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A PRELIMINARY EXPERIMENT ON REGRESSION OF HUMAN OVARIAN TUMOR TRANSPLANTS IN ATHYMIC NUDE MICE FOLLOWING A SINGLE EXPOSURE TO ^{60}Co RADIATION

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The thymus defective, nude mouse mutant (FLANAGAN 1966) offers promising possibilities in several aspects of cancer research. This mouse mutant is both hairless and athymic. Due to lack of thymus, the T-cell compartment of the immune apparatus is absent, and the animals do not reject implants of foreign tissue. Malignant tissue from foreign species as well as normal skin grafts have been grown successfully by several investigators (RYGAARD 1973).

At Norsk Hydro's Institute for Cancer Research and The Norwegian Radium Hospital we have been experimenting with implants of various types of human tumors obtained directly from the operating room of the hospital. In the present introductory investigation emphasis is placed on development of technical procedures for utilization of the nude mouse in studies of radiation effects on human tumor implants under physiologic growth conditions. As these mice lack hair, the growth of subcutaneously implanted tumors can be followed and measured with a reasonable degree of accuracy, and they can relatively easily be treated with local irradiation.

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The present work is also aimed at providing the necessary foundation for a full-scale experiment on the time course of regression of human ovarian tumor transplants following a single, local exposure to ^{60}Coradiation.

Material and Methods

Tumor pathology. The tumor used (AN–LE) was a poorly differentiated adenocarcinoma of the ovary, taken at operation from a 55-year-old postmenopausal woman, suffering from ovarian cancer, stage IV. She had received no previous therapy.

Pathology and ultrastructure of this tumor are described fully in a report on the response to chemotherapy of AN–LE transplants in nude mice (DAVY & MOSSIGE, to be published).

Mice. The animals were purchased from Gamle Bomholtgård, Denmark. This nude strain is in the process of being backcrossed to a BALB/c/A/Bom background. The animals are kept in a separate room at 27°C with automatically regulated 12 hour dark and light periods, under conventional, but very strict conditions. Viability is variable and about half of the mice survive for several months.

Implantation and measurement of tumor growth. Tumor tissue from the operating theatre was transferred directly to the animal house where representative pieces were cut into small bits (ca 1 to 2 mm^3) and implanted subcutaneously on the dorsal surface of the mouse. After a lag period of two months the implants began to grow. Once a week the tumor cross-section of individual animals was measured by means of calibrated calipers.

When the tumors had attained a cross-sectional area of about 75 mm^2, some of the animals were killed and their tumor tissue reexamined histologically. AN–LE has been maintained in six passages to date. Six female mice from the third passage were used in this preliminary investigation.

Irradiation procedure, radiation quality and dosimetry. The animals were not anaesthetized during irradiation, to avoid affecting the oxygen supply to, and hence the radiation response of, the tumor tissue.

Thin-walled perspex mouse holders (4), were made (Fig. 1) the internal diameter of which just allowed the mouse to enter. A large hole (8) was made in the cranial end of the tube so the animal could breathe freely. A piston arrangement in the 'tail'-end (6, 7) positioned the animal firmly in the holder. By inserting a wad of cotton-wool (5) in the holder just opposite the tumor, the latter was made to protrude through an opening in the tube (9), cut to fit the tumor region.

A 5 000 Ci ^{60}Co therapy unit (TEM, Mobaltron 80) was used as radiation source (1). The tumors were irradiated at a focus-skin distance (FSD) of 80 cm and positioned

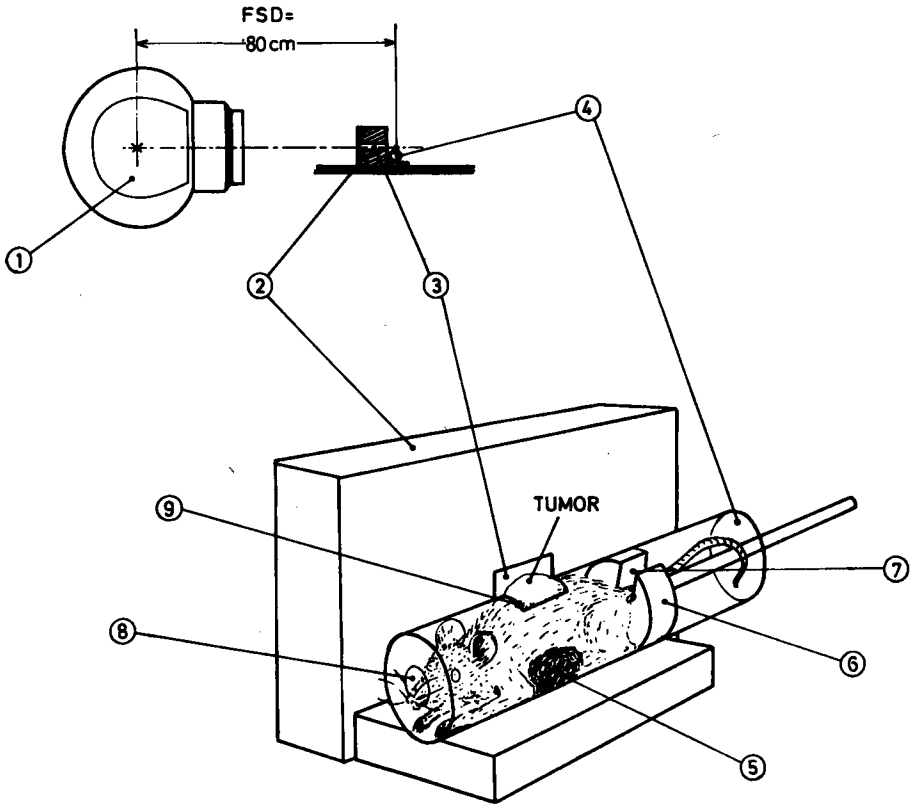

Fig. 1. Set-up for irradiation of tumor transplants in nude mice. The mouse is placed in a thin-walled perspex tube (4). A hole in the cranial end of the tube (8) allows the mouse to breathe freely. A piston arrangement (6) with a slit to fit the tail (7) immobilizes the mouse in the holder. A wad of cotton-wool (5) just opposite the tumor makes the tumor protrude out of an opening (9). A ^{60}Co Mobaltron (1) aligned horizontally irradiates the mouse locally at a FSD = 80 cm through a 7 mm × 12 mm aperture (3) in an 8 cm thick lead block (2).

for irradiation, as shown in Fig. 1, by means of the beam defining light of the therapy unit. A 7 mm × 12 mm brass-lined hole (3) through an 8 cm thick lead block served as a beam defining aperture (2).

The depth dose distribution for the 7 mm × 12 mm radiation field at FSD = 80 cm was determined from ionization chamber measurements in a tissue equivalent perspex phantom. Measurements made with thermoluminescence discs (TLD 100 LiF, Ribbon, Harshaw) placed at the ionization maximum, varied within a few per cent of the corresponding ionization chamber readings of 105 rad/min. The homogeneity of the dose distribution across the 7 mm × 12 mm radiation field was demonstrated by densitometer readings of exposed films. The dose outside the radiation field was of the order of 0.5 per cent.

Since the radiosensitivity of the skin of these animals is unknown, and furthermore,

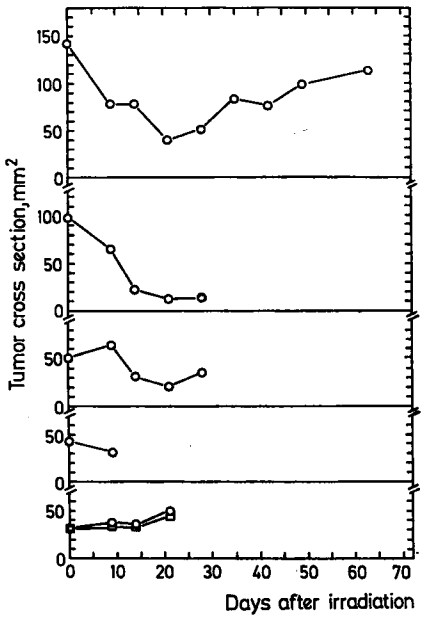

Fig. 2. Tumor cross section plotted as a function of the time (days) elapsed after irradiation. From top to bottom: 1 575, 1 180, 750 and 375 rad and unirradiated controls.

since the aim of the present introductory experiment was to study tumor regression following large single doses, it was considered desirable to achieve a certain reduction of the dose to the skin relative to that applied to the tumor. Although dosimetry in depths less than that corresponding to ionization maximum poses problems, this part of the depth dose distribution was utilized to provide a skin sparing effect.

The tumors were irradiated by applying two oppositely directed, tangential radiation fields (see Fig. 1). Dose distribution patterns in the tumors, calculated from the measured depth dose curve, showed the surface dose of the tumors to be approximately 70 per cent of the maximum dose delivered in the center of the tumor.

Results and Discussion

The tumors of four animals were irradiated. The average doses to the entire tumor were calculated to be 375, 750, 1 180 and 1 575 rad, respectively. The tumors in two animals were not irradiated and served as controls. The changes in the tumor cross sections of unirradiated controls and of irradiated tumors were recorded weekly as a function of the time elapsed after irradiation.

No complications due to radiation-induced skin reactions or other side effects were noted during the time period the animals were studied following irradiation. As a consequence, in our subsequent work tumor transplants are being irradiated

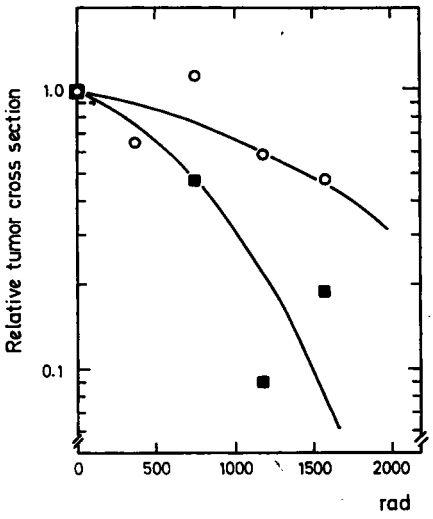

Fig. 3. Relative tumor cross sections plotted as a function of dose at two different times after irradiation, viz. 9 (○) and 21 (■) days.

in one tangential field, behind an aqueous 'bolus' which positions the entire tumor at depths equal to or greater than that of the ionization maximum.

The animal whose tumor was given a dose of 375 rad died on the 10th day after irradiation. The mouse treated with 1 575 rad was followed over a period of two months after irradiation.

Fig. 2 shows the changes in the tumor cross sections of irradiated tumors and unirradiated controls as a function of the time elapsed after irradiation.

These data suggest: (1) Single exposure of AN–LE transplants results in regression of the tumors, particularly for doses exceeding 1 krad. (2) Tumor regression, following a dose of 750 rad or more, appears to continue over a period of about 3 weeks. (3) For times in excess of about 3 weeks following a 1 575 rad exposure the tumor cross section increases steadily.

The data presented in Fig. 2 are insufficient to allow conclusions to be drawn about how the relative growth rate of the tumor cross section depends on the absolute tumor size. On the assumption that the relative growth rate as derived from the control tumours is representative for all tumor sizes dealt with in the present work, the data presented in Fig. 2 may be used to calculate relative tumor cross sections for given doses and given times after irradiation.

Such dose-response curves are presented in Fig. 3. Here relative tumor cross sections are plotted as a function of dose, for two given times after irradiation, viz. 9 and 21 days. Curves of this nature demonstrate clearly the potentiating effect of dose as well as of the time elapsed after irradiation. However, it seems more important that curves of this nature, when based on a full-scale experiment, may shed light on the kinetics involved in the tumor regression following irradiation, and whether or not the overall kinetics depend on the time elapsed after irradiation.

Acknowledgements

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SUMMARY

Human ovarian tumor transplants (AN–LE) are grown in the thymus defective nude mouse mutant (nu/nu BALB/c/A/Bom). Procedures for local irradiation of the tumors with ^{60}Co-radiation are described. An introductory test of the usefulness of these transplants in studies of radiation effects on human tumors is performed by investigation of the time course of regression of AN–LE transplants in 6 mice, following single exposures to 375, 750, 1 180, and 1 575 rad, respectively.

ZUSAMMENFASSUNG

Transplantate menschliche Ovarialtumoren (AN–LE) wurden in Thymus defizienten „nude" Maus Mutanten (nu/nu BALB/c/A/Bom) implantiert. Die Methoden für Lokalbestrahlung der Tumoren mit ^{60}Kobalt werden beschrieben. In einem einführenden Versuch wird der Nutzen dieser Transplantate für Studien über Strahleneffekte auf menschliche Tumoren untersucht. Dazu wird der zeitliche Verlauf der Regression von AN–LE Transplantaten in 6 Mäusen nach Einzelexponierung auf 375, 750, 1 180 oder 1 575 rad untersucht.

RÉSUMÉ

Des transplants de tumeur ovarienne humaine (AN–LE) se sont développés dans des souris mutantes nues à thymus anormal (nu/nu BALB/c/A/Bom). Les auteurs décrivent la technique d'irradiation locale des tumeurs avec le rayonnement du ^{60}Co. Ils ont fait une expérimentation préliminaire concernant l'utilité de ces transplants pour l'étude des effets des radiations sur les tumeurs humaines en étudiant l'évolution, en fonction du temps, de la régression des transplants AN–LE sur 6 souris après des expositions uniques à 375, 750, 1 180, et 1 575 rad respectivement.

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