DIFFERENT EFFECT OF MOUSE HEPATOCARCINOMA CELLS WITH DIFFERENT METASTATIC POTENTIAL ON HOST IMMUNE SYSTEM

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ABSTRACT

Objective: To investigate the effect of mouse hepatocarcinoma cell line HCa-F (high metastatic potential) and HCa-P (low metastatic potential) on immune system of the host. Methods: Mice were subcutaneously implanted with HCA-F (F) or HCA-P (P) cells. Cell cycle of lymphocytes from metastatic lymph nodes of tumor-bearing mice was studied by flow cytometry. Fas-L expression of F and P cells was detected immunohistochemically. Apoptosis of macrophages in metastatic lymph nodes was examined by TUNEL methods. Results: For the Hca-F cell bearing mice, the proliferating peak of lymphocytes appeared on 14th day post-inoculation and then decreased rapidly, while for the Hca-P cell bearing mice, the peak appeared on the 7th day post-inoculation and then kept at high level. The expression of Fas-L in F cells was stronger than that in P cells (P<0.01). Apoptosis occurred in macrophages near metastatic F cells in lymph nodes of tumor-bearing mice. Conclusion: Carcinoma cells with high metastatic potential may suppress immune reaction of host through inducing apoptosis of macrophages (one of the important antigen present cells) by Fas-L-Fas interreaction. And the expression of Fas-L in tumor cells may be associated with high metastatic ability to lymph nodes.

Key words: Liver neoplasm, Neoplasm metastasis, Immune reaction

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Metastasis is the most lethal attribute of cancer[1-3] and lymph nodes are often the first organ to develop metastasis and also a bridgehead for further metastasis[4,5]. A pair of synogenetic mouse hepatocarcinoma cell lines Hca-F and Hca-P present specific potential of lymphogenetic metastasis when inoculated subcutaneously in 615 mice. Hca-F shows a high metastatic potential (>80%), while Hca-P a low one (<30%)[6]. Some researches have showed that there is no difference in metastatic rate between Hca-F bearing and Hca-P bearing nude mice whose T lymphocytes are inactive[7], which indicates that T lymphocytes play an important role in suppressing tumor metastasis. The potential of tumor cells to induce apoptosis of host immune cells is the main way for them to escape from being killed by immune cells. Whether there is a direct effect of these two cell lines on immune system of the host is uncertain. This experiment employed flow cytometry to study the cell cycle of lymphocytes from metastatic lymph nodes of tumor-bearing mice and discussed the molecular mechanism of the effect of tumor cell on immune system of the host.

MATERIALS AND METHODS

Animals, Cell Lines and Flow Cytometry

In our laboratory 64 inbred 615-mice maintained were equally divided into two groups. The Hca-F and Hca-P tumor cell lines preserved in our laboratory were inoculated at 2×10⁶ in 32 mice subcutaneously in each group. On the 7th, 14th, 21st, and 28th day post-inoculation, 3 mice from each group were killed, and their lymphocytes were collected and detected for growth fraction with flow cytometry. The process of flow cytometry was as follows[8]: the lymph nodes were minced and centrifuged at 3000 rpm, and the supernatant
was discarded. After repeated washing, cells were suspended in PBS. The lymphocytes at 10⁵/100 μl were stained for 30 minutes by Propidium Iodide. Flow cytometry was performed on a FACSscan cytometer with LYSYII software. The fluorescence of 10⁴ cells was analyzed for each sample. The other 40 mice were terminated on the 28th day post-inoculation, and their lymph nodes were H.E. stained and examined under microscope by paraffin sections. The lymph nodes metastatic rates of Hca-F and Hca-P tumor cells were calculated.

Immunohistochemistry

The expressions of Fas-L (Santa Cruz, USA) in the tumor cells of inoculated area (less necrosis) of 10 Hca-F cell bearing mice and 10 Hca-P cell bearing mice, and metastatic Hca-F cells in lymph nodes were detected by standard immunohistochemistry[9]. The semiquantitative estimation of cancer cells was classified into 4 categories by assessing the percentage of stained tumor cells: 0, <2%; 1, 2-25%; 2, 26-50%; 3, 51-75%; and 4, >75% cells.

In Situ DNA Fragmentation

We examined DNA fragmentation of the tumor cells of inoculated area of Hca-F cell bearing mice and Hca-P cell bearing mice, and metastatic Hca-F cells in lymph nodes, by the method of Zhu et al.[10]. After desparaffin, these slides were pre-treated with 20 mg/L proteinase K for 30 min, and then incubated with terminal deoxynucleotidy transferase and fluorescein labeled dUTP containing nucleotide mixture (TUNEL reaction mixture, in situ Cell Death Detection Kit/POD, Boehringer Mannheim, Germany) in a humid atmosphere at 37°C for 30 min. TUNEL reaction mixture without terminal transferase served as negative control.

RESULTS

Lymph Nodes Metastatic Rate and Flow Cytometry

On the 28th day post-inoculation, the lymph node metastatic rate of Hca-F was 80% (16/20), whereas that of Hca-P was 10% (2/20). The growth fraction of lymphocytes from lymph nodes of mice transplanted with Hca-F and Hca-P tumor cells was examined using flow cytometry (Figure 1). For the Hca-F cells, the proliferating peak of lymphocytes appeared on 14th day post-inoculation and then decreased rapidly, and for the Hca-P cells, the peak appeared on the 7th day post-inoculation and then kept at high level.

Immunohistochemistry

The expression of Fas-L protein in Hca-F cells was significantly higher than that in Hca-P cells (P<0.01, Table 1, Figures 2).

<table>
<thead>
<tr>
<th>Tumor cells</th>
<th>Grade</th>
<th>Average rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumor of Hca-P (n=10)</td>
<td>1</td>
<td>78</td>
</tr>
<tr>
<td>Primary tumor of Hca-F (n=10)</td>
<td>2</td>
<td>219</td>
</tr>
<tr>
<td>Metastatic tumor of Hca-F (n=10)</td>
<td>0</td>
<td>138</td>
</tr>
</tbody>
</table>

In situ DNA Fragmentation

Few positive Hca-F and Hca-P cells were observed. Positive signals appeared in the macrophages around Hca-F cells (Figure 3).

DISCUSSION

Some studies have showed that the immunogenicity of Hca-F cells is far below that of Hca-P cells[11]. We detected the cell cycle of lymphocytes from lymph nodes of tumor-bearing mice, and found that For the Hca-F cells, the proliferative peak of lymphocytes appeared on 14th day post-inoculation and then decreased rapidly,