Studies of a Transplantable Rat Pheochromocytoma

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Abstract

A transplantable rat pheochromocytoma in New England Deaconess Hospital (NEDH) was first described by Warren in 1972. It is characterized by the documented association with systolic hypertension and the known presence of increased urinary metanephrines and vanilmandelic acid in **tumor-bearing** animals. The present report describes features of the transplantable tumor, our laboratories have noted, in five tumor transplantations starting in 1974. **Tumor-bearing** animals survive 49 ± 5 days and die much sooner than aging, **non-tumor-bearing** litter mates. Gross measurements confirm the rapid growth of the primary tumor, although at autopsy, histologically proven metastatic foci are rarely seen. Polycythemia with or without increased erythropoietin (ESF) levels were not detected. Electronmicroscopic studies confirmed the presence in tumor tissue of the previously described intracytoplasmic granules. Further studies on this **endocrine-associated** transplantable tumor are warranted and feasible.

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Introduction

In 1972, Warren and Chute described a transplantable pheochromocytoma in NEDH (New England Deaconess Hospital) rats [1]. Warren et al. had previously noted a high incidence of these tumors in elderly irradiated parabiotic NEDH rats [2]. The transplantable tumor Warren described, was reported to usually kill the animals in a period of 30–60 days and only occasionally reached a size of 1.5 cm in diameter and 1 g of primary tumor weight [1]. Concurrent studies confirmed that systolic blood pressure rose to an average of 167 mm Hg [1]. Additional observations noted that in tumor-bearing animals vanilmandelic acid and urinary metanephrines were sharply elevated [1]. In 1973, the same laboratory noted that high intracellular concentrations of primary catecholamines were found in the transplanted tumor tissue [3]. Additional electronmicroscopic studies determined that two types of intracellular tumor granules, containing norepinephrine and epinephrine-like material, were present in two distinct cell populations [3]. When similar tumor tissue was appropriately grown in vitro, the cells were characterized by elongated branching processes and resembled neuronal type cells which have been cultured from neuroblastomas and sympathetic ganglia [3, 4]. In 1974, this tumor and a supply of breeding animals were generously made available to us by Warren. We herein describe our observations to date. This experimental tumor model provides an added useful means of studying relationships to a comparable tumor state in man.

Materials and Methods

In 1974, young adult male and female tumor-bearing and healthy NEDH rats were provided the Experimental Surgery Department of the Roswell Park Memorial Institute by Warren et al. As previously described, similar care was taken in the breeding and care of the stock animals and in the transplantation of the tumor [1, 3]. A tumor mass of about 1x1x3 mm was transplanted by trocar subcutaneously in the interscapular region. At the beginning of these studies, the tumor was in its first through fifth transplant generation. The studies reported herein, were those completed through 1975. 59 untreated animals were observed until death. Gross and microscopic studies were completed to determine tumor weight, histological appearance and the detectable presence of metastatic foci in organs and soft tissue by light microscopy. Microscopic inspection for skeletal metastases was not performed. 15 non-tumor-bearing animals were also employed in added studies. Additional tumor-bearing and non-tumor-bearing animals were sacrificed randomly for 30–60 days after tumor transplantation for special studies. These investigations included electron microscopic examination of suitably prepared specimens, peripheral hematocrit (vol%) determination, and erythropoietin (ESF) assay of serum, tumor and/or kidney extracts [1, 3, 5]. Quantitative results were expressed as the mean $\pm$ SEM (standard error of mean).

Results

Non-treated tumor-bearing animals survived 49 ± 5 days from tumor implantation until death in 59 cases. Non-tumor-bearing animals all lived longer than 60 days, and can readily be maintained for over 6–12 months under similar conditions. The mean gross tumor size at death was 2.3x1.1x0.8 cm. At the time of death, tumor-bearing animals had lost 27 ± 3 % of the initial body weight compared to non-tumor-bearing healthy animals. Fresh, wet gross tumor weight at the time of death was 1.1 ± 0.2 g. Careful gross and microscopic assessment on the 59 animals confirmed one instance of pulmonary metastases. Focal necrotic and secondary changes in the local tumor were grossly evident at the site of tumor implantation.

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Table I. Erythropoietin values in animals with a transplantable pheochromocytoma. Values shown are $\pm$ standard error of mean for five animals in each group.

Fluid or tissue studied Mean % ⁵⁹Fe uptake

normal pheochromocytoma

animal bearing animal

% %

Serum 0.76 ± 0.2 0.95 ± 0.6

Extract of tumor – 0.93 ± 0.02

Kidney extract 1.59 ± 0.3 0.91 ± 0.4

Kidney supernatant 2.73 ± 0.98 1.64 ± 0.53

The primary tumor histologically exhibited relatively large cells, some pleomorphism, and invasion of the adjacent musculature. Electron microscopic studies confirmed the previously described tumor-related intracytoplasmic granules, not found in healthy NEDH rat adrenal medullary tissue. Selected sacrificed tumor-bearing and healthy animals all exhibited no anemia (Hct < 30 vol%). Polycythemia was also not detected (Hct > 55 vol %).

Erythropoietin (ESF) values are illustrated in table I. Tumor-bearing animals did not have significant ESF elevations. Suitably prepared extracts of the primary tumor did not exhibit increased ESF activity. In contrast, in tumor-bearing animals ESF activity in kidney extracts was

significantly ($p < .05$) reduced. Random histological inspection of kidneys from tumor bearing animals failed to reveal the presence of any metastatic foci. As shown in table I, supernatant extract ESF assays were also decreased in the pheochromocytoma bearing animals. The difference, however, was not statistically significant. Inspection for non-tumor renal or cardiac lesions failed to reveal any significant degenerative changes.

Discussion

We feel our results confirm to a significant degree those of Warren et al. described in 1972 and 1973 [1, 3]. The NEDH pheochromocytoma tumor can be, with care, successfully transplanted into young NEDH rats. The tumor, as judged by gross measurements, increases to a measurable degree over time. Although tumor-bearing animals showed significant weight loss, they were not anemic. The weight loss did not correlate with the presence of widespread metastatic disease. Warren et al. in their original communications did not describe widespread gross metastatic foci [1]. However, in their report they documented the presence of significant hypertension, and described in a few host animals who survived longer, the presence of significant renal disease and myocardial damage [1]. From the present study, the precise cause of death remains uncertain, although it appears more likely a result of hypertension [1, 3]. Our studies were not designed to confirm earlier observations. We have noted, however, that the animals do die earlier, and not in association with widespread metastases. Selective cardiovascular measurements are in order in the future, and will be completed. To differentiate histological degenerative aging changes normally present in NEDH rats, will require controlled serial studies with blood pressure measurements. Additional endocrine measurements of a functioning pheochromocytoma normally seen clinically and previously described in detail by Warren must also be repeated [1]. However, on the basis of our electronmicroscope studies on the tumor tissue, we are able to confirm the presence of intracytoplasmic granules thought to represent both norepinephrine and epinephrine type granules [1,2,3,4]. Pheochromocytoma in clinical practice is occasionally associated with polycythemia and increased ESF activity [5]. Our present studies failed to detect elevated ESF activity or polycythemia in this animal model. The kidney is normally thought to be the source of ESF stimulation, even in association with abnormal adrenal function [5]. The decreased ESF activity in kidney extracts of pheochromocytoma-bearing animals is curious and not explainable in terms of excess ESF stimulation as judged by hematocrit levels or ESF serum activity. Although this may be tumor associated, additional studies are required for confirmation and will be performed.

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