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Akinori Shiraishi* George W. Wooley†

*Okayama University,
†Cornell University,

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Abstract

The human tumor H. Ep. #3 maintained in rats could be transferred for 1-8 generations in treated guinea pigs. H. Ep. #3 grew in the subcutaneous and intramuscular sites in each host at the same time. The treatment with the combination of X-ray 250 r. and 80 mg/kg of cortisone turned out to be the optimal conditioning studied. The number of tumor takes averaged 95.7-100 per cent in the subcutaneous site in guinea pigs treated with optimal conditioning, but in the intramuscular site, the number of tumor takes was 65.2-93.8 per cent. Host mortality varied from 4.2-37.5 per cent in the hosts treated with optimal conditioning. The subcutaneous tumor weights in hosts treated with optimal conditioning averaged 3.3 gm, and their intramuscular tumor weights averaged 5.6-6.2 gm. Tumor weights in hosts treated with only cortisone averaged 1-2 gm in both subcutaneous and intramuscular sites. Histological findings. for the original tumors were found to be the same as that for the successful transplanted tumors in the guinea pigs. The malignancy of the tumor was evaluated by the criteria of anaplasia, invasion, rapidity of growth, and ease of maintenance of transplants. There was no metastasis found in any organs.

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THE HUMAN TUMOR IN CONDITIONED GUINEA PIGS*

Akinori SHIRAISHI** and George W. WOOLLEY

Sloan-Kettering Institute for Cancer Research and Sloan-Kettering Division, Cornell Medical College, Cornell University, New York, U. S. A.

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Many reports on successful heterologous transplantation of tumors have appeared in recent years. This success was achieved by depositing tumor cells in certain protected sites, by using embryos, or by using cortisone and irradiation to circumvent immunological response in the host.

In 1938, and in subsequcnt years, various types of human and animal tumors were successfully transplanted to the anterior chamber of the eye of the guinea pig and other animals by GREENE and his associates[8-11]. Successful growth of human tumors in the guinea pig's eye was also reported by SCHILLING et al.[21], KREMENTZ et al.[14], NEWTON[20], EICHWALD[6], TOWBIN[35], DYER and KELLY[5].

The brain of mice, rabbits and guinea pigs was used for heterologous transplantation of tumors by SHIRAI in 1929[22]. Human cancers were successfully transferred to the brain of guinea pigs and other animals by GREENE[12.13].

In 1950, the membranous cheek pouch of the hamster was introduced as a site for heterotransplantation of tumors by LUTZ and associates[15.16], and human cancers were transferred to the cheek pouch and succssfully grown by TOOLAN[25.27]

MURPHY[17.18] reported in 1912 that mammalian tumors can grow in embryonated eggs, although with some variation in success, especially when using human tumors. In 1955, DAGG, KARNOFSKY et al.[2.3.4] successfully transferred human tumors in the chorioallantoic membrane of chick embryos and yolk sacs by serial passage.

In 1914 MURPHY[19] showed that mammalian tumors survived longer in rats repeatedly irradiated than in non-irradiated rats. In recent years TOOLAN[24.25] showed that a number of human tumors can be grown successfully on subcutaneous implantation in irradiated rats and hamsters. The animals were given a total dose of 200—300 r. and were implanted with tumors 1—5 days after the

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** Visiting Research Fellow: Department of Internal Medicine, Okayama University Medical School, Okayama, Japan

last radiation treatment, and then serial transfer was successfully done. Furthermore, in 1953 TOOLAN[26-33] reported successful transplantation of human cancers to the subcutaneous tissue of weanling rats or hamsters treated with radiation and cortisone or with cortisone only. By TOOLAN's reports[28] about 90 per cent of the implanted tumors were growing 12—20 days after implantation following injection of cortisone. By selection TOOLAN obtained a number of strains of human tumors which were transferred successively to cortisone-treated hamsters and rats. The tumors consisted of a human soft part sarcoma of unknown origin, some human epidermoid carcinomas, and a human embryonal rhabdomyosarcoma. Some of the transplanted tumors were lethal due to the cachectic state of the hosts. No growth of human tumors was ever seen in a normal untreated hamster or rat.

There have been many other reports on succcssful heterotransplantation of these tumors in other conditioned hosts. For instance, GALLILY and WOOLLEY[7] and TAKAYAMA and WOOLLEY[23] reported successful heterotransplantation of the human epidermoid carcinoma H. Ep. #3 in mice treated with X-rays and cortisone.

The guinea pig as a host for tumor heterotransplantation has been almost neglected, except for the eye and the brain. The following paper is a description of a successful serial transplantation of H. Ep. #3 to the subcutaneous and intramuscular sites in conditioned guinea pigs.

MATERIALS AND METHODS

In this experiment 127 young female guinea pigs about 250 gm. body weight were used. They were obtained from Rockland Farms, New York. Following TOOLAN's method, X-ray and cortisone were used for conditioning.

The transplanted tumor was the human epidermoid carcinoma, H. Ep. #3, which was established and maintained by TOOLAN in 1953 from a metastatic epidermoid carcinoma, Grade 3, primary in the buccal mucosa of a human patient. In our experiment the tumor was usually taken from rats treated with X-ray and cortisone.

One hundred and three guinea pigs received one single total body dose of 100, 200, 250, or 300 roentgen 1—4 days before implantation of tumor. Two X-ray machines were used. Each machine was rated at 180 KVP, and 25 m. amp., and was equipped with Cu and Al filters of 0.5 mm. and 1.65 mm. thickness respectively. The machines were placed above and below the animals at a distance of 40 cm. The dose rate was 86 r/min.

Cortisone acetate* was injected subcutaneously into 115 animals including

* Cortisone Acetate (Cortone, Merck) kindly supplied through the courtesy of Merck, Sharp and Dohme, West Point, Pa.

103 X-ray irradiated animals at a dose of 40 mg/kg, 60 mg/kg or 80 mg/kg, soon after implantation of tumor tissues, and then given on every other day for 5—6 times. Other 12 animals are pairfed controls and grafted tumors without pretreatment.

The human tumor used initially in this experiment was 11—12 day old tumors of H. Ep. ♯3 grown in rats treated with X-rays and cortisone. From the first generation in guinea pigs and thereafter, 11—15 day old tumors grown in conditioned guinea pigs were used.

The tumor was aseptically extirpated from the hosts, minced with scalpels and prepared as a suspension in 0.85% saline solution. To each 100 ml. of this saline solution was added 10,000 units penicillin and 100 mg. of streptomycin. One-half ml. of the suspension of tumor tissue containing about 400 mg. of tumor was injected subcutaneously into the right flank and intramuscularly into the left leg in each host simultaneously. Tumor growth rate was measured as the external tumor size and the tumor weight. And animal weight change, percentage of tumor takes, and host mortality were also examined. In addition, a histological examination of the human tumors growing in guinea pigs was made. All instruments and glasswares were sterilized, and the tumor suspension for transplantation was made and injected aseptically into conditioned guinea pigs.

RESULTS

H. Ep. ♯3 tumors which were implanted subcutaneously and intramuscularly in the guinea pigs grew progressively for at least 2 weeks. After a period of 2 weeks, the tumors became so regressive, and sometimes necrotic, that it was found advisable to use an 11—15 day old tumor for transplantation into the next generation. Usually tumors grown in guinea pigs were regressing 3 weeks after implantation.

In general, there was no remarkable difference in the growth rate measured as the external tumor size of tumors when these were transplanted subcutaneously as compared with tumors transplanted intramuscularly. However, there was a variation in the external tumor size in the X-ray and cortisone-treated guinea pigs as compared with guinea pigs treated with cortisone only. The tumors grew larger in the X-ray and cortisone-treated hosts, as shown in Table 1.

The animal body weight varied depending on the tumor growth in the host especially when treated with different conditioning, as shown in Table 1.

The tumor weight in the host treated with X-ray and cortisone was slightly greater in the intramuscular site than in the subcutaneous site; and in the host treated with cortisone only the tumor weight in both sites was small, as shown in Table 1.

Table 1. Population Data Derived from H. Ep. #3 Tumor-Bearing, Conditioned Guinea Pigs for 15 Days

1 Total No. Measurments

Treatment	No. of Guinea Pigs
X-ray*1), Cortisone*2)	41
cortisone	12
non-conditioned	12

2 Animal Weight Change (in increase %)

Treatment	days after implantation				
	3	6	9	12	15
X-ray, cortisone	2.3	13.9	13.7	13.1	2.7
cortisone	4.6	7.4	14.2	20.5	21.3
non-conditioned	5.7	9.2	16.7	18.0	21.2

3 Takes of H. Ep. #3 (per cent)

a) Subcutaneous

Treatment	days after implantation	
	7	14
X-ray, cortisone	95.7	100.0
cortisone	50.0	50.0
non-conditioned	0	0

b) Intramuscular

Treatment	days after implantation	
	7	14
X-ray, cortisone	65.2	93.8
cortisone	16.7	16.7
non-conditioned	0	0

4 Host Mortality (per cent)

Treatment	days after implantation	
	7	14
X-ray, cortisone	4.2	37.5
cortisone	0	0
non-conditioned	0	8.3

5 Tumor Size (external mean diameter mm.)

a) Subcutaneous

Treatment	days after implantation			
	6	9	12	15
X-ray, cortisone	13.9	17.3	21.2	24.5
cortisone	10.4	12.8	12.2	11.9

b) Intramuscular

Treatment	days after implatation			
	6	9	12	15
X-ray, cortisone	16.8	19.9	26.0	27.0
cortisone	10.1	12.5	15.6	13.2

6 Tumor Weight (gm.)

a) Subcutaneous

Treatment	days after implantation	
	12	15
X-ray, cortisone	3.3	4.7
cortisone	1.1	1.2

b) Intramuscular

Treatment	days after implantation	
	12	15
X-ray, cortisone	5.6	6.2
cortisone	1.8	1.2

*1). X-ray 250 r., *2). 80 mg. /kg. of cortisone

The external tumor size and animal weight change were measured every two days for 15 days, and the tumor weight in the sacrificed host was measured on the 12th day and 15th day after implantation.

Tumor takes were high in the host treated with X-ray and cortisone almost 100 per cent. On the other hand, tumor takes were low in the host conditioned by cortisone only. The number of tumor takes in the latter was 50 per cent on the 7th day as well as on the 14th day after implantation. In the non-conditioned host, there were no tumor takes.

The highest mortality for the host treated with 250 r. and 80 mg/kg of cortisone was 37.5 per cent on the 14th day after implantation. In the host treated with cortisone only and non-conditioned, the host mortality was 0—8.3 per cent.

The transplantability of H. Ep. # 3 in guinea pigs, as summarized in Table 2, shows that when the guinea pigs are treated with 100—300 r. and 40—80 mg/kg of cortisone tumors grow for 3—8 generations. In the guinea pigs treated with 250 r. and 80 mg/kg of cortisone, the serial heterotransplantation of H.

Table 2. The Transplantability of H. Ep. # 3 Tumor in Guinea Pigs

Conditioning		Site	Generations of Growth	Number of Guinea Pigs
X-ray (r.)	Cortisone (mg/kg)			
300	80	s. c. & i. m.	5	20
250	80	s. c. & i. m.	8	41
0	80	s. c. & i. m.	1	12
200	60	s. c. & i. m.	4	24
100	40	s. c. & i. m.	3	18
0	0	s. c. & i. m.	0	12

The sites of inoculation were subcutaneous=s. c.; and intramuscular=i. m.

Ep. # 3 was successful through eight generations. Therefore, the treatment with the combination of 250 r. and 80 mg/kg of cortisone should be considered as the optimal conditioning investigated.

A histological examination was made of the tumors serially grown in guinea pigs. It was confirmed by means of the histological findings of the tumor grown in guinea pigs that the tumor was the same as the original tumor grown in rats.

DISCUSSION

It might be of importance in the interpretation of our results to survey the tumor growth rate in different transplantation sites, histological findings, regression, the effect of conditioning and tumor growth on the host, the adequate treatment of the host, and the malignancy of the tumor.

H. Ep. # 3 tumors implanted in subcutaneous and intramuscular sites in the treated guinea pigs grew rapidly in two weeks, and could be successfully transferred to 1—8 generations. Intramuscular tumors showed greater growth as compared to the subcutaneous tumors. It was conceivable that the reason for greater growth with intramuscular transplants was due to a richer blood supply in the intramuscular site; this was proved by a histological examination of the tumor transplant. In the histological findings of 4—6 day old tumors profuse hemorrhage and island-like tumor tissues could be seen among the hemorrhagic muscle tissues. This was not as prevalent in the early tumors in the subcutaneous site.

In general, histological examinations of H. Ep. # 3 tumor showed the findings of epidermoid carcinoma similar to that in the original and transplanted tumors. In the histological findings of the serially transplanted tumors many mitosis usually appeared, but when the tumor underwent regression, mitosis were reduced.

It was noted that the regression of the tumor grown in the treated host occurred in the third week after implantation when no further injections of cortisone were given. Therefore, long-continued injections of cortisone were not given in this experiment.

The hosts showed various mortalities according to the different treatments. Moreover, the host mortality was extensively affected by various conditions; for example, the guinea pig's disease, if present, and other sometimes unknown factors. The mortality of the hosts treated with the conditioning, believed optimal, 250 r. and 80 mg/kg of cortisone was 4.2 per cent on the 7th day and 37.5 per cent on the 14th day after implantation.

Our results showed that tumor takes rate was almost 100 per cent in the hosts treated with optimal conditioning, aud tumors grown in these hosts were

serially transferred for 8 generation. In the hosts without treatment, there were no tumor takes. Consequently, the necessity of the treatment with the combination of X-ray and cortisone was evident for heterotransplantation of H. Ep. #3 tumors into guinea pigs.

It is not yet fully understood how to inhibit the immunological response of the hosts to heterologous transplants by various types and regimes of conditioning. The main effect of cortisone is ascribable to its generally accepted ability to reduce antibody formation. It has been confirmed that prior irradiation of the hosts[1] partially breaks down resistance to heterologous transplants.

Among the various cricteria[34,36] used to evaluate the malignancy of tumors grown in conditioned hosts, these following criteria -- anaplasia, rapidity of growth and ease of maintenance of transplants -- were useful for evaluating the tumor grown in the conditioned guinea pigs, and although evident, no metastasis could be found in the host.

SUMMARY

The human tumor H. Ep. #3 maintained in rats could be transferred for 1—8 generations in treated guinea pigs. H. Ep. #3 grew in the subcutaneous and intramuscular sites in each host at the same time.

The treatment with the combination of X-ray 250 r. and 80 mg/kg of cortisone turned out to be the optimal conditioning studied.

The number of tumor takes averaged 95.7—100 per cent in the subcutaneous site in guinea pigs treated with optimal conditioning, but in the intramuscular site, the number of tumor takes was 65.2—93.8 per cent. Host mortality varied from 4.2—37.5 per cent in the hosts treated with optimal conditioning.

The subcutaneous tumor weights in hosts treated with optimal conditioning averaged 3.3 gm, and their intramuscular tumor weights averaged 5.6—6.2 gm. Tumor weights in hosts treated with only cortisone averaged 1—2 gm in both subcutaneous and intramuscular sites.

Histological findings for the original tumors were found to be the same as that for the successful transplanted tumors in the guinea pigs.

The malignancy of the tumor was evaluated by the criteria of anaplasia, invasion, rapidity of growth, and ease of maintenance of transplants. There was no metastasis found in any organs.

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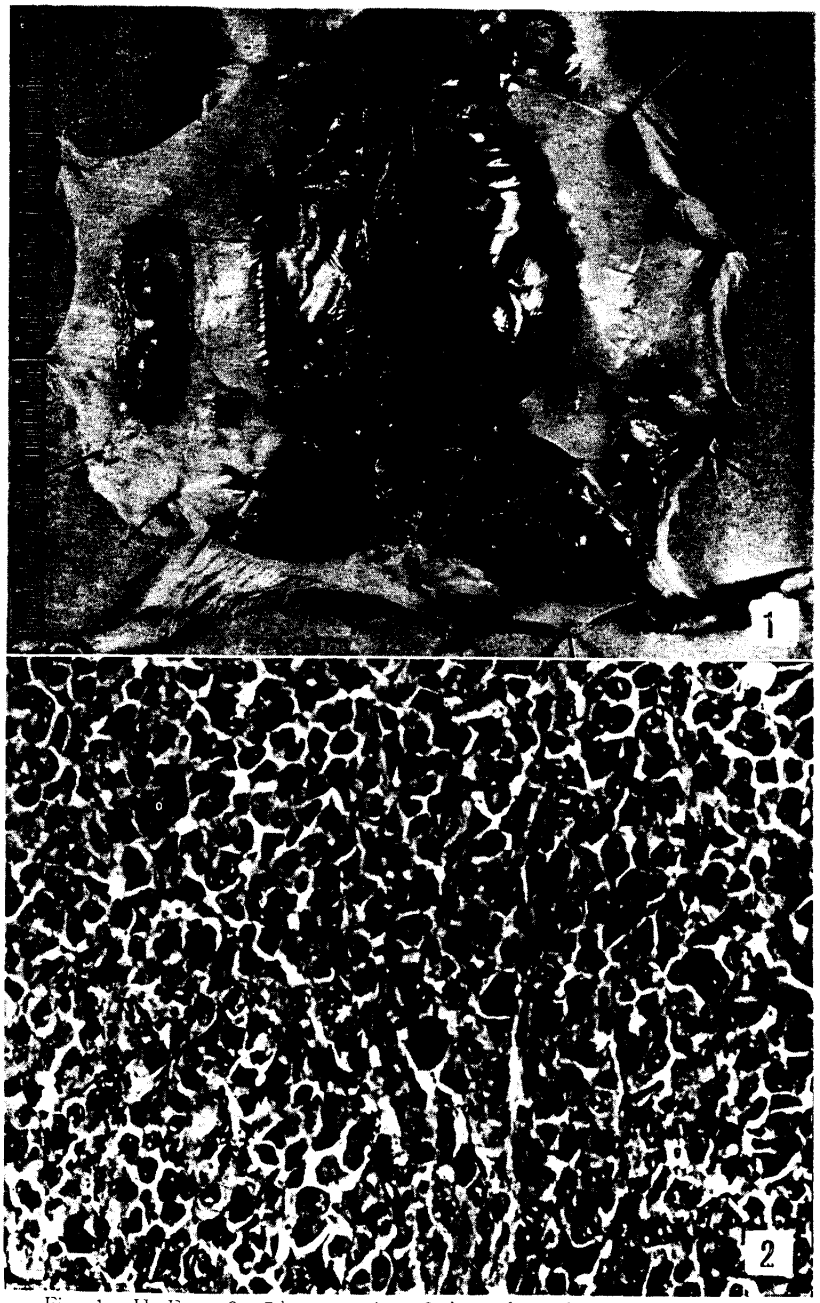

Fig. 1. H. Fp. #3, 5th generation, 9 days after subcutaneous and intramuscular implantation into guinea pig treated with X-ray and cortisone.

Fig. 2. H. Ep. #3, 11 day old tumor grown in treated rat and implanted into guinea pig (original tumor).

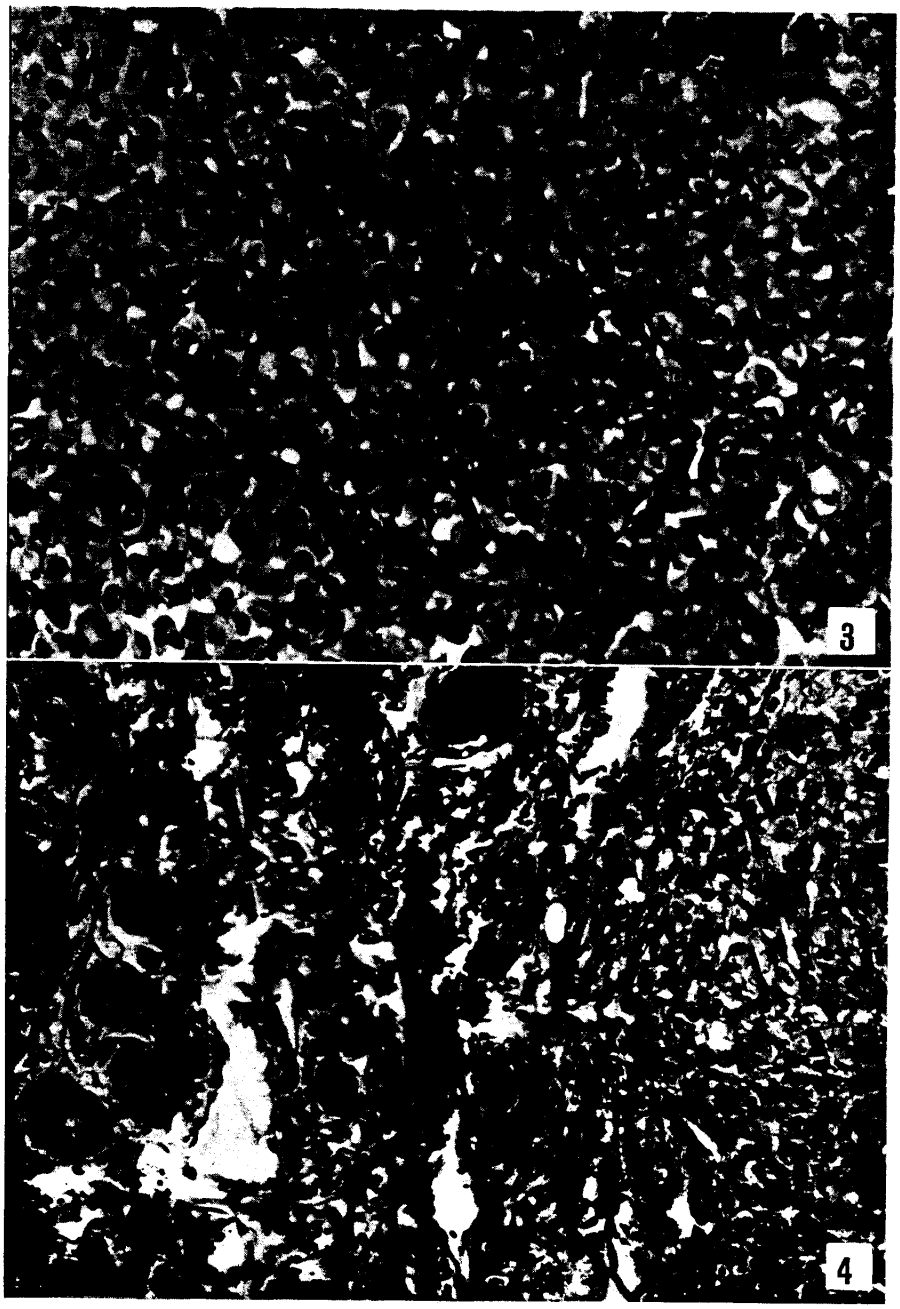

Fig. 3. H. Ep. ♯3, 7th generation, 9 days after intramuscular implantation into treated guinea pig. Mitotic figures are prominent and general morphology is similar to the original tumor.

Fig. 4. H. Ep. ♯3, 7th generation, 9 days after subcutaneous implantation into treated guinea pig. The tumor has invaded the subcutis.