Brief Communication: Lack of Correlation Between Malignancy and Sensitivity to Killing by Concanavalin A 1,2

Fa-Ten Kao 3 and Henry Harris 4,5

SUMMARY—Eight mouse cell lines and seven hybrid clones, both malignant and nonmalignant, were tested for possible correlations between malignancy and sensitivity to killing by concanavalin A (Con A). These cells included six highly malignant and two less malignant cell lines, two malignant and three nonmalignant hybrid clones derived from five different crosses, and two malignant hybrid clones segregated from a cross between malignant and nonmalignant cells. The malignancy of the cells was determined by their ability to grow progressively in irradiated, syngeneic newborn mice and to kill the host. All these cells, except one, showed similar sensitivities to killing by Con A. No correlation could be established between the malignancy of a cell line or of a hybrid clone and its sensitivity to the lectin.—J Natl Cancer Inst 54: 767–768, 1975.

DIFFERENCES BETWEEN TRANSFORMED AND NONTRANSFORMED CELLs in their agglutinability by lectins have been clearly demonstrated. In general, transformed cells are more readily agglutinable than nontransformed cells (1). Furthermore, “flat” revertants that have lost some transformed properties have been isolated from transformed cell populations by treatment with the plant lectin concanavalin A (Con A) at lethal concentrations (2). The differential sensitivity of cells to Con A killing might therefore be used as an in vitro marker, not only for distinguishing between malignant and nonmalignant cells but also for selecting nonmalignant hybrids from fused cell populations. This report describes experiments in which a variety of mouse cell lines and malignant and nonmalignant hybrids derived from them were tested to explore a possible correlation between malignancy and sensitivity to killing by Con A.

MATERIALS AND METHODS

The mouse cells and the hybrids were cultivated in Eagle’s minimum essential medium containing 10% fetal calf serum. Single cell platings were done by the procedure of Ham and Puck (3). Two hundred cells of each type were inoculated into each of a series of 35-mm Falcon plates and placed for 6 hours in a CO2 incubator. Various concentrations of freshly prepared Con A (Sigma Chemical Co., St. Louis, Mo.) were then added to each plate, and the cells incubated for 7–10 days. To determine the number of colonies surviving the Con A treatment, the plates were fixed and stained. This procedure yielded the most consistent results as compared with the behavior of these cells treated with Con A for 1, 2, or 3 days.

Eight mouse cell lines and seven hybrid clones were tested. These included six malignant and two less malignant cell lines, two malignant and three nonmalignant hybrid clones, and two malignant hybrid clones segregated from a cross between malignant and nonmalignant cells. The clonal hybrid cells were isolated from various fusion experiments involving six different crosses. The origins and properties of these cells and hybrids were described in (4–8). The hybrid clones were stored in liquid nitrogen and thawed for use.

The tumorigenicity of each cell type was determined previously by injection of the cells into irradiated, syngeneic newborn mice. Malignant cells grow progressively in vivo and eventually kill the host animal, whereas nonmalignant cells fail to develop a tumor with comparable inocula of cells. The A9 and B82 cells are far less tumorigenic than their malignant derivatives A9HT and B82HT and the other highly malignant cells used in this study. Only after injection of large numbers of cells will A9 and B82 produce tumors in a small proportion of mice given the injections.

Mouse cell lines

T A3 Hauschka (T A3 Ha): highly malignant cells derived from a spontaneous mammary carcinoma; this subline shows reduced expression of H-2 isoantigens.

T A3 HaB: a clonal subline of TA 3 Ha selected for resistance to 6-thioguanine and lacking inosinic acid pyrophosphorylase activity; also highly malignant.

A9: an L cell derivative without inosinic acid pyrophosphorylase activity and resistant to 8-azaguanine; low tumorigenicity.

B82: an L cell derivative lacking thymidine kinase activity and resistant to 5-bromodeoxyuridine; low tumorigenicity.

A9HT: a highly malignant cell derivative selected from A9 by passage through the animal.

B82HT: a highly malignant cell derivative selected from B82 by passage through the animal.

MM: highly malignant mouse melanoma cells derived from a melanotic tumor of the C57 BL mouse.

EL4: highly malignant Moloney virus-induced lymphoma of the C57 BL mouse.

M5WBS: highly malignant 3-methylcholanthrene-induced sarcoma of the A.SW mouse.

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Mouse/Mouse hybrid clones

TAaHaB X B82 clone 1: nonmalignant.
A9 X B82HT clone 2: nonmalignant.
A9 X EAT clone S1: malignant; a segregant from a cross between the less malignant A9 and the highly malignant Ehrlich ascites tumor cell, selected by passage of the hybrid cells through the animal.
A9 X EAT clone S3: malignant; another segregant tumour line.

TAaHaB X EL clone 2: highly malignant carcinoma-lymphoma-hyphaema hybrid.
TAaHa X MSWBS clone 2: highly malignant carcinoma-sarcoma hybrid.

A9HT X C57BLy clone 7: nonmalignant clone from a cross between the malignant A9HT and a normal diploid lymphocyte of the C57 BL mouse.

RESULTS AND DISCUSSION

Table 1 presents the plating efficiencies of the various cell lines and their hybrids, malignant and nonmalignant, after growth in the continuous presence of Con A. Although the highly malignant TAaHaB cells displayed higher sensitivity to Con A killing than the less malignant A9 and B82 cells, no consistent relationship between malignancy and sensitivity to Con A killing could be demonstrated in the other malignant and nonmalignant cells or their hybrids.

The two malignant and the three nonmalignant hybrids behaved similarly in their responses to Con A, and this was also true for the two segregant malignant clones derived from a malignant X less malignant cross. The less malignant A9 and B82 cells and their respective highly malignant derivatives A9HT and B82HT were almost the same in their response to the Con A treatment. The TAaHa cells were slightly more resistant to Con A killing than were the TAaHaB cells. However, under the present growth conditions, TAaHaB cells attached more firmly to the plates than did TAaHa cells, and this property might have contributed to the slight difference observed in their responses to the Con A treatment.

The response of the mouse melanoma MM cells to Con A was unusual in that, unlike the other cells and hybrids tested, there was a progressive increase in killing with increasing doses of Con A. Other cells showed a rather sharp end point.

Thus apparently there is no direct correlation between the malignant behavior of a cell and its sensitivity to killing by Con A. More tests should be done to see if in vitro techniques, purporting to distinguish between malignant and nonmalignant cells, actually do so in these cells in which malignancy has been scored in vivo. We have tested some of these cells, both malignant and nonmalignant, for their ability to grow in 0.33% agar and found that this property also failed to distinguish them.

The high sensitivity of the TAaHaB cells to killing by Con A may be useful as a marker for selection of more resistant cells after mutagenic treatment or for selection of resistant hybrids from crosses between TAaHaB and resistant cells.

REFERENCES

(2) OZANNE B: Variants of simian virus 40-transformed 3T3 cells that are resistant to concanavalin A. J Virol 12: 79-89, 1973
(7) ———: The analysis of malignancy by cell fusion. V. Further evidence of the ability of normal diploid cells to suppress malignancy. J Cell Sci 15:177-183, 1974
(8) ———: The analysis of malignancy by cell fusion. VI. Hybrids between different tumour cells. J Cell Sci 16:189-198, 1974

Table 1.—Relative and absolute plating efficiencies of malignant and less malignant or nonmalignant cells and hybrids treated with Con A at various concentrations

<table>
<thead>
<tr>
<th>Cell</th>
<th>Malignancy</th>
<th>Relative plating efficiency (%) at Con A concentrations (μg/ml)</th>
<th>Absolute plating efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>TAaHaB</td>
<td>Malignant</td>
<td>100*</td>
<td>100*</td>
</tr>
<tr>
<td>A9</td>
<td>Less malignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>B82</td>
<td>Less malignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A9HT</td>
<td>Malignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>B82HT</td>
<td>Malignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MSWBS</td>
<td>Malignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TAaHaB X MSWBS</td>
<td>Malignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TAaHaB X EL</td>
<td>Malignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A9 X B82HT</td>
<td>Nonmalignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>TAaHa X B82</td>
<td>Nonmalignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A9HT X C57BLy</td>
<td>Nonmalignant</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>A9 X EAT S1</td>
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</tr>
<tr>
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</tr>
<tr>
<td>MM</td>
<td>Malignant</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* All relative plating efficiencies close to the control values (within 10% deviation) are scored as 100%.