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Replating efficiency of metastatic melanoma cells from lymph node and subcutaneous sites does not predict patient survival†

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The efficiency of replating of cells from primary colonies grown in semisolid medium has been used to detect and quantitate self-renewal *in vitro*. A positive correlation has been found by others between the replating efficiency of cells from myelogenous leukemia and patient survival. In the current study we measured primary and secondary replating efficiency of metastatic melanoma cells from subcutaneous tissues or lymph nodes of twelve patients and related these results to patient survival from time of biopsy. No relationship was found between primary and secondary plating efficiency nor for primary or secondary replating efficiency and survival. These results suggest that colony-forming melanoma cells grown under anchorage-independent conditions do not identify a stem cell population important for survival distinct from highly proliferative cells. These studies do not, however, rule out the possibility that a non-clonogenic transitional cell population exists in the tumor.

Introduction

Biological and clinical features of tumors have been used extensively to predict patient response and survival [16, 23]. The importance of cellular proliferation to these outcomes has been amply demonstrated using multiple myeloma as the model [9]. Recently, an enormous amount of effort has been spent in studying the growth of colony-forming cells under anchorage-independent conditions as a predictor for therapeutic responsiveness [17, 25]. We have shown, for example, that this system can help in the selection of drugs for further development and to some degree for individual patient benefit [1, 17, 24].

Studies of colony-forming cells from normal hematopoietic tissue have demonstrated that pluripotent cells can be isolated which are highly proliferative and exhibit self-renewal capacity [27, 28]. The most convenient method to assess self-renewal using human tissue has been to measure the efficiency of replating cells from primary colonies [10]. McCulloch and co-workers [4, 6] have performed a series of studies demonstrating that cells from acute myelogenous leukemia (AML) self-renew. Additionally, they have shown [4, 16] that the efficiency of self-renewal, but not primary colony formation, was correlated with clinical complete response in patients with AML.

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Investigations of the replating properties of human solid tumors have been limited to cells from ovarian, bladder and melanoma tumors and self-renewal has been demonstrated [3, 11, 26]. We have described assay systems for the measurement of the growth and replating efficiency of cells from metastatic melanoma tissue [18, 26]. We have also shown that different sizes of human primary melanoma colonies replate with similar efficiency [21]. In this study, we compared the primary and secondary plating efficiencies of melanoma colony formation of soft agar with patient survival and no relationship was found.

Materials and methods

Patients

Twelve patients with documented metastatic melanoma tumors were selected for study. All biopsies were from patients with subcutaneous or lymph node metastases as the predominant form of their diseases [2] (informed consent was obtained using a protocol approved by University of Arizona Institutional Review Board).

Culture of cells

We have described the culture of melanoma colony-forming tumor cells elsewhere [18, 26]. All cultures to determine primary and secondary plating efficiency were performed in microtiter wells as previously described and were highly reproducible [18]. The coefficient of variation of plating efficiency was between 5 and 19 per cent in replicate samples. The number of cells in primary and secondary colonies was calculated using a nomogram which we have developed, which relates cell and colony size to total number of cells [20]. Plating efficiencies were expressed as follows:

$$PE_1 = \frac{\text{number of primary colonies} > 60\,\mu\text{m diameter}}{\text{total number of viable melanoma tumor cells}}$$

$$PE_2 = \frac{\text{number of secondary colonies} > 60\,\mu\text{m diameter}}{\text{total number of viable melanoma tumor cells from primary colonies}}$$

Statistics

The PE_1, PE_2 and PE_2/PE_1 values were related to survival of the patient from time of biopsy using a simple linear regression [20].

Results and discussion

We first examined the relationship between primary and secondary plating efficiency (figure 1) and no clear relationship was demonstrated (correlation = 0·11, $R^2 = 0{\cdot}01$). PE_1 and PE_2 were each plotted against survival from time of biopsy (figure 2). An analysis of the relationship is shown in table 1. No relationship was found between PE_1 PE_2 or PE_2/PE_1 of metastatic melanoma tumor cells at the time of biopsy of subcutaneous or lymph node tissue and subsequent survival of the patient. Detailed studies of the growth of rat mammary adenocarcinoma cells in semi-solid medium have also demonstrated that the proliferative expression of cells in this system is not related to the ability to metastasize spontaneously [22].

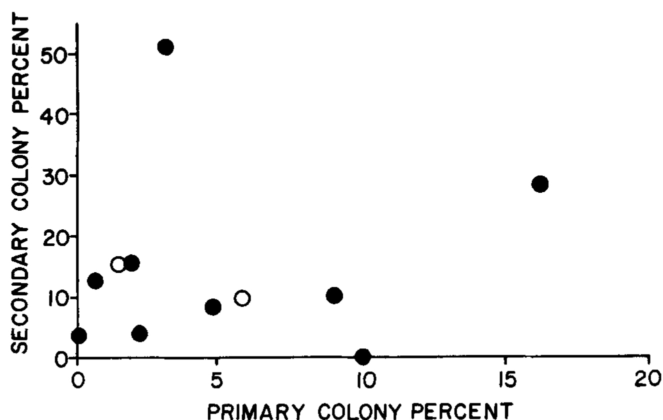

Figure 1. Relationship of frequency of primary and secondary plating efficiencies. ●, Dead; ○, alive.

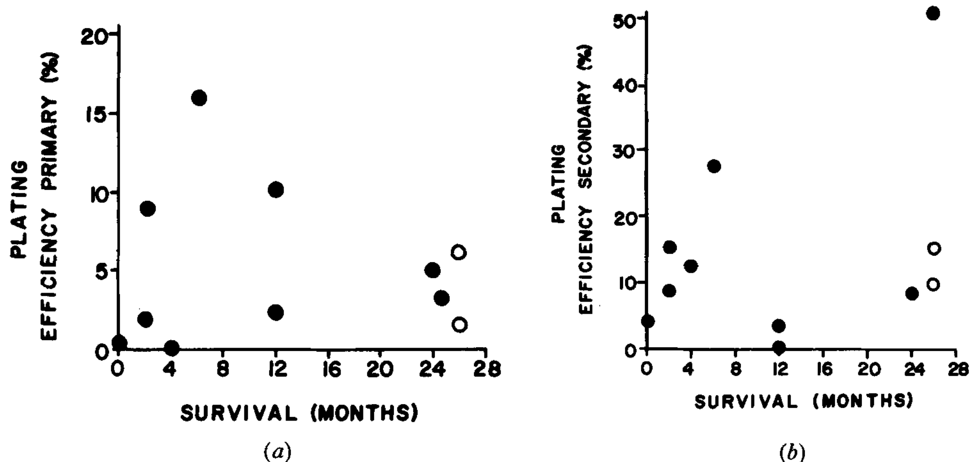

Figure 2. Plating efficiency and relation to patient survival from data of biopsy. A, Primary; B, secondary. ●, Dead; ○, alive.

Table 1. Analysis of relationship of plating efficiency to patient survival.

Variable	Coefficient	Global χ^2	P
PE_1	0·1040	1·13	0·2886
PE_2	−0·0019	0·01	0·9317
PE_2/PE_1	0·0167	0·27	0·6007

The range of PE_1 and PE_2 was large and, therefore, the failure to show a relationship was not related to a narrow spectrum of values. An alternative explanation for the results is heterogeneity of plating efficiencies among biopsy sites. In three patients we measured self-renewal plating efficiencies from two different biopsy sites. In two, the PE_1s and PE_2s were similar, whereas in the third, the PE_1s were similar but the PE_2s disparate. Many more samples would need to be tested before this alternative conclusion could be made. These results are in contrast to those reported by Buick *et al.* [4] for cells from acute myelogenous leukemia in which PE_2 (but not PE_1) was an excellent and, in fact, the best predictor of several biological properties for clinical response.

What might be responsible for these differences between tumors? McCulloch *et al.* [15] and others have demonstrated that a clonal hierarchy with a large n (number of levels of transitional cells) exists for myelogenous leukemia, mimicking its tissue of origin. In contrast, we have shown that metastatic melanoma has extensive self-renewal properties and $n = 1–2$, suggesting that there is no clonal hierarchy for cells that form colonies [21]. The melanoma cells that do not form colonies may represent the transitional cells and there may be a smaller hierarchy for melanoma with fewer transitional and other cells [19, 21]. This reflects the natural history of melanocytes in which no cellular hierarchy in tissue has been demonstrated. An alternative explanation may be that the culture system may only support subpopulations of cells which have achieved autonomous growth or extreme sensitivity to components within serum at low concentrations. This supposition cannot be proved or disproved at present.

What of other solid tumors? The self-renewal properties of cells from ovarian and bladder cancers were measured and $n = 4–6$ was calculated [11, 12, 14], but no relationship of PE_1 or PE_2 to clinical parameters has yet been published.

The present work and our other studies of the proliferative characteristics and self-renewal properties of metastatic melanoma imply the following about the growth of human melanoma cells in semi-solid conditions [3, 19, 21]: (1) the assay measures a cell capable of self-renewal; (2) PE_1 and PE_2 are unrelated; (3) PE_1 and PE_2 are not predictors for patient outcome; and (4) a limited hierarchy exists for clonogenic melanoma cells.

The results from ovarian and bladder cancer have been used to develop a stem cell model of human solid tumor growth [5, 7, 13]. This strategy has been widely cited and is being used to model drug resistance in human tumor cells. Our data suggest that the proportion of stem cells may vary greatly for different types of tumors, and clinical strategies may need to reflect these differences.

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