

# Effect of cell fusion on metastatic ability of mouse hepatocarcinoma cell lines \*

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**Subject headings** carcinoma, hepatocellular; cell lines; cell fusion; neoplasm metastasis; lymphatic metastasis

## Abstract

**AIM** To study the effect of cell fusion on metastatic ability of mouse hepatocarcinoma cells and the factors involved in the process of metastasis.

**METHODS** By the method of successively increasing the concentrations, cell fusion and limit dilution, 8-Ag resistant cells were selected, and HGPRT-Hca-P cells and eight cloned hybridoma cells were obtained. To observe their metastatic ability, they were inoculated into mice foodtaps and the drainage lymph nodes were examined under microscope.

**RESULTS** The end concentration of 8-Ag which was used to select HGPRT deficient Hca-P cells was 30mg/L. All the cells selected died in HAT culture medium in one week. Fused cells appeared approximately 9 days later. They were round, transparent and a little larger than their parental cells. Eight clones of hybridoma cells were obtained and named as PSH1-PSH8. The metastatic rate of HGPRT-Hca-P cells and PSH7 cells was 28.6% and 71.4% respectively, the difference being significant ( $P < 0.05$ ). The metastatic rate of other clones was no more than 20% and there was no significant difference from HGPRT-Hca-P cells ( $P > 0.05$ ).

**CONCLUSION** In normal mice splenic lymphocytes, there are some factors that could inhibit tumor metastasis, however, there are some other factors accelerating tumor cells to metastasize. The establishment of PSH7 provides an experimental model which could be used to study the factors involved in metastasis.

## INTRODUCTION

It was known during the 1970s that the malignancy of hybridoma cells decreased when tumor cells were fused with normal cells and the malignant phenotype was suppressed obviously or diminished. But since 1980, in many laboratories, the increased invasive and metastatic abilities of hybridoma cells have been found when tumor cells were fused with lymphocytes or macrophages which had ambulant ability<sup>[1-5]</sup>. These results implied that there were some factors that could increase the metastatic ability of tumor cells and raised a hypothesis that tumor cells used the ambulant mechanism of normal cells<sup>[6]</sup>. In this study, cell fusion was used to study the factors involved in the process of metastasis.

## MATERIALS AND METHODS

### Animals

A total of 615 inbred mice were provided by the Department of Pathology of Dalian Medical University.

### Tumor cell lines

Mouse hepatocarcinoma cell lines Hca-P(P) with low lymphatic metastatic ability were established and stored as described previously<sup>[7]</sup>.

### Hypoxanthine-guanine phosphoribosyltransferase deficient (HGPRT-) cells

In order to select HGPRT- cells, P cells were converted into 8-Ag resistant by growing in successively increased concentration of 8-Ag as described previously<sup>[8]</sup>. The initial concentration was 3mg/L. If alive tumor cells had the dominant position, the concentration was increased successively, from 3 mg/L to 6 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, and 30 mg/L. To test the sensitivity of selected cells to HAT, 8-Ag resistant cells which had been cloned by limit dilution method were inoculated into HAT culture medium. A week later, Trypan blue repelling test was used to judge the vitality of cells and the 100% dead cells which kept alive in normal culture medium were proliferated. The cells were taken as parental cells in fusion. To measure their metastatic ability, HGPRT-Hca-P cells were inoculated into foodtaps of 615 mice<sup>[7]</sup>. The animals were sacrificed 28 days later, and original tumor, the ipsilateral popliteal, inguinal and axillary lymph nodes were removed, fixed in 10% formalin, made into paraffin section, stained with HE

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and observed under microscopy.

### Fusion

Spleen lymphocytes of normal mice were prepared routinely. HGPRT<sup>-</sup>-Hca-P cells and normal spleen lymphocytes were taken as parental cells of fusion. Solution of fusion was 50% PEG-4000. The average cell numbers of each well in 96 well plate used for fusion were  $3 \times 10^5$  and the ratio of lymphocytes to tumor cells was 8:1. While fused cells appeared, they were suspended in  $2 \times$ HAT culture medium, then were cultured at 37°C in a humidified atmosphere (950 mL/L air, 50 mL/L CO<sub>2</sub>). The medium was changed every three days and two weeks later it was substituted by normal medium. Subsequently, the obtained hybridoma cells were cloned by limit dilution method. To measure the metastatic ability of hybridoma cells, Hca-P cells and HGPRT<sup>-</sup>-Hca-P cells were inoculated into foodtaps of 615 mice respectively as described above.

### RESULTS

The end concentration of 8-Ag which was used to select HGPRT deficient Hca-P cells was 30mg/L. All of the cells proliferated after being cloned died in HAT culture medium within one week, suggesting that they were HGPRT<sup>-</sup> cells.

Fused cells appeared approximately 9 days later. They were round, transparent and were a little larger than their parental cells. Eight clones of hybridoma cells were obtained by using limit dilution method and were named PSH1-PSH8. It was shown by the histological examination that the metastatic ability of PSH7 increased but the rest decreased. The metastatic ability of PSH7 was significantly higher than that of HGPRT<sup>-</sup>-Hca-P cells, but there was no significant difference between HGPRT<sup>-</sup>-Hca-P and Hca-P which were not treated with 8-Ag ( $P < 0.05$ , Table 1). Because the sample size was small, exact probabilities in  $2 \times 2$  table of Chi-square test was used to analyze the data.

**Table 1 Metastatic rate of cells**

Cells	Number of experimental animals	Number of metastatic animals	Metastatic rate (%)	<i>P</i>
HGPRT <sup>-</sup> -Hca-P	14	4	28.6	0.24>0.05
Hca-P	16	3	18.8	
PSH1	8	1	12.5	
PSH2	8	0	0	
PSH3	8	1	12.5	
PSH4	10	2	20	
PSH5	8	0	0	
PSH6	10	1	20	
PSH7	14	10	71.4	0.027<0.05
PSH8	8	0	0	

### DISCUSSION

The method of successively increasing concentration was often used to select resistant cells. In this study, it was used to select 8-Ag resistant Hca-P cells. The critical concentration was 30 mg/L under which most cells died. As the concentration was increased, the survived and proliferated cells were 8-Ag resistant cells. These cells could be transplanted into normal culture medium. In order to prevent HGPRT<sup>-</sup> cells from turning into HGPRT<sup>+</sup> cells, it was necessary to treat them with 8-Ag now and then or always keep them growing in culture medium containing 8-Ag.

Cell fusion has been extensively used in the study of phenotypic expression and regulation of malignant cells. It has been known that the metastatic ability of hybridoma cells could decrease or increase while certain normal cells were fused with nonmetastatic or low metastatic cells. In those experiments, the metastatic ability of hybridoma cells increased only when the parental cells were ambulant cells, such as lymphocytes or macrophages and the hybridoma cells obtained certain characters.

Both Hca-F and Hca-P cells isolated from mouse hepatocarcinoma cells had different metastatic ability only to lymph nodes but not to other organs. Because the metastatic rate of P cell was 18.8% and metastatic phenotype was stable, it is of great advantage to study the changes and related mechanism of metastatic ability of hybridoma cells which were obtained from the fusion of Hca-P and spleen cells of mice.

The metastatic rate of HGPRT<sup>-</sup>-Hca-P cells was still lower than 30% and there was no significant difference from that of Hca-P cells. The metastatic ability of most hybridoma cells kept stable or decreased, in contrast, that of PSH7 increased up to 71.4%, being significantly different from that of HGPRT<sup>-</sup>-Hca-P cells. Because hybridoma cells had the nature of their parental cells, it is important to study the factors which affect tumor metastatic ability by cell fusion. Hca-P as one of the parental cells had the character of stable metastatic ability to lymph nodes. Therefore no matter how the metastatic ability changed, it was caused by lymphocytes. The metastatic ability of seven hybridoma cell lines decreased, while only one increased. These results suggested that the lowering trend was dominant, which was probably due to tumor suppressive gene existing in normal cells, furthermore, there were some other factors that could enhance tumor metastatic ability.

There were many similar aspects between metastasis of tumor cells and ambulence of lymphocytes. Both of them could enter circulation by passing endothelia of vessels, proliferate in drainage lymph nodes, and enter peripheral tissue by immi-

gration. Furthermore, organ preference of tumor metastasis was similar to the homing of lymphocytes. The relationship between homing receptor and tumor metastasis was discovered recently<sup>[9]</sup>. Here comes the question: do the hybridoma cells with increased metastatic ability use some special mechanism of lymphocytes or macrophages, such as ambulant mechanism and homing receptor. It is suspected that probably tumor cells metastasize to special organs by means of some structures like homing receptor and a certain mechanism like the homing of lymphocytes. The results of our study show that there must be something existing in spleen lymphocytes that accelerates tumor cells to metastasize. The establishment of PSH7 has provided an experimental model which could be used to study the factors involved in metastasis.

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