ENHANCEMENT OF NORMAL AND NEOPLASTIC MAMMARY GROWTH BY CROSSBREEDING BETWEEN STRAINS OF FEMALE AND MALE MICE WITH HIGH MAMMARY GROWTH POTENTIALS

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Summary

Based on our previous results that among 4 strains of mice SHN and GR/A showed the highest mammary growth potentials in females and males, respectively. Effects of crossbreeding on normal and neoplastic mammary growth were studied in (SHN x GR/A)F_1 virgin female mice. F_1 mice were higher than the parental strains in the end-bud formation and the ductal growth of mammary glands at 60 days of age and at tumorous age, respectively. While there was little difference between F_1 and both parental strains in the onset age of the first mammary tumors, mammary tumorigenic potential was apparently higher in the former than in the latters. This would be the first report that demonstrated directly the contribution of mammary growth potential of males to that of female offspring.
(Key Words: Females, Heterosis, Males, Mammary Growth, Mammary Tumorigenesis)

Introduction

Nagasawa et al. (1987[b]) found in four strains of mice with different lactational performance that there was a marked difference in mammary growth potential between females and males in each strain. In this paper, mammary growth was examined in F_1 female offspring between female and male strains with high mammary growth potentials as a possible step to evaluate directly the contribution of male mammary growth potentials to that of female offspring.

Materials and Methods

Animals

Virgin female SHN/Mei and GR/AMei strains of mice (Nagasawa and Furukoshi, 1985; Mori et al., 1986; Nagasawa and Konishi, 1987; Nagasawa *et al.*, 1987[a,b,c]) maintained in our laboratory by the strict brother x sister mating and their virgin female F_1 hybrid, (SHN x GR/A) F_1, were used. They were kept in teflon cages (18 x 30 x 15cm) with wood shavings, 5-6 each, maintained in a windowless animal room which was air-conditioned (22-24°C and 55-75% relative humidity) and artificially illuminated (14 hours of light from 5:00 AM to 7:00 PM) and provided with a commercial diet (Lab MR Breeder; Nihon Nosan Kogyo KK, Yokohama, Japan) and tap water *ad libitum*.

Normal and neoplastic mammary growth

On the morning (7:00-9:00 AM) of 60 days of age, some mice in each group were weighed and killed by decapitation under the light ether anesthesia and blood was collected from the trunk. The bilateral third thoracic mammary glands were immediately removed and used for the wholemount evaluation.

Remaining mice in each group were checked for palpable mammary tumors every seven days until 14 months of age when all F_1 mice developed mammary tumors. At autopsy when the first mammary tumors appeared, the bilateral third thoracic mammary glands were also used for the wholemount evaluation.

The wholemount preparations of mammary glands were checked under 10-fold magnification

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and mammary gland area bounded by a line joining the periphery of mammary ducts and mammary rating graded from 1 to 7 in increments of 1 were used as the indices of the growth of mammary duct and end-bud systems, respectively. In each parameter, the mean in the bilateral glands represented the value for the individual.

Endocrine organ weights and ovarian histology

At autopsy, anterior pituitary, adrenals and ovaries were also removed and weighed. Ovaries were further examined histologically.

Serum levels of prolactin and growth hormone

Collected blood was left at room temperature for 5-6 hours, kept overnight at 4°C and centrifuged. Serum was frozen and stored at −20°C. Prolactin and growth hormone (GH) levels were determined by radioimmunoassay.

Statistics

Statistical significance of difference among groups in mammary tumorigenesis was evaluated by analysis of variance considering simultaneously both the incidence and the onset age of mammary tumors. For other parameters, Duncan's multiple range test was used.

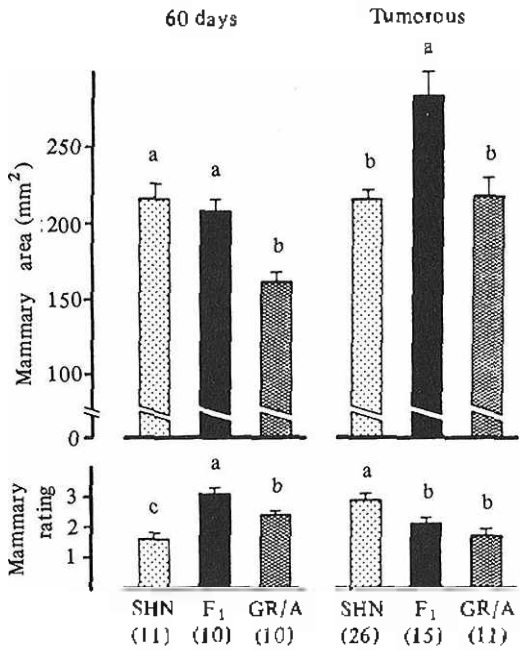

Figure 1. Normal mammary gland growth in each group at 60 days and tumorous ages (Mean ± SEM). Number of mice examined is in the parentheses. [a-c]Means with the different superscripts are different at $p < 0.05$ or 0.01.

Results

Body and endocrine organ weights (table 1) and ovarian histology: At 60 days of age, body weights of SHN and F_1 mice, between which no difference was observed, were significantly greater than that of GR/A ($p < 0.01$). There was no significant difference among groups in anterior pituitary weight. Adrenal weight of F_1 mice was intermediate between those of the parental strains. Ovarian weight was significantly higher in F_1 than in both parental strains ($P < 0.01$).

TABLE 1. BODY AND ENDOCRINE ORGAN WEIGHTS IN EACH GROUP AT 60 DAYS OF AGE (MEAN ± SEM)

Group	Number of mice	Body weight (g)	Endocrine organ weights (mg)		
			Anterior pituitary	Adrenals	Ovaries
SHN	11	23.1±0.3[a]	2.1±0.2	11.9±0.4[a]	13.4±0.6[a]
F_1	10	23.2±0.3[a]	1.6±0.2	8.6±0.3[b]	16.0±0.6[b]
GR/A	10	20.1±0.2[b]	1.7±0.1	7.3±0.2[c]	9.9±0.6[c]

[a-c]Means with the different superscripts are significantly different at $p < 0.01$.

While there was no intrinsic difference in the ovarian structure among groups, both corpora lutea and follicles looked more functional in F_1 mice than in the parental strains.

Mammary gland growth (figure 1): On 60 days, F_1 mice were similar to SHN and significantly higher than GR/A in mammary area ($p < 0.01$). Mammary rating was significantly higher in F_1 mice than in SHN and GR/A ($p < 0.01$ and 0.05, respectively).

In tumorous mice, mammary area was significantly higher in F_1 mice than in both parental strains ($p < 0.01$), while F_1 mice was intermediate between SHN and GR/A in mammary rating.

Mammary tumorigenesis (figure 2): Mammary tumorigenesis was significantly higher in SHN and F_1 mice than in GR/A ($p < 0.05$) and F_1 mice were intermediate between the parental strains in mammary tumorigenesis until 11 months of age. However, F_1 mice apparently surpassed both

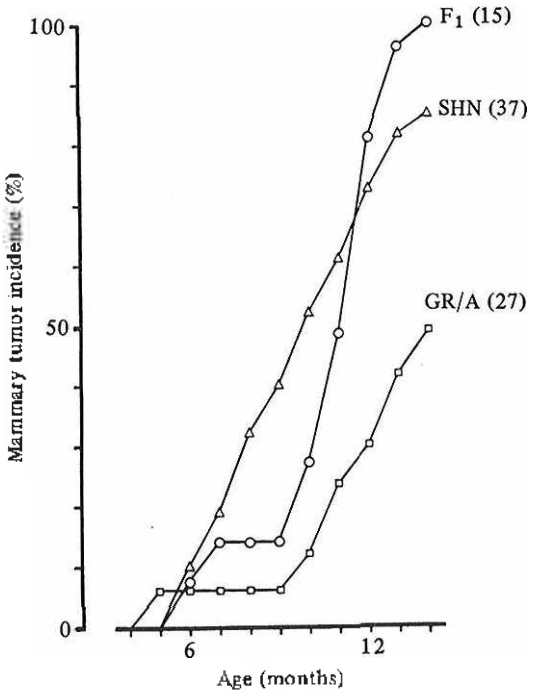

Figure 2. Cumulative incidence of mammary tumors in each group. Number of mice examined is in the parentheses.

TABLE 2. SERUM LEVELS OF PROLACTIN AND GH IN EACH GROUP (MEAN + SEM)

Group	Prolactin (ng/ml)	GH (ng/ml)
SHN	869, 774 (2)[a]	31, 21 (2)
F_1	616 ± 68 (3)	31±13 (3)
GR/A	803 ± 63 (5)	21 ± 3 (5)

[a]Number of estimates.

strains in mammary tumorigenesis after 12 months of age, although the difference was not statistically significant.

Serum levels of prolactin and GH (table 2): There was no significant difference among groups in serum levels of prolactin and GH.

Discussion

Several studies have been done on the genetic improvement of lactational performance in both the farm and the experimental animals and it is now generally considered that lactational performance of the crossbred female offspring was intermediate between those of the parents (Warwick and Legates, 1979). In all of these studies, however, mammary growth potentials of males were estimated indirectly by those of their sisters or daughters. The present experiments using males, of which high mammary growth potential was directly proved, showed an apparent heterotic effect on mammary growth in the female offspring. Furthermore, the effect was found to be different according to the age of the offspring, on mammary end-buds or lobulo-alveoli during youth and on mammary duct system in older (tumorous) mice. The preceeding mammary growth is essential for the subsequent lactation and the male contributes genetically to mammary growth potential of offspring. Nevertheless, it is impossible to estimate directly mammary growth potential of males in the farm animals and even in the most experimental animals. Thus, mouse strains used in this study would be invaluable models to clarify the mechanism(s) of contribution of males to the female offspring for mammary growth and further for lactation.

In this study, little difference was observed between F_1 mice and their parental strains in the circulating levels of pituitary mammotropic hormones, prolactin and GH, despite an apparent difference in mammary growth among groups. These are in good agreement with the previous observations (Nagasawa et al., 1967, 1976; Sinha, et al., 1976; Nagasawa and Yanai, 1981; Sinha, 1981) and they stress again more importance of mammary gland susceptibility to hormones rather than their levels acting on the glands (Nagasawa et al., 1986). However, the higher secretion of ovarian hormones may partly contribute to the heterotic effects of crossbreeding on mammary growth as estimated by more functional state of ovaries in F_1 mice than in the parental strains.

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