BIOGERONTOLOGY

Epithalon Decelerates Aging and Suppresses Development of Breast Adenocarcinomas in Transgenic *HER-2/neu* Mice

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Female transgenic FVB/N mice carrying the breast cancer gene *HER-2/neu* received epithalon (Ala-Glu-Asp-Gly) in a dose of 1 mg subcutaneously 5 times a week to from the 2nd month of life to death. Epithalon prolonged the average and maximum lifetimes of mice by 13.5 ($p<0.05$) and 13.9%, respectively. The peptide prolonged the average lifetime of animals without neoplasms (by 34.2%, $p<0.05$). Epithalon decelerated the development of age-related disturbances in reproductive activity and suppressed the formation of neoplasms. The peptide decreased the incidence of breast adenocarcinomas, lungs metastases (by 1.6 times, $p<0.05$), and multiple tumors (by 2 times). Epithalon 3.7-fold increased the number of mice without breast tumors ($p<0.05$), while the number of animals with 6 or more breast tumors decreased by 3 times ($p<0.05$). Epithalon prolonged the lifetime of mice with breast tumors by 1.4 times ($p<0.05$). These results indicate that Epithalon possesses geroprotective activity and inhibits breast carcinogenesis in transgenic mice, which is probably related to suppression of HER-2/neu expression.

Key Words: *transgenic mice; HER-2/neu; breast cancer; peptide Epithalon*

Gerontological and oncological studies are performed on animals with modified genome (knockout and transgenic animals) [5,9]. Transgenic and knockout mice with short or long lifetime allow evaluating the role of genes involved in aging in the pathogenesis of age-related diseases, including cancer. The relationship between aging and carcinogenesis attracts much recent attention, which is associated with lengthening of life expectancy and increase in the proportion of elderly people in many countries [4,5,8]. Breast cancer is one of the most abundant malignant tumors and the leading cause of cancer-dependent death in women [12].

Transgenic mice carrying the *HER-2/neu* gene that belongs to the family of tyrosine kinase receptors for epidermal growth factor are characterized by high incidence of breast tumors and short lifetime [3,13]. Recent studies demonstrated that hormones and peptides from the pineal gland suppress the development of spontaneous and chemical carcinogen-induced breast tumors in laboratory rodents. Epithalamin, a preparation from the pineal gland, suppresses the growth of spontaneous breast tumors in female C3H/Sn and SHR mice and 7,12-dimethylbenz[a]anthracene-induced breast tumors in female rats [7,11]. Targeted synthesis

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of physiologically active peptides regulating functional activity of the pineal gland opened new vistas to their introduction into medical practice.

Tetrapeptide Epithalon was synthesized on the basis of amino acid analysis of Epithalamin [10]. Long-term treatment with Epithalon prolongs the lifetime, inhibits the growth of spontaneous neoplasms in female CBA mice [6], and suppresses the development of breast adenocarcinomas in female FVB/N mice transfected with the breast cancer gene *HER-2/neu*. Here we studied the effects of Epithalon on the lifetime and development of breast tumors in these mice.

MATERIALS AND METHODS

Experiments were performed on homozygote *HER-2/neu* transgenic FVB/N mice obtained from the Italian National Research Center for Aging and maintained at the Department of Carcinogenesis and Oncogerontology (N. N. Petrov Institute of Oncology). The animals were kept at 22±2°C under a 12-h light/dark regimen and received food and water *ad libitum*. Transgenic females (n=48) were randomly divided into 2 groups. Control animals received subcutaneous injections of 0.1 ml 0.9% NaCl starting from the 2nd month of life to natural death (5 times a week). Experimental mice were subcutaneously injected with Epithalon in a dose of 1 μg. The peptides were synthesized by E. I. Grigor'ev at the St. Petersburg Institute of Bioregulation and Gerontology. The animals were monthly weighted, and the amount of consumed food was estimated. The development of breast tumors, their localization, and size were estimated weakly by palpation. Cytological assays of vaginal smears from mice aging 3, 6, and 9 months were performed daily for 2 weeks. Dead animals were subjected to macro- and microscopic examination after autopsy.

The results were analyzed by exact Fischer test, Wilcoxon test, Mann—Whitney test, Student's t test, Newman—Keuls test, and ANOVA.

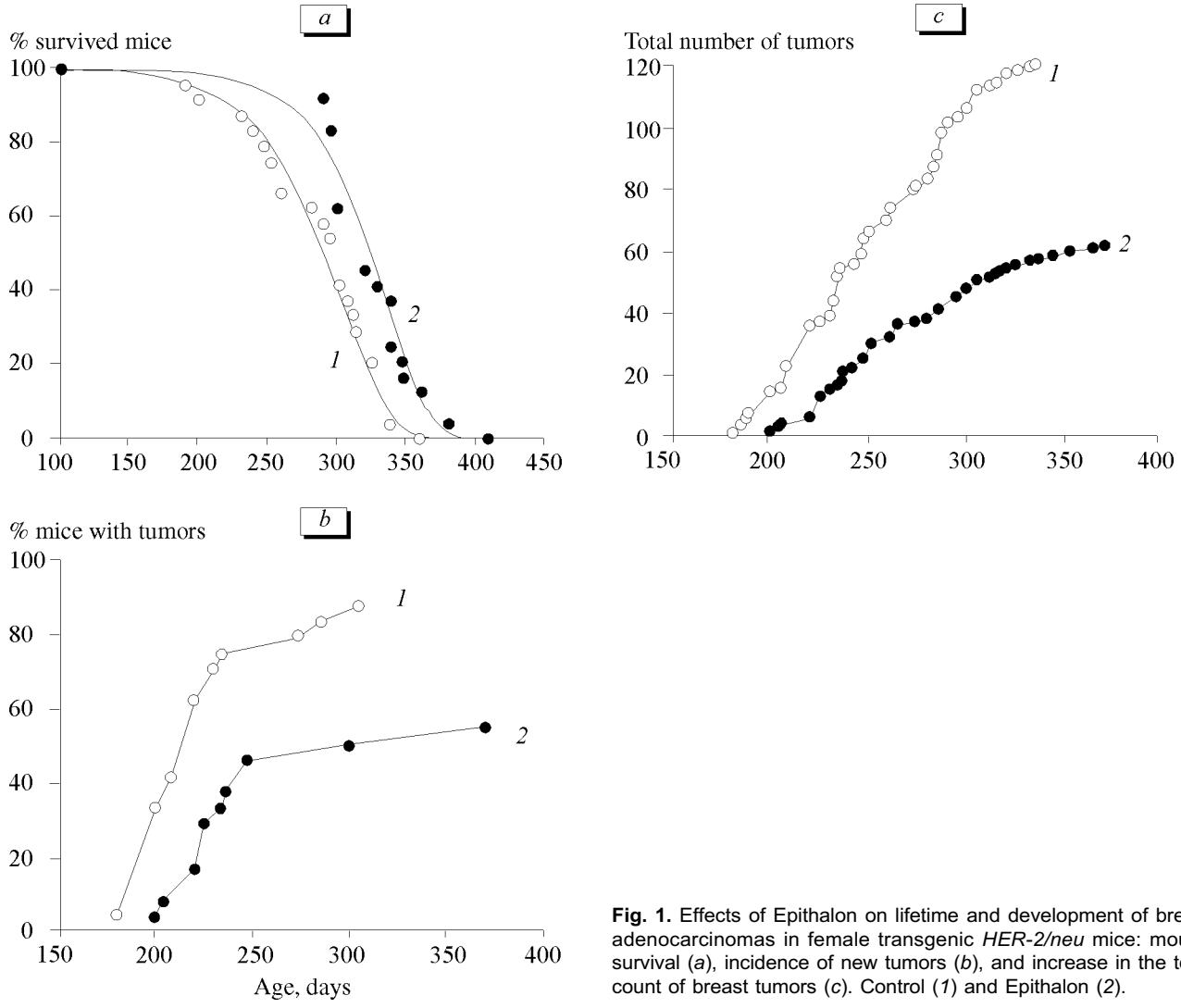

Fig. 1. Effects of Epithalon on lifetime and development of breast adenocarcinomas in female transgenic *HER-2/neu* mice: mouse survival (*a*), incidence of new tumors (*b*), and increase in the total count of breast tumors (*c*). Control (*1*) and Epithalon (*2*).

RESULTS

Epithalon prolonged the average and maximum lifetimes of mice carrying the breast cancer gene *HER-2/neu* by 13.5 and 13.9%, respectively, compared to the control (Table 1). The peptide produced a rightward shift of the survival curve (Fig. 1, *a*). Control and experimental animals consumed the same amount of food, and their body weight increased similarly with age. Cytological assay of vaginal smears showed that the number of mice with short estrous cycle (less than 5 days) decreased with age (72 and 42% animals aging 3 and 9 months, respectively). However, the number of animals with irregular and persistent estrous cycles increased from 0 to 52% on the 3rd and 9th months of life, respectively. Epithalon abolished the age-related decrease in the number of mice with short estrous cycles (58 and 61% on months 3 and 9, respectively). Irregular estrous cycles were observed only in 8% animals aging 9 months. These results indicate that Epithalon decelerates aging of the reproductive system in mice.

Breast tumors in control and Epithalon-treated mice were first detected on days 180 and 200 of life, respectively (Table 1). The dynamics of breast tumor growth did not differ between the control and peptide-treated mice to the 7th month of life. Then, the incidence and total count of neoplasms markedly decreased in animals treated with Epithalon (Fig. 1, *b*, *c*). The incidence of breast adenocarcinomas in mice carrying the *HER-2/neu* gene and receiving Epithalon was lower than in control animals ($p<0.05$). Moreover, in animals treated with Epithalon the incidence of lung metastases decreased by 1.6 times (Table 1). The number of Epithalon-treated mice without breast tumors was 3.7-fold higher than in the control ($p<0.05$), while the number of animals with 6 or more breast tumors significantly decreased (Table 1). The size of breast adenocarcinomas did not differ between the control and experimental mice. Epithalon 1.4-fold prolonged the average lifetime of animals with breast tumors, which indicates that this preparation inhibited the growth of neoplasms. It should be emphasized that Epithalon prolonged the average lifetime of mice without neoplasms by 34.3% (Table 1). Therefore, Epithalon suppresses the development of breast adenocarcinomas and possesses geroprotective activity.

Our previous experiments with RT-PCR showed that in mice injected with Epithalon the intensity of *HER-2/neu* mRNA expression in breast tumors decreased 3.7-fold compared to the control [1]. It can be hypothesized that suppression of tumor growth is related to continuous treatment with Epithalon and considerable inhibition of oncogene expression.

Our results indicate that continuous treatment with Epithalon decelerates aging of the reproductive system and inhibits breast carcinogenesis in transgenic *HER-2/neu* mice. During continuous administration this effect is more pronounced than after courses of treatment with Epithalon [1]. Our results are consistent with published data on suppression of spontaneous tumor growth in long-living CBA mice treated with this tetrapeptide [6]. Moreover, previous studies showed that Epithalamin inhibits the development and growth of spontaneous, carcinogen-induced, and transplanted tumors [2,11].

TABLE 1. Effects of Epithalon on the Lifetime and Development of Breast Adenocarcinomas in Female Transgenic *HER-2/neu* Mice (*M*±*m*, *n*=24)

Parameter		Control	Epithalon
Lifetime, days	*M*±*m*	289.0±9.3	328.0±6.6*
	max	360	410
Mice with breast tumors, %		21 (87.5)	13 (54.2)*
Development of the 1st tumor, days		180	200
Latency of breast tumors, days		251.0±3.6	268.0±5.5*
Lifetime of mice with tumors, days		298.0±8.9	346.0±8.8*
after development of the 1st tumor		76.0±7.6	103.0±9.7*
Total number of tumors		120	61
Number of tumors per 1 mouse in each group		5.00±0.52	2.50±0.54*
Number of mice with lung metastases (%)		11 (45.8)	7 (29.2)
Lifetime of mice with lung metastases, days		304.0±16.1	351±6*
Average lifetime of mice without tumors, days		228.0±15.1	306.0±4.9*
Number of mice with 6 and more tumors (%)		12 (50.0)	4 (16.7)*

Note. *$p<0.05$ compared to the control.

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