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CLONOGENIC CELL SURVIVAL CURVES FOR HUMAN MELANOMA XENOGRAFTS USING AGAR DIFFUSION CHAMBER AND LUNG COLONY ASSAYS

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Summary.—Studies using 2 different cloning assays to grow colonies and measure cell survival after treatment of human melanoma xenografts are reported and reviewed.

Clonogenic cell survival curves were constructed for 5 melanoma xenografts with a clinically relevant range of drugs, using a soft agar diffusion chamber assay. Cells in colonies were similar to human melanoma cells in morphology, histochemistry, ultrastructure and antigenicity and xenograft tumours could be grown from the colonies.

The survival curves were compatible with the known clinical patterns of response of malignant melanoma. The sensitivities of the xenografts correlated with the response of the same tumour in the patient when assessable.

In 2 xenograft lines, it proved possible to grow colonies in the lungs of immune deprived mice. Studies of drug sensitivity using this lung colony assay agreed closely with the soft agar assay.

It is concluded that the measurement of clonogenic cell survival can be a valuable endpoint in the assessment of the response of some xenograft tumours to therapy. The agreement between the 2 assays in which colonies were grown under widely different conditions decreases the likelihood that cells cloned represent an atypical subpopulation.

THE sensitivity of human tumour xenografts to therapy is most commonly estimated from measurements of tumour volume after treatment. However, volume changes are the result of a complex interaction between numerous factors including local oedema, host cell infiltration and removal of cell debris as well as cell death and cellular repopulation. The immune response of the host animal to the tumour may also influence tumour regrowth.

An alternative endpoint for tumour response is provided by assays which allow the growth of colonies from tumour cell suspensions. These methods may be used to construct clonogenic cell survival curves. They are less likely to be influenced by host immune responses, which may be particularly valuable in the study of xenografted tumours. Several such assays have now been described including a soft agar in diffusion chamber (ADC) assay (Smith *et al.*, 1976), an *in vitro* soft agar assay (Courtenay & Mills, 1978) and, most recently, an *in vivo* lung colony assay (Thomas, 1979).

Cloning assays have been used to study the radiosensitivity of several human tumour xenografts (Courtenay & Mills, 1976; Smith *et al.*, 1978; Guichard *et al.*, 1977) and the chemosensitivity of a few xenografts (Smith *et al.*, 1976; Courtenay & Mills, 1978). In this paper, we briefly outline the results which have been obtained using the ADC and lung colony assays to study a series of human melanoma xenografts and describe experiments in which the 2 methods have been compared.

Human melanoma xenografts

Human melanoma biopsies were implanted and serially transplanted in CBA mice, immune-deprived by thymectomy, cytosine arabinoside pre-treatment and whole body irradiation. Ten transplantable lines were studied and have been shown to retain the histology, histochemistry, antigenicity, karyotypes and ultrastructure of human melanomas. Two of these lines showed a decrease in pigmentation on serial passage and there was evidence for increasing growth rate and mitotic frequency in some lines (Selby *et al.*, 1980*a*).

Agar diffusion chamber (ADC) assay

Details of this method were given by Smith *et al.* (1976). Cell suspensions prepared from human tumour xenografts were mixed with soft agar to a final concentration of 0·3% and incubated in diffusion chambers in the peritoneal cavities of pre-irradiated C57B mice. Colonies were counted under a dissecting microscope after approximately 3 weeks incubation.

Colonies were grown from all of the 10 melanoma xenograft lines with plating efficiencies ranging from 0·04 to 75%. There was a linear relationship between the number of cells plated and the number of colonies scored for all of the lines. Plating efficiencies increased in 5 of the lines after serial passage. Cells in colonies were compatible with their tumours of origin in morphology, histochemistry, ultrastructure and antigenicity. Xenograft tumours were grown from colonies implanted back into immune-deprived mice in 2 cases (Selby *et al.*, 1979*a*, 1980*b*).

Clonogenic cell survival curves were constructed for 5 melanoma xenografts treated with melphalan, methyl CCNU, adriamycin and dacarbazine (Selby *et al.*, 1980*b*). Individual melanoma xenograft lines had individual spectra of chemosensitivity to this range of drugs and different melanomas could differ greatly in sensitivity to the same drug. Some, but not all, were resistant to methyl CCNU and dacarbazine; most were moderately sensitive to melphalan while all were resistant to adriamycin. This overall pattern of response is compatible with the known clinical behaviour of malignant melanoma. A correlation was demonstrated between the sensitivities of 2 tumours when treated with melphalan as xenografts and in the patient.

Survival curves were exponential for methyl CCNU and melphalan which is in keeping with data from animal tumours and cell lines. However, plateaus were observed at high doses of dacarbazine which was probably because of resistant sub-populations of cells in 2 tumour lines.

There was no evidence that artefacts resulting from alterations of cell yield after treatment influenced the results and assays were performed 18 h after treatment, before the onset of cell repopulation in the tumours. No changes in the susviving fractions of clonogenic cells were observed between 3 and 24 h after drug treatment.

Lung colony assay

Methodological details were given by Thomas (1979). Cell suspensions prepared from human tumour xenografts were injected into the tail veins of CBA mice immune-deprived by thymectomy, cytosine arabinoside pretreatment and whole body irradiation. Lungs were removed 3 to 4 weeks later and fixed in Bouin's fixative for 48 h. Visible lung colonies were then counted under a dissecting microscope (Fig. 1).

Colonies grew from 2 of the 6 melanoma xenografts which were tested and cloning efficiencies were approximately 1 and 0·1%. A linear relationship was observed between cells injected and colonies counted. Cell survival after treatment with methyl CCNU, melphalan, adriamycin and Cobalt-60 gamma irradiation was measured in these tumours (Thomas, 1979).

In one series of experiments, the lung

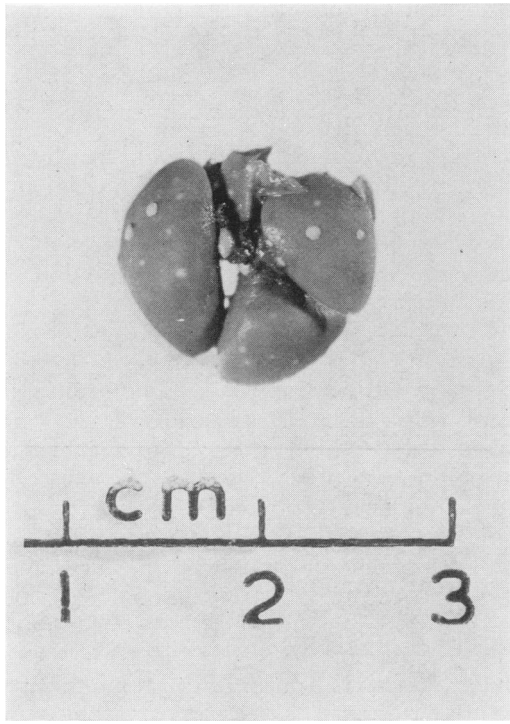

FIG. 1.—Colonies of cells from human melanoma xenograft HX 34 growing in the lungs of immune-deprived CBA mice.

colony assay was used to estimate the relative sensitivity of a melanoma xenograft (designated HX34) when the tumour was grown and treated either as subcutaneous implants or as lung colonies. It was shown that the cell survival curve for lung colonies (approx. 0·5–1 mm) treated with melphalan was steeper than that of subcutaneous tumours (approx. 2 cm). This probably reflects better drug penetration into the small lung colonies as has been shown for small xenograft tumours growing in lymph nodes (Selby *et al.*, 1979*b*). A similar difference was shown to exist for treatment with Cobalt-60 irradiation although here the apparent radioresistance of the larger tumours probably reflected their large hypoxic fractions, estimated at 65% (Thomas, 1979).

Comparison of the assays

The growth conditions for cells in any clonogenic cell assay are very different from those prevailing in the tumour *in situ*. Not only are nutritional factors different but in many assays, including the soft agar assays, supporting stroma is absent. It may be argued that the cells which form colonies in any assay are an atypical subpopulation, suited to the growth conditions of that assay but not representative of the stem cells of the whole tumour. If this subpopulation of cells were of different chemosensitivity to the whole population, then the cell survival curves would be a misleading estimate of response to treatment. This limitation is unlikely to apply when cloning efficiencies are very high, but is particularly important when only a small proportion of cells form colonies. The likelihood that assays select for atypical subpopulations of cells is probably less if similar cell survival curves result from the use of different cloning assays in which colonies are grown under widely different conditions.

In previous studies, it was shown that cell survival measurements after treatment of a human tumour xenograft were

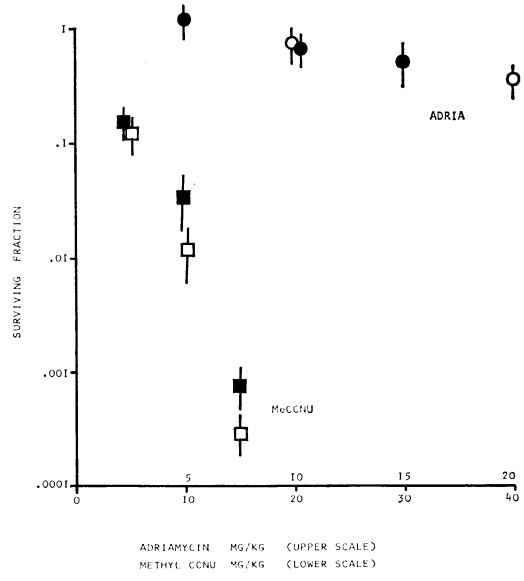

FIG. 2.—Clonogenic cell survival curves for HX34. Methyl CCNU: ■ = colonies in ADC; □ = lung colonies. Adriamycin: ● = colonies in ADC; ○ = lung colonies.

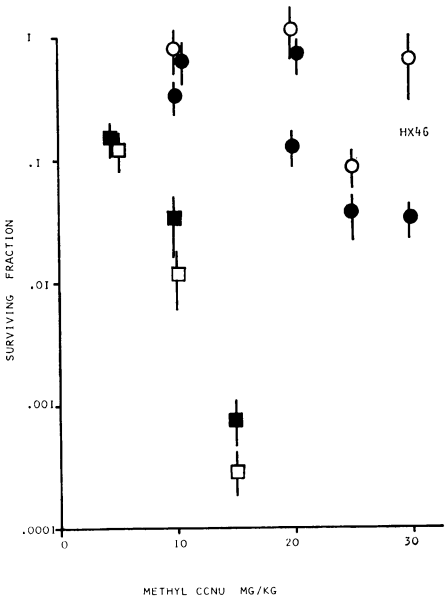

FIG. 3.—Clonogenic cell survival curves for two xenografts treated with methyl CCNU. HX 34: ■ = colonies in ADC; □ = lung colonies, HX 46: ● = colonies in ADC; ○ = lung colonies.

similar whether the colonies were grown in soft agar *in vitro* or in intraperitoneal diffusion chamber (Smith *et al.*, 1976; Smith *et al.*, 1978; Courtenay & Mills, 1978). In the present study, 2 human melanoma xenografts (designated HX34 and HX46) were treated with methyl CCNU or adriamycin and cell suspensions prepared 18 h after treatment. Surviving fractions were estimated simultaneously by growth in the ADC assay and the lung colony assay. The results of 4 experiments are shown in Figs. 2 & 3. The horizontal axes are approximately normalized to the LD_{10} dose of each drug (dose lethal to 10% of CBA mice). In HX34, the 2 assays agreed closely in experiments with methyl CCNU and adriamycin (Fig. 2). The data for HX46 treated with methyl CCNU are more scattered (Fig. 3) but no consistent difference is seen between the assays.

Conclusions

The data outlined and reported here confirm the feasibility of using clonogenic cell survival to assess the response of human tumour xenografts and suggest that useful information may be obtained in this way. The overall agreement with known clinical response patterns for malignant melanoma and the similarity between results obtained with the different assays support the value and reliability of these techniques.

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