Eur J Cancer Clin Oncol, Vol. 19, No. 8, pp. 1175–1178, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Letter to the Editor

Spontaneous Alteration in Growth Rates of Two Human Melanoma Xenografts. Concurrent Changes in Chemosensitivity

ØYSTEIN FODSTAD, EINAR K. ROFSTAD, KJELL M. TVEIT and ALEXANDER PIHL
Norsk Hydro's Institute for Cancer Research and The Norwegian Cancer Society, Montebello, Oslo 3, Norway

HUMAN tumor xenografts in athymic mice frequently increase their growth rates during the first 3–5 transplantations [1–3], probably as an adaptation to the new environment. On subsequent transplantations they have been found to retain constant growth rates, even for several years [4, 5]. However, we demonstrate here that spontaneous alterations in the growth rates of xenografts may occasionally occur and that such changes may be associated with altered sensitivity to some but not necessarily all chemotherapeutic drugs. The results emphasize that in studies on serially transplanted human tumor xenografts it is essential to monitor the growth rates, particularly when xenografted tumors are used for chemosensitivity studies.

The growth rates of two melanoma xenografts at different passages are shown in Fig. 1. It is seen that the xenograft MMX-G.E. (left panel), when small, grew almost exponentially and that later the growth rate tapered off as expected. The tumor volume doubling times (TDs) remained constant from the second to the ninth passage, corresponding to a period of 14 months. However, the growth rate of the next passage measured, P_{12}, was slightly but definitely increased and that of P_{17} was markedly increased. From then on the growth rate again remained constant, so far (P_{34}) for a period of more than 36 months. Apparently most of the change in the growth rate occurred between P_{12} and P_{17}, i.e. during a period of about 6 months. The increase in growth rate was associated with a decrease in the lag-time.

In the case of MMX-T.H. (Fig. 1, right panel) a marked change in growth rate occurred during a 3-month period (from P_6 to P_{10}). After this the growth rate remained constant, so far for a period of 22 months. In this xenograft there was a large difference in the lag-time between P_6 and P_7.

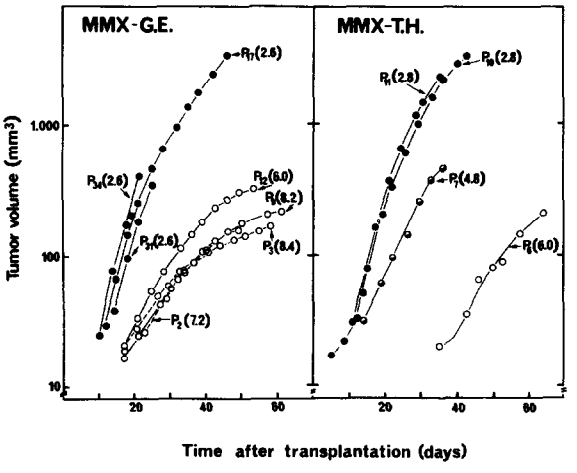

Fig. 1. Growth at different passages of two xenografted malignant melanomas. Transplantation and measurement procedures are as described in [4]. *Numbers in parentheses give the time, in days, required to double mean tumor volume* (30 → 60 mm³).

The increased growth rate of the two xenografts was not associated with substantial differences in histological and ultrastructural appearance. Morphologically dissimilar cell populations could not be discerned. In the case of MMX-G.E., where a series of examinations had been carried out also before the increase in growth rate, it was found that the enhanced growth rate was not accompanied by changes in the pattern of lactate dehydrogenase isoenzymes, the chromosome

Accepted 14 February 1983.

number (Table 1) or the fraction of clonogenic cells, as measured by a soft agar assay [6].

Cytofluorometric measurements showed (Fig. 2) that the change in growth rate of MMX-G.E. was associated with an increase in the fraction of cells in S phase. The growth parameters obtained by analysis of such data are summarized in Table 1. The fraction of cells in S phase had increased approximately 3-fold and concurrently the fraction of cells in G_1/G_0 had decreased slightly. The fraction of cells in $G_2 + M$ and the DNA content of the cells in G_1/G_0, expressed as the DNA index, were unchanged. The estimated growth fraction was about three times greater at the high than at the low growth rate [7]. Concurrently, the cell loss factor decreased appreciably. The results indicate that the observed increase in growth rate involved at least these two factors.

Table 1. Characteristics of the malignant melanoma xenograft MMX-G.E. before and after increase in growth rate

	Before	After
Doubling time *in vivo* (days)*	8.0	2.6
Cells in G_1/G_0 (%)	80	70
Cells in S (%)	5	16
Cells in G_2 + M (%)	15	14
Growth fraction†	0.16; 0.20	0.56; 0.71
Cell loss factor†	0.70; 0.79	0.12; 0.37
DNA index‡	2.9	2.9
Chromosome number: modal	43	46
range	29–58	37–54
Plating efficiency in soft agar§	0.6–0.8	0.4–0.8

*Time required to double volume of tumor of 30 mm^3 [4].
†The figures were calculated on the basis of cell cycle parameters, previously measured on a slow-growing and a fast-growing melanoma xenograft respectively [7].
‡DNA index of diploid cells = 2.0.
§Percentage of viable cells plated.

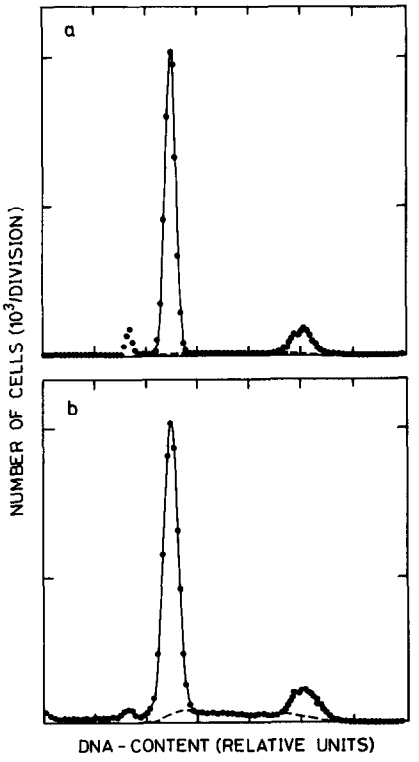

Fig. 2. DNA histograms of the melanoma xenograft MMX-G-E- before (a) and after (b) change in growth rate. The first, small peak is due to non-parenchymal mouse cells.

In the case of the second melanoma, MMX-T.H., a histogram obtained after the growth change exhibited two distinct peaks, suggesting the existence of at least two different tumor cell populations.

Since we have measured in an early passage the sensitivity of MMX-G.E. to four cytostatic drugs, the spontaneous increase in growth rate offered an opportunity to study in one specific tumor line the relationship between growth rate and chemosensitivity. The effect of DTIC on the growth rate of early (P_3) and late (P_{32}) passages is shown in Fig. 3. It is evident that the fast growing, late passage was significantly more sensitive to DTIC than was the early passage. Similar data were obtained with CCNU (Table 2). These results are consistent with the finding that DTIC and CCNU act more strongly on proliferating than on normal resting bone marrow cells [8, 9].

Xenograft MMX-G.E. responded poorly to vinblastine. On the basis of its mechanism of

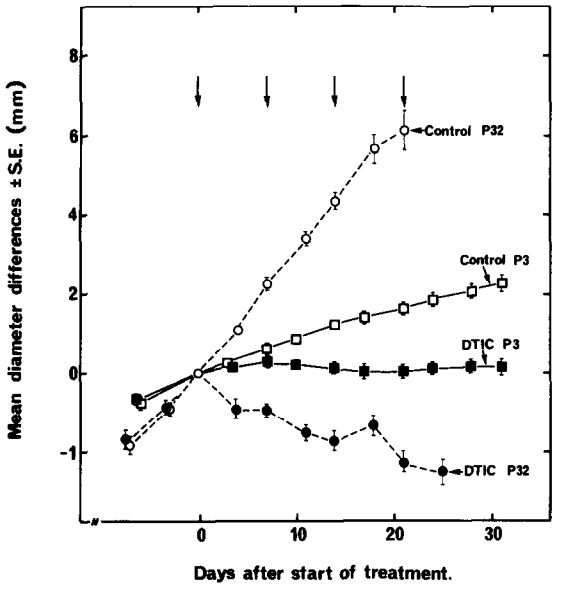

Fig. 3. The effect of DTIC on the growth of MMX-G.E. examined at an early (P_3) and a late (P_{32}) passage in athymic mice. The treated and the control groups of mice consisted of 8–10 animals. Each symbol represents the mean diameter difference ±S.E. of the tumors, as compared with the mean diameter at the start of treatment. DTIC (250 μg/kg i.p.) was given at the times indicated by the arrows.

Table 2. Chemosensitivity of the malignant melanoma xenograft MMX-G.E. before and after increase in growth rate

Drug*	Growth delay† Before	After
CCNU	2.7	>6.0
DTIC	2.6	>7.5
Vinblastine	0.5	0.7
Abrin	1.6	1.5

*The doses and schedules were as in [4].

†$\frac{TD_{treated} - TD_{control}}{TD_{control}}$, estimated as in [4].

action, vinblastine might be expected to have a stronger effect when the growth rate and the growth fraction of the xenograft are increased. This was, however, not the case (Table 2). The results indicate that the cells were inherently resistant to vinblastine and that their response to this drug was not influenced by the increase in growth rate. Likewise, no increase in the sensitivity of the xenograft to the protein synthesis inhibitor abrin occurred when the growth rate increased. This is consistent with our previous finding that normal proliferating bone marrow cells were no more sensitive to abrin than were normal resting bone marrow cells [10].

In the case of the second melanoma, MMX-T.H., the chemosensitivity had not been measured before the increase in growth rate. Measurements after the increase showed that this xenograft, while resistant to DTIC and abrin, was now exceptionally sensitive to CCNU and vinblastine. The sensitivities to CCNU (growth delay >9.8) and to vinblastine (growth delay >6.6) are the highest ones observed in our laboratory so far. The results suggest that also in this case the increase in growth rate had resulted in changes in the chemosensitivity.

These results emphasize that although the growth rate of tumors influences their sensitivity to some drugs, other factors also affect the sensitivity and may prevail over the growth rate. This is also evident from a previous study where we compared the sensitivity of *different* tumors to various drugs. Thus we found that for the five melanoma xenografts examined, the sensitivity to CCNU, DTIC and procarbazine was actually inversely related to their growth rates [11].

It is clinically accepted that cancers frequently alter their growth rates. The explanation usually offered is that such changes involve a selection of a subpopulation of tumor cells, caused by host factors or by the treatment. In the experiments reported here there was no positive evidence that a selection of a subpopulation had taken place. The spontaneous growth change observed suggests the possibility that such changes in patients' tumors may also sometimes be spontaneous and not necessarily induced by extra-tumoral factors.

Acknowledgements—The authors wish to thank Drs Jan Vincents Johannessen and Tore Lindmo for valuable help and advice. The technical assistance of Unni Rønning and Anne Due is gratefully acknowledged.

REFERENCES

1. HOUGHTON JA, TAYLOR DM. Growth characteristics of human colorectal tumours during serial passage in immune-deprived mice. *Br J Cancer* 1978, **37**, 213–223.
2. MATTERN J, WAYSS K, HAAG D, TOOMES H, VOLM M. Different growth rates of lung tumours in man and their xenografts in nude mice. *Eur J Cancer* 1980, **16**, 289–291.
3. SELBY PJ, THOMAS JM, MONAGHAN P, SLOANE J, PECKHAM MJ. Human tumour xenografts established and serially transplanted in mice immunologically deprived by thymectomy, cytosine arabinoside and whole-body irradiation. *Br J Cancer* 1980, **41**, 52–61.
4. FODSTAD Ø, AASS N, PIHL A. Assessment of tumour growth and of response to chemotherapy of human melanomas in athymic, nude mice. *Br J Cancer* 1980, **41** (Suppl. IV), 146–149.
5. POVLSEN CO, VISFELDT J, RYGAARD J, JENSEN G. Growth patterns and chromosome constitutions of human malignant tumors after long-term serial transplantation in nude mice. *Acta Pathol Microbiol Scand Sect A* 1975, **83**, 709–716.
6. TVEIT KM, FODSTAD Ø, OLSNES S, PIHL A. *In vitro* sensitivity of human melanoma xenografts to cytotoxic drugs: correlation with *in vivo* chemosensitivity. *Int J Cancer* 1980, **26**, 717–722.
7. ROFSTAD EK, FODSTAD Ø, LINDMO T. Growth characteristics of human melanoma xenografts. *Cell Tissue Kinet* 1982, **15**, 545–554.
8. STEEL GG, STEPHENS TC. The relation of cell kinetics to cancer chemotherapy. In: ADOLPHE M, ed. *Advances in Pharmacology and Therapeutics*. Oxford, Pergamon Press, 1978, Vol. 10, 137–145.

9. VALERIOTE F, VAN PUTTEN L. Proliferation-dependent cytotoxicity of anticancer agents: a review. *Cancer Res* 1975, **35**, 2619–2630.
10. FODSTAD Ø, PIHL A. Synergistic effect of ricin in combination with daunorubicin, *cis*-dichlorodiammineplatinum (II) and vincristine in systemic L1210 leukemia. *Cancer Res* 1982, **42**, 2152–2158.
11. FODSTAD Ø, AASS N, PIHL A. Response to chemotherapy of human malignant melanoma xenografts to cytotoxic drugs. *Int J Cancer* 1980, **26**, 717–722.