Transplantable Line of Amelanotic Hamster Melanoma Resulting from Abrupt Depigmentation

Several lines of transplantable hamster melanomas have been described during the last decade[1]. Among these were 2 lines obtained in this laboratory from DMBA-induced amelanotic and heavily pigmented melanoblastomas[1]. Tissue from the seventeenth transplant generation of the pigmented tumour was inoculated s.c. into 4 old female and 1 young adult male hamsters. 8 days later the transplanted tumour became palpable in the male animal only. The nodule grew rapidly and reached a size of 1 × 1.5 cm at a time when the transplants in the female hamsters were still hardly noticeable. At autopsy 2 small intensely black tumours and another, much larger, whitish growth with black pigmentation at one pole were detected in the male animal. Pale fragments from the whitish tumour were successfully transplanted and a new transplantable subline was established.

Materials and methods. The biological and morphological properties of the new tumour line were studied in more than 150 male and female animals from our colony of non-inbred Syrian hamsters (*Mesocricetus auratus*). The transplantations were made s.c. or i.p. with viable tumour tissue 1–1.5 mm in diameter. The latency, takes, rate of tumour growth and time of ulceration, as well as behaviour and deaths of animals were registered weekly or daily for representative groups. All tumours were examined microscopically after fixation in 10% neutral formalin and the fixatives of LILLY and ZENKER. Paraffin slides were stained with hematoxylin-eosin, Azan, GÖMÖRI's and PAS reactions. Fresh viable tissues from the tumour were incubated in L-tyrosine[2] and D,L-DOPA[3] with subsequent staining for melanin[4].

Results. Some properties of the new tumour line are listed in the Table and compared with the properties of the original pigmented melanoma.

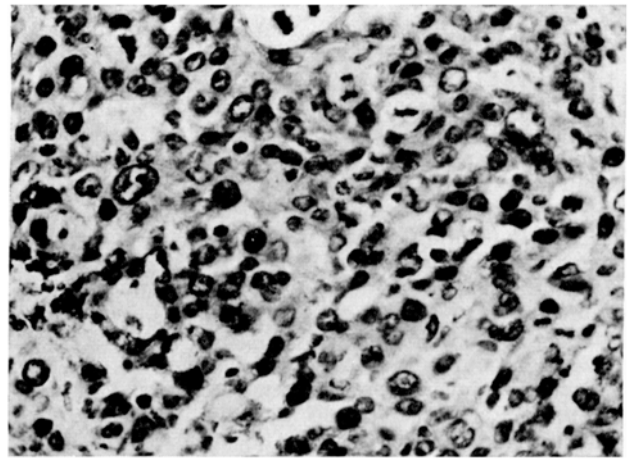
Microscopically there was a tendency towards the retaining of the alveolar pattern of the pigmented line, although the diffuse pattern was more frequent (Figure). The tumour cells possessed an oval or rounded form with weakly stained cytoplasm which contrasted with the dark nuclear sap, containing coarse chromatin granules and 1–3 prominent nucleoli. Giant mononuclear and multinuclear cells were sparsely distributed. Mitoses in the new line were frequent as compared to those of the primary tumour. Stromal collagenous and reticuline fibres surrounded the cell nests while in the diffuse tumours these were scarce. The number of the stromal cells varied according to the extent of inflammation and necrosis. A few capillaries and many lymphatics could be detected in all tumours examined.

Discussion. Depigmentation of spontaneous melanomas during their transplantation has been observed in mice[5,6] and hamsters[7,8]. Our report indicates that depigmentation may occur also in DMBA-induced melanomas. The abrupt depigmentation described here appears to be rather exceptional since the amelanotic lines, in most cases, were obtained after long-term selection of weakly pigmented areas[5–8]. The negative tyrosinase and DOPA oxidase reactions, as well as the loss of melanin was followed by a pronounced acceleration of the growth and mitotic rate, shortening of the latent period and animals survival time after transplantation. The question whether depigmentation is followed by some further qualitative changes in the tumour is still open to discussion. FORTNER et al.[9] have reported that pigmented melanomas differed from their amelanotic descendants by the presence of melanin only. Results on GREENE's melanoma[7] have confirmed this view, but some authors[5,8] found various changes such as an acceleration of the growth rate after depigmentation. Data on the activity of melanin-forming enzymes in animal melanomas[10] indicate that most of these tumours

Some properties of s.c. transplanted melanoma line in syrian hamsters before and after abrupt depigmentation[a]

Tumour properties	Melanotic line	Amelanotic line
Takes (%)	85–90	100
Latency. Mean ± S.D. (days)	16.5 ± 2.1	4.88 ± 0.75
Tumour weight. Mean ± S.D. (g)[b]	0.217 ± 0.06	18.86 ± 4.45
Animal survival time	2.5–3.5 months	25–35 days
Metastases	Lymph nodes, pulmo	None detected
Pigment	Abundant	None visible
DOPA-oxidase reaction	Strongly positive	Negative
Tyrosinase reaction	Strongly positive	Negative

[a] During the first to the thirty-ninth transplant generation of the pigmented line and amelanotic subline. [b] 10 animals/group with transplants from the thirty-ninth generation at the twenty-second day after transplantation.



Pattern and cytology of a typical tumour from the new line. Hematoxylin-eosin staining.

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were tyrosinase and DOPA-oxidase negative after depigmentation.

In conclusion, the tumour described here represents an interesting example of tumour progression subsequent to abrupt depigmentation of a heavily pigmented melanoma.

Zusammenfassung. Im Anschluss an die plötzliche Depigmentation eines stark pigmentierten Transplantationsmelanoms des Goldhamsters wurde ein neuer pigmentloser Tumorzellstamm erhalten. Er befindet sich in seiner 39. Transplantationsgeneration. Der Tumor dieses Stammes läuft bei allen Tieren nach einer Latenzperiode von 4–5 Tagen an und führt auf Ende des ersten Monats zum Exitus. Die mikroskopische Struktur ist durch alveoläre und diffuse Anordnung von ovalen und runden, pigmentlosen Zellen und viele Mitosen charakterisiert. Zytochemisch zeigen die Tumorzellen negative DOPA-Oxydase und Tyrosinase-Reaktionen.

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Enhancement of Radioresistance in Mice Simultaneously Exposed to Dimethyl Sulfoxide Vapor and X-Irradiation

Dimethyl sulfoxide (DMSO) has been well recognized as a good radioprotective agent[1,2]. In a recent report[3], it was shown that topical applications of DMSO to mice prior to lethal amounts of X-irradiation offer considerable protection. This drug also influences the post-irradiation avoidance behavior of these animals[4]. In all these experiments, the experimental subjects either received the drug orally, by i.p. injection, or by contact with their skin. Increasing interests on these results encouraged us to test the effect of DMSO vapor on the radiosensitivity of mice[5,6]. This report deals with another aspect of the radioprotective effect of DMSO vapor.

Methods. CF_1 mice, 50–60 days old, were used. Each group of 60 animals were housed in standard plastic cages (10 mice in a cage). The experiment started about 1 week after the mice arrived from the supplier. Irradiation techniques were the same as described in references [5] and [6]. The total dosages received by the mice varied between 750–900 R (at approximately 80–85 R/min) in the first experiment.

In the second experiment, 3 main experimental procedures were followed: the animals were exposed to DMSO or H_2O vapor during irradiation only, 10 min before irradiation, or 10 min after irradiation.

Each experimental procedure was repeated 3 consecutive times. Each time 120 mice were divided into 2 groups, 60 DMSO and 60 water treated. The water-treated animals were exposed to 850 R versus 900 R for the DMSO treated mice; thus the reliability for comparing mortality in 2 groups increased considerably. Mortality observations took place daily until all the mice in the control group treated with water were dead.

Results and discussion. Sham-irradiated but DMSO or water-treated control mice showed no mortality during the experiment, regardless of the duration or time of their exposures to the vapors. For the first experiment, Figure 1 shows the survival percentage of 2 groups of 60 mice each exposed to DMSO or H_2O vapors and X-rays simultaneously. The DMSO mice were exposed to 900 R whole-body while the control water group was irradiated with only 850 R. Highest mortality was observed between the sixth and eleventh post-irradiation days. Statistical significance of the results was obvious between the seventh day ($0.02 < p < 0.05$) and the eighth day post-irradiation ($0.01 < p < 0.02$). In Figure 2 (second experiment), the curves represent the daily survival of 3 groups of mice (60 in each) exposed to DMSO vapor either during (group I), 10 min before (group II), or 10 min after (group III) radiation exposure. The control water group was exposed to water vapor during irradiation only. Total radiation dose for all 4 groups was 750 R. All the mice in the water-treated control group were dead by the eleventh day after irradiation while 88% of the first group, 66% of the second group, and 39% of the third group still survived. It is clear from these results that the protective effectiveness was highest when the animals were exposed simultaneously to DMSO vapor and irradiation and lowest when the exposure to the drug vapor took place after irradiation. While other investigators did not indicate any effect on mortality of mice treated with DMSO after irradiation, we observed that the protection of DMSO vapor was still quite pronounced when the drug was applied even after radiation exposure (Figure 2).

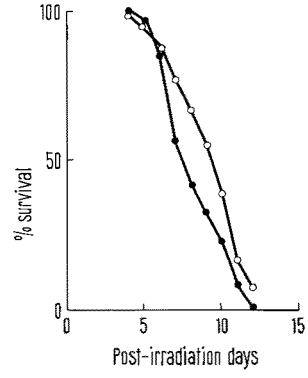

Fig. 1. Post-irradiation survival percentage of 2 groups exposed to H_2O and DMSO vapor and 2 different doses of X-irradiation. o——o DMSO treated and 900 R, ●——● H_2O treated and 850 R.

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