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Growth of Solid Tumor Cells in Clonogenic Assays: a Prognostic Factor?*

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Abstract—*Clonogenic assays can be used to study several characteristics of hemopoietic and solid tumor cells. Treatment of ovarian cancer patients, anticancer drug development and studies of tumor cell biology (self-renewal and cytogenetics) seem to be facilitated by these techniques, in spite of their limitations. This paper reviews data showing that good* in vitro *clonal growth might be a poor prognostic factor. These results are remarkable but only few patients have been studied and detailed analyses looking at other prognostic factors are lacking. However, studies of* in vitro *growth characteristics of hemopoietic malignancies have shown similar results. Further follow-up should substantiate these initial findings in solid tumors.*

INTRODUCTION

In vitro colony formation by human solid tumor cells can be obtained using several techniques [1-3]. Claims have been made that the use of such methods might allow the prediction of which drug to use or avoid when treating an individual patient [4]. This first retrospective analysis has been somewhat confirmed by other authors in a prospective trial [5]. For ovarian cancer it seems that treatment according to *in vitro* results can offer substantial survival advantages for relapsing patients [6], and that treatment with the three best *in vitro* drugs induces a higher remission rate [7]. For most other tumors the clonogenic assays, as presently used, are an inefficient and problematic tool for individualized treatment planning [8]. They might, on the other hand, be an interesting method for developing new anticancer drugs [9].

These assays are not limited to predictive drug testing. Their potential for studies of tumor biology should be exploited in a way similar to the one that has allowed such considerable progress in understanding haematopoiesis [10]. For example, studies have been done to characterize the self-renewal characteristics of ovarian carcinoma [11] and melanoma [12], and they can be of help in the study of tumor cytogenetics [13].

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In vitro growth characteristics of haemopoietic malignancies have been shown to correlate with remission outcome in acute leukemias [14-16] and seem to allow a better characterization of the myelodysplastic syndromes that will progress into acute leukemia [17, 18]. In this paper I will review presently available data from my own studies in breast cancer and other early results from various authors. These data tend to indicate that growth of tumor cell colonies *in vitro* might be a prognostic factor; 'no growth' might not reflect technical problems only but might also have biological significance.

BREAST

In December 1983 we studied 33 consecutive samples put into culture until May 1982. Growth characteristics of the seven pleural effusions from patients with advanced metastatic disease had no correlation with survival. Patients whose primary breast cancer did not grow *in vitro* had a survival of 21+ months (10/12 patients alive). Primary tumor samples that grew *in vitro* came from patients whose median survival was 16 months (9/14 patients dead, $P < 0.05$). We attempted an analysis of the relationship between known prognostic factors and growth of clonogenic cells *in vitro*. Tumor size and presence or absence of metatases did not correlate with the number of samples growing, but had the expected influence in shortening patient survival. Estrogen receptor (ER) status did not correlate to colony growth, and in this series the 17 ER-positive patients had

the same median survival (21 months) as the nine ER-negative patients. Nodal status seemed to be the only factor that might possibly correlate with clonal growth. Five of six patients with four or more positive nodes had colonies growing from their primary tumor specimen, against 9/20 patients with 0–3 nodes. This difference fails to reach statistical significance and it should be noted that 6/9 node-negative patients had colony growth. Patients with four or more positive nodes had a median survival of 12 months, compared with 21 for the others [19].

Forty patients with stage I, II and III breast cancer and a median follow-up of only 367 days suffered from seven recurrences. Regression analysis showed a trend ($P=0.10$) for a relationship between short disease-free survival and increasing colony counts. Eight of 16 patients with stage IV cancer relapsed and they all had more that ten colonies per assay dish. The other eight stage IV patients were still alive and their samples grew less than ten colonies [20].

BLADDER

Forty-six patients suffering from transitional cell carcinoma of the bladder were studied at various time intervals after the first endoscopic tumor resection. Ten of 11 patients who had histopathologic evidence of recurrence on follow-up had had growth of tumor colonies in the initial examination. No tumor recurrence was seen in 35 patients, 20 of whom had also had *in vitro* tumor growth. Further follow-up will be necessary to see whether this latter group of patients is at high risk of relapse [21].

GASTRIC

Twenty of 44 specimens (35 solid and nine fluids, 15 from pretreated patients) from various sites showed growth of more than five colonies per plate. These patients had a median survival of 5 months, and the 24 other patients had a median survival of 6 months [22].

HEAD AND NECK

Twenty-seven patients with positive cultures (five or more colonies) were divided into two groups: those with cloning efficiencies greater and less than 0.02%. Six of 11 patients with high cloning efficiencies were dead whereas only 1/16 patients with low cloning efficiencies was dead within 3 months from diagnosis. This was statistically significant at $P<0.01$ by the chi-square test [23].

Another study tends to confirm these data, as at the time of the report 8/14 patients with a cloning efficiency greater than 0.005% had relapsed or died, compared to 3/15 patients with a lower cloning efficiency. Those two groups differed with a P value of 0.04 [24].

OVARY

Twenty-nine specimens of ovarian carcinoma showed the following correlations with patient survival. All four patients with good growth (colonies of good quality and some larger than 250 μm) died, with a mean survival of 74 days. Five of 11 patients with poor growth (cluster formation, or clusters and colonies of less than 150 μm in diameter generally showing signs of deterioration) were dead, with a mean survival

Table 1. Clonal growth and patient outcome

Tumor	No. of patients		Reference
Bladder	46	growth: 10/30 relapse no growth: 1/16 relapse	[19]
Breast			
Primary	26	growth: 9/14 are dead, median survival 16 months no growth: 2/12 are dead, median survival 21+ months	[20]
Stage I-III	40	growth = short relapse free survival (trend; $P=0.10$)	
Stage IV	16	growth: 2.6 deaths/1000 person-days (8 dead patients) no growth: 0 deaths/1000 person-days (8 live patients)	[21]
Gastric	44	growth: 5 months median survival (20 patients) no growth: 6 months median survival (24 patients)	[22]
Head and neck	27	growth: 6/11 died at three months from diagnosis no growth: 1/16 dies at three months from diagnosis	[23]
	29	growth: 8/14 relapsed or died no growth: 3/15 relapsed or died	[24]
Ovary	29	good growth: 4 patients, mean survival 74 days poor growth: 11 patients, mean survival 103 days no growth: 14 patients, mean survival 203 days	[25]

time of 103 days, and 8/14 patients whose sample showed no growth were dead, with a mean survival time of 203 days [25].

MISCELLANEOUS

In 19 patients with tumors of several origins a preliminary study showed that rapidly evolving tumors yielded cells demonstrating the highest cloning rate [26].

DISCUSSION

This brief review indicates that good *in vitro* growth of tumor cell colonies is a poor prognostic factor. Only one study fails to show a correlation. This is a remarkable finding in view of the heterogeneous nature of the patients reported in most of these studies: pretreated and newly diagnosed patients of all stages are mixed.

Attempts have been made to correlate the *in vitro* clonal growth characteristics of these tumors to other known predictive parameters. Tumor grade has been shown to influence colony growth of breast carcinomas [27] and gastric cancer [28], and contradictory data is available for head and neck cancer [23, 24]. TNM status does not seem to correlate with *in vitro* growth of breast cancer [19] but increased stage is related to high cloning efficiency in squamous cell carcinoma of the head and neck [24]. Estrogen receptor status is generally said not to correlate to *in vitro* growth characteristics of breast cancer [19, 20, 29]. At the present stage, in view of the small numbers of patients studied, it is premature to draw any definitive conclusions from these observations.

Another prognostic aspect of clonogenic assays of human solid tumors is the study of bone marrow metastases. Patients with neuroblastoma have been shown to have an association between the number of colonies formed by their marrow metastases and their clinical course [30].

Further patient accrual with careful consideration of all other prognostic variables will be necessary to see if the growth pattern of adequate samples from cancer patients is an independent prognostic factor. The relationship between the growth fraction of tumors as determined by the labeling index [31] and results of the clonogenic assays remains to be determined. Clonogenic capacity of tumors might partially be a reflection of this characteristic.

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