity to hepatoma cell line CBRH7919 without any stimuli. On basis of ultrastructural observations though mast cells contacted with hepatoma cells, obvious cell conjugate formations were not found, and the cytoplasmic membrane of cancer cells remained intact in the contacting sites. These observations suggested that mast cells are capable of suppressing hepatoma cells by releasing some cytotoxic factors.

We demonstrated here that mast cells can suppress the expression of c-*myc* mRNA in hepatoma cells. That the coding product of c-*myc* gene combined with DNA may promote replication of DNA.[5] The activation and expression of c-*myc* gene has a good with the mitogenesis and proliferation of cancer cells. The phenomenon that mast cells suppressed the c-*myc* gene expression in hepatoma cells indicated that mast cells can lead to the changes of cellular biological character of hepatoma cells at molecular levels.

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