Characterization of the Elastase 1-Simian Virus 40 T-Antigen Mouse Model of Pancreatic Carcinoma: Effects of Sex and Diet

Daniel S. Longnecker, Elna T. Kuhlmann, and Daniel H. Freeman, Jr.

Department of Pathology [D. S. L., E. T. K.], and Department of Community and Family Medicine [D. H. F.], Dartmouth Medical School, Hanover, New Hampshire 03756

ABSTRACT

Elastase 1-simian virus transgenic mice, strain Tg(Ela-1, SV40E) Br118, were studied to characterize the development of pancreatic neoplasms. The incidence of pancreatic carcinomas was compared in groups of male and female mice fed one of three diets chosen because of their effect on the development of pancreatic carcinomas in other animal models. Male mice developed more exocrine carcinomas than female mice and their tumors were larger. Groups fed chow had fewer exocrine carcinomas than groups fed purified diets. The level of fat in the latter diets, 5 versus 20% corn oil, did not alter tumor incidence. An unexpectedly high incidence of islet cell tumors was found in all dietary groups, with a higher incidence in females than in males.

INTRODUCTION

Carcinoma of the exocrine pancreas ranks high as a cause of death from cancer in the United States, fourth among males and fifth for both sexes (1). The failure to diagnose this cancer early and treat it successfully highlights the need to understand its causes so that we might design better approaches to its prevention.

Mouse models for pancreatic carcinogenesis have been developed recently by introducing viral or human oncogenes under the control of a pancreas-specific promoter into fertilized mouse ova. Virtually all affected progeny of mice bearing elastase 1 promoter and ras or simian virus 40 early gene constructs developed exocrine pancreatic carcinomas whereas a myc construct was nontumorigenic. The development of the transgenic mouse models of pancreatic carcinogenesis illustrates the potential importance of specific genetic changes in this cancer (2-4).

The transgenic mice that were used in this project were produced by microinjection of linear DNA containing the rat elastase-SV40 early region fusion gene into the pronuclei of fertilized ova (3, 5). The elastase promoter/enhancer was selected to target the pancreas, and the SV40 early genes to obtain expression of the “transgene” will be influenced by diet. This descriptive background is needed in order to allow the model to be used for further study of the mechanisms of dietary and hormonal influences on pancreatic carcinogenesis and for evaluation of chemotherapeutic or dietary chemopreventive agents.

Goals of our studies of the ELSV model include (a) characterization of the transgenic mouse model of pancreatic carcinogenesis in regard to enhancement by dietary fat and other diets; and (b) evaluation of the effect of hormones including estrogen and androgen on the development of pancreatic carcinomas. We report here the results of studies using three diets and comparing tumor incidence in male and female mice.

MATERIALS AND METHODS

Transgenic Mice. Founder pairs of Tg(Ela-1, SV40E) Br118 strain mice that are homozygous for the elastase promoter-SV40 gene construct were obtained from the laboratory of Dr. Ralph Brinster through contact with Dr. Richard Palmiter. The line was derived from C57 × SJL F1 hybrids. The mice were housed at 21°C with a 12-h light, 12-h dark cycle, 2-5/1000 shoebox cage, on aspen shavings. They were given food and water ad libitum. Mice were checked daily to monitor health and were weighed weekly. The mice were weaned at the age of 3–4 weeks and fed one of three diets until they were 26 weeks of age. This duration was chosen because preliminary experience suggested that most mice would survive for 26 or more weeks, and because we wished to compare tumor incidence at a standard interval. Mice that became moribund before 26 weeks were autopsied. Two mice fed a high fat diet were inadvertently allowed to survive 32 and 35 weeks.

Diets. The mice were fed chow (RMH 3000; Agway, Waverly, NY) until weaning and then placed on one of three diets. NIH 07 chow (Ziegler Brothers, Gardners, PA), AIN-76A (which contains 5% corn oil and 20% casein), or a modified AIN-type diet with 20% corn oil and 20% casein (Teklad, Madison, WI). The high fat diet was formulated to maintain isonutrient content in regard to calories. NIH 07 chow contains 5–6% fat according to analyses supplied with various lots.

Autopsy. At autopsy, the major focus was on the pancreas which was weighed and fixed completely for histological study. Large tumors were sampled rather than being embedded completely. Other organs were examined grossly, and abnormal-appearing tissues were sampled for histological study. Representative samples of liver and lung were taken when these organs appeared normal. Tissues for light microscopy were fixed in Bouin’s solution, sectioned, and stained with hematoxylin and eosin or special stains as indicated. The slides were evaluated without knowledge of dietary group.

Statistical Analysis. The effect of diet and sex on the incidence of exocrine carcinomas, islet cell tumors, and body and pancreas weights were evaluated by looking for outliers, nonnormality, and other peculiarities in the data for weights and frequencies. The latter were subjected to univariate and logistic regression analysis (7). In each case, the logistic regression analysis and the simple contingency table analysis agreed so the latter is reported. For the pancreas weights, we conducted a two-factor analysis of variance. No interactions were present. A Tukey’s multiple comparison test was used to identify which levels of pancreas weight differed. The standard deviations for pancreas weights were relatively large, so a log normal distribution analysis was also conducted.

RESULTS

Exocrine tumors varied in size from less than 1 mm to 3 cm in greatest diameter. In order to achieve consistency in classi-
PANCREATIC CARCINOMA IN ELSV TRANSGENIC MICE

Fication, exocrine tumors measuring more than 3 mm in diameter were classified as carcinomas, and smaller lesions with the same cytological features were classified as carcinoma in situ. One of two mice autopsied after 19 weeks had an exocrine carcinoma and the other had an islet cell carcinoma. Among animals in which metastases from an exocrine carcinoma were found, the smallest primary carcinoma was 1.5 cm in diameter.

Male mice develop a higher incidence of exocrine carcinomas than females (Table 1; P < 0.001). The incidence among males fed purified diets was 71% and among females it was 32%. Mice fed a diet with 20% corn oil had a slightly higher incidence and multiplicity of exocrine carcinomas, but these were not significantly greater than the group fed AIN76A (Table 1). The result was similar when the two mice that survived for 32 and 35 weeks were excluded.

Animals fed either of the purified diets developed a higher incidence of exocrine carcinomas than those fed NIH 07 chow (P = 0.007). The exocrine carcinomas had metastasized to the liver in three mice. Two of these were fed 20% corn oil and one was fed the AIN76A diet. Metastases were sometimes detected only by histological examination, and the true incidence is undoubtedly underestimated because of the limited sampling of grossly normal liver and lung.

The overall incidence of islet cell tumors was 77%, and the incidence was higher in female than in male mice (Table 2; P = 0.006). The incidence of islet cell tumors was not affected by diet and was 76.2, 77.3, and 77.3% among rats fed AIN76A, 20% corn oil, and chow diets, respectively. Three of the islet tumors in mice in had metastasized to the liver. Islet cell hyperplasia was noted in 61% of the mice and was also more frequent in female than in male mice.

The pancreas of male mice weighed more than those of females (P = 0.028, natural scale; P = 0.006, log scale). This difference reflects the higher incidence of exocrine tumors in males and the larger body size of males. These factors were not offset by the higher incidence of endocrine tumors in females. The largest exocrine tumors measured up to 3 cm in diameter and were larger than the largest islet cell tumors which seldom exceeded 1 cm in diameter. Mice fed the high fat diet weighed more than those fed the chow diet (P = 0.025) but did not differ from mice fed AIN76A.

DISCUSSION

In the ELSV transgenic mouse model, the nature of the gene construct predicts and the reported histological changes confirm that the exocrine carcinomas arise from acinar cells. Thus, this model seems more similar to chemically induced models in the rat, in which acinar tumors predominate, than it is to the hamster model, in which duct-like tumors are most common. The high incidence of islet cell tumors which we have encountered was not predicted on the basis of the transgene construct, although production of pancreatic islet β-cell tumors by using a gene constructed with the insulin promoter/enhancer and the SV40 oncogene has been reported (8). We have described the islet tumors and the accompanying islet cell hyperplasia in greater detail in a separate report (9) but the reason for their development is not known. Islet tumors are not a feature of the azaserine-induced rat model which we have studied extensively (10).

We have reported the effect of several dietary modifications on the development of pancreatic carcinomas in the azaserine model in rats (10, 11). The rate of progression of carcinogen-induced foci and nodules to invasive and metastasizing carcinomas can be influenced by a variety of dietary factors including fat content. We have shown that high fat diets enhance the development of foci, nodules, and carcinomas (12–14) in both 4-month and 1- to 2-year studies. Two other groups have reported that high fat diets enhance the development of pancreatic carcinomas in carcinogen-treated hamsters (15, 16) and rats (16). Thus, the ELSV mouse model appears to differ from chemically induced rodent models in regard to the magnitude of effect of a 20% corn oil diet. The incidence of exocrine carcinomas was not significantly increased in mice fed the high fat diet. Perhaps experiments of shorter duration should be undertaken in order to examine the effect of the high fat diet on earlier stages of tumor development when the incidence of carcinomas in the control group is less than 50%.

We have repeatedly observed that azaserine-treated rats fed a purified diet develop a higher incidence of pancreatic carcinomas than chow-fed rats (17). This difference also pertains in ELSV mice and demonstrates that dietary modulation of the development of exocrine carcinomas is feasible in this model. The basis of the difference is not known in either species.

Other genes have been introduced into mice by transgenic techniques, and one model provides evidence of modulation by diet of the expression of the transgene. The gene for rat growth hormone (rGH) was linked to the metallothionein (MT) promoter in this model. Mice bearing the MTrGH gene construct made excessive amounts of growth hormone and grew larger than controls, but GH production was stimulated 10-fold further when zinc, a natural inducer of MT genes, was added to the diet (18).

Male rats develop a higher incidence of pancreatic cancers than females in the azaserine model (10). The same trend in the sex ratio is found in human pancreatic cancer. We have presented evidence that testosterone promotes the growth of foci and nodules in rats (19) and that estrogen inhibits their growth (20). It has also been demonstrated that the same carcinoma grows faster when it is transplanted into male compared to female rats (21, 22). Hayashi and Takahashi (23) have reported that testosterone influences the incidence of adenomas that develop in 4-hydroxyaminoquinoline-1-oxide-treated rats. Other workers have noted a sex difference in incidence of carcinomas in carcinogen-treated guinea pigs (reviewed in Ref. 11) and of spontaneous acinar cell lesions in rats (24). These

<table>
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<th>Sex</th>
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<th>Diet</th>
<th>Body wt (g)</th>
<th>Pancreas (g)</th>
<th>Cancer (%)</th>
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Table 1 Incidence and multiplicity of exocrine tumors in transgenic mice on NIH 07 chow, and 5 or 20% fat purified diet

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differences are consistently in the direction of a higher incidence of advanced lesions in male than testosterone-treated animals, and a lower incidence in females. Thus, there is a background of data suggesting that testosterone stimulates the growth of preneoplastic lesions or neoplasms in the exocrine pancreas, and possibly that estrogen inhibits the growth of such lesions. The presence of a male-predominant sex difference in the incidence of exocrine cancers in the ELSV mice suggests that this model may follow the same pattern of hormonal influence. In contrast, it appears that female ELSV mice are more prone than males to develop islet cell tumors. In humans, the incidence of insulinomas is similar in the sexes, although glucagonomas is more common in females (25). Hanahan (8) did not comment on the relative incidence of islet cell tumors in male and female transgenic mice bearing a recombinant insulin/SV40 construct.

The descriptive data presented here redefine the characterization of the ELSV transgenic mouse model of pancreatic carcinoma. New details that emerge include the male > female sex difference in the incidence of exocrine cancers, demonstration that the rate of exocrine tumor development can be affected by diet, and the occurrence of an unpredictable, high incidence of islet cell tumors. These findings should be considered in the design of future studies with this model.

REFERENCES

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