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Characterization of Human Soft-Tissue Sarcoma and Malignant Glioma Xenografts with Particular Reference to Spontaneous Changes During Serial Passages

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Introduction

Transplantation of malignant human tumors into immunodeficient nude mice is a good experimental model for studying the characteristics and growth behavior of these tumors. A prerequisite for reliable investigations is the stability of the biological characteristics of the human xenografts during serial passages.

Materials and Methods

Over a ten-year period between October 1980 and May 1990, 144 human soft-tissue sarcomas and 273 malignant gliomas were transplanted into nude athymic mice at the Department of Radiation Oncology, University of Essen, FRG. The soft-tissue sarcomas contained the histologic subgroups: 29 liposarcomas, 28 malignant fibrous histiocytomas, 21 leiomyosarcomas, 12 spindle-cell sarcomas, 11 neurofibrosarcomas, 10 chondrosarcomas, 7 synovial sarcomas, and 26 other or unclassified sarcomas. In the malignant glioma group, there were 72 astrocytomas grade III/IV and glioblastomas, 61 astrocytomas grade III, 34 astrocytomas grade II, 21 oligodendrogliomas grade III, and 85 other or unclassified gliomas. To characterize the tumor lines, we used histologic examinations, flow cytometry, electrophoretic enzyme analysis of the lactate dehydrogenase and glucose-6-phosphate dehydrogenase, and determination of the tumor volume-doubling times.

The "take rate" was defined as the proportion of tumor lines that grew once on nude mice, the "establishment rate" was defined as the proportion of tumor lines that showed progressive growth in nude mice for a minimum of three passages.

Results

Soft-Tissue Sarcoma

For soft-tissue sarcomas we found a take rate of 54% and an establishment rate of 35%, i.e., 50 out of 144 tumors were established in nude mice. The histological grading of the tumors played an important role for establishment in nude mice. Depending on the grading, there was an increasing establishment rate. G1, G2 and G3 tumors were established in nude mice in 4%, 26% and 52% of the transplants studied, respectively, so we could see a selection for undifferentiated tumor lines. The histologic subgroup did not play a marked role in this respect; a preference was observed for neurofibrosarcomas (establishment rate 55%), malignant fibrous histiocytomas (50%), and leiomyosarcomas (43%) (table 1).

The DNA content was euploid in 30% of the soft-tissue sarcoma xenografts studied, and aneuploid in 70%. Changes in the DNA index were seen more frequently in the aneuploid group; a change rate of 30% was found, with a median in the 15th passage.

Table 1. Soft-tissue sarcoma. Establishment rate according to histologic subgroup

Histologic type	Total (n)	Establishment rate	
		n	%
Neurofibrosarcoma	11	6	55
Malignant fibrous histiocytoma	28	14	50
Leiomyosarcoma	21	9	43
Liposarcoma	29	10	35
Spindle-cell sarcoma	12	4	33
Synovial sarcoma	7	2	29
Chondrosarcoma	10	2	20
Others and unclassified sarcoma	26	3	12
Total	144	50	35

No correlation was found between tumor volume-doubling times and the DNA index. For a leiomyosarcoma, for instance, an increase in the tumor volume-doubling time was seen after the 24th passage, but the DNA index was unchanged up to the 48th passage (fig. 1). A similar situation was observed for a xenografted malignant fibrous histiocytoma with changing tumor volume-doubling times parallel to a constant euploid DNA content. Electrophoretic analyses of the lactate dehydrogenase and the glucose-6-phosphate dehydrogenase showed a loss of the LDH 1 and 2 band in 46% and a loss of the human glucose-6-phosphate dehydrogenase band in 36% of the soft-tissue sarcoma lines.

Malignant Glioma

For malignant gliomas, the take rate and establishment rate were much lower as compared to the soft-tissue sarcoma group: 19% and 7%, respectively. Depending on the grade of differentiation, we found decreasing establishment rates with decreasing grades of malignancy: for glioblastomas, gliomas III to IV, and astrocytomas III to IV together an establishment rate of 19% was observed, whereas the other subgroups showed lower rates (table 2). The two grade-II astrocytoma lines could not be established permanently in nude mice; after the third and seventh passages, growth of the tumor lines ceased.

Initially, 86% of the xenografted tumor lines were euploid and 14% aneuploid. Changes in the DNA content were seen in the euploid sub-

Table 2. Malignant glioma. Establishment rate according to histologic subgroup

Histologic type	Total (n)	Establishment rate	
		n	%
Astrocytoma III/IV Glioblastoma	72	14	19
Astrocytoma III	61	2	3
Astrocytoma II	34	2	6
Oligodendroglioma III	21	1	5
Others and unclassified glioma	85	0	0
Total	273	19	7

group only, the change rate being 36%, with a median in the 15th passage. The DNA index of a xenografted human astrocytoma III/IV, for instance, showed a spontaneous change from diploid to tetraploid after the 25th passage, which did not result in any change in the tumor volume-doubling time (fig. 2).

Loss of the lactate dehydrogenase 1 and 2 band and loss of the human glucose-6-phosphate dehydrogenase band was observed in 17% and 0%, respectively.

For all transplanted tumor lines (soft-tissue sarcomas and malignant gliomas), examinations of the correlation between the tumor volume-doubling time and the DNA index, the tumor volume-doubling time and grading, and the DNA index and grading, showed no significance. A change in the biological characteristics occurred mostly in higher passages, and up to the tenth passage about 84% of the transplanted tumor lines appeared stable with regard to the criteria mentioned above.

Discussion and Conclusions

During serial passages, spontaneous changes in the human characteristics and the tumor volume-doubling times were observed. These findings are in agreement with the results of other investigators [1-3], although other reports in the literature show no or only sporadic changes in the growth behavior of the xenografted tumors [4-6].

In our experience with xenografted human soft-tissue sarcomas and malignant gliomas in nude mice, changes in the biological characteristics of these tumors during serial passages were proved especially above the fifteenth passage. This indicates that after a few passages, or before any experiment with xenografted human tumors, regular controls and determinations of the biological characteristics are necessary in order to check routinely for the presence of human tissue in an animal model such as the nude mouse.

We recommend tumor transplantation into previously total body-irradiated nude mice, characterization in the third passage, repetition of these characterization studies after three passages (especially before planning an experiment), starting experiments not before the fourth passage and repeated cryoconservation every third to fifth passage in order to obtain a stem of unchanged tumor lines for subsequent experiments.

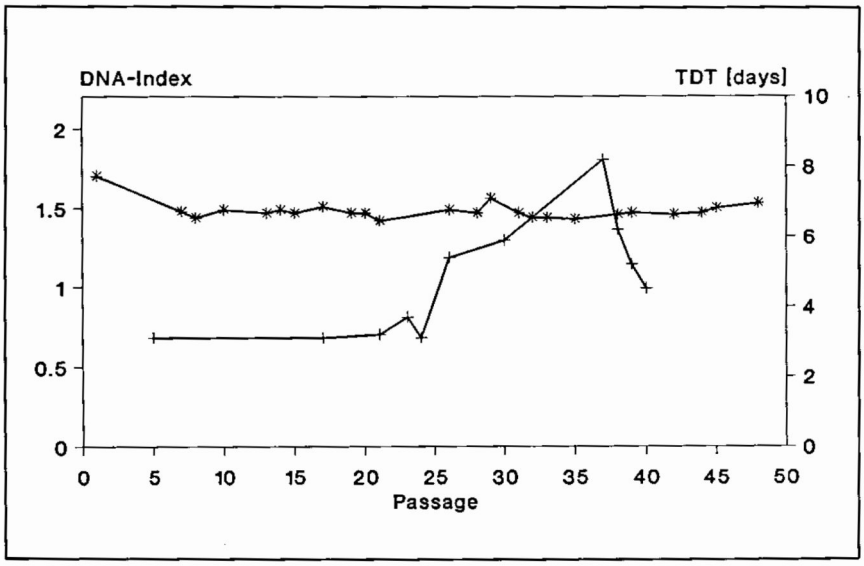

Fig. 1. DNA index and tumor doubling time for serial passages of a leiomyosarcoma.

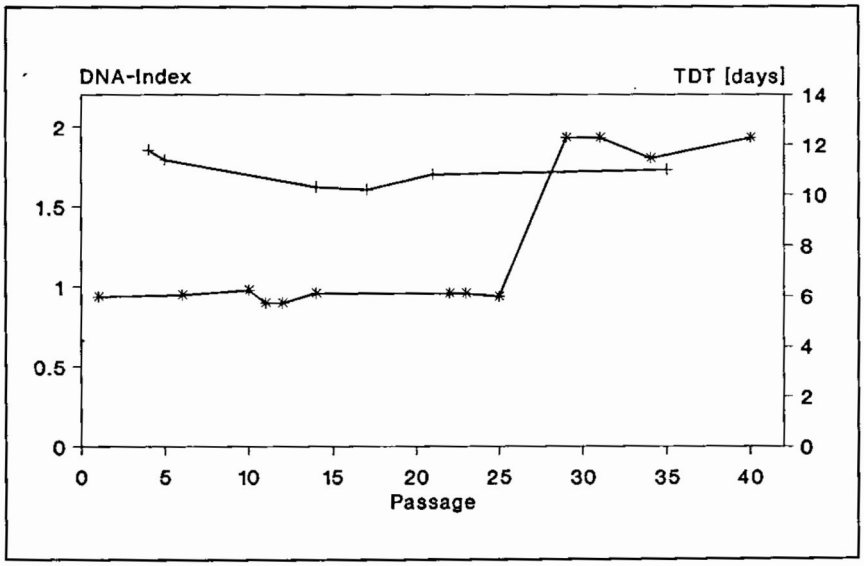

Fig. 2. DNA index and tumor doubling time for serial passages of an astrocytoma III/IV.

Summary

Between 1980 and 1990, 144 human soft-tissue sarcomas and 273 malignant gliomas were transplanted into nude athymic mice at the Department of Radiation Oncology, University of Essen. Histologic examinations, tumor volume-doubling times, flow cytometry, and electrophoretic enzyme analysis of lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (GPD) were used to characterize the tumor lines. For soft-tissue sarcomas and malignant gliomas, we found an establishment rate of 35% (50/144) and 7% (19/273), respectively, with a preference for high-grade tumors. Referring to the criteria mentioned above, 32% and 44% of the established soft-tissue sarcoma and glioma lines showed changes in tumor characteristics mainly in higher passages, with a median in passage 15 for both tumor entities. However, up to the tenth passage, about 84% of the transplanted tumor lines appeared stable. For experiments using human tumor xenografts, routine determinations of the biological characteristics of the tumor lines are necessary.

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