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RESEARCH ARTICLE

Biomarkers of aging, life span and spontaneous carcinogenesis in the wild type and HER-2 transgenic FVB/N female mice

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Abstract FVB/N wild type and transgenic HER-2/neu FVB/N female mice breed at N.N. Petrov Research Institute of Oncology were under observation until natural death without any special treatment. Age-related dynamics of body weight, food consumption and parameters of carbohydrate and lipid metabolism, level of nitric oxide, malonic dialdehyde, catalase, Cu, Zn-superoxide dismutase, vascular endothelial growth factor were studied in both mice strains. The parameters of life span and tumor pathology were studied as well. Cancer-prone transgenic HER-2/neu mice developed in 100 % multiple mammary adenocarcinomas and died before the age of 1 year. Forty tree percent of long-lived wild type mice survived the age of 2 years and 19 %—800 days. The total tumor incidence in wild type mice was 34 %. The age-associated changes in the level of serum IGF-1, glucose and insulin started much earlier in transgene HER-2/neu mice as compared with wild type FVB/N mice. It was suggested that transgenic HER-2/neu involves in initiation of malignization of mammary epithelial cells but also in acceleration of age-related hormonal and metabolic changes in turn promoting mammary carcinogenesis.

Keywords Biomarkers of aging · Life span · Spontaneous carcinogenesis · HER-2/neu · FVB/N mice

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Introduction

There is a wide choice of inbred, outbred and genetically modified phenotypically and genetically characterized strains of rodents (mice and rats) commonly used as models in aging and cancer studies (Ingram and Jucker 1999; Anisimov 2001; Nadon 2006; Anisimov et al. 2013; Liao and Kennedy 2014). During recent years the FVB/N mice have been one of most frequently used for generation of transgenic mouse models. This mouse strain is characterized by high fertility, large dimensions of the pronuclei in fertilized zygote facilitating transgenic inoculation into embryo, and fairly good survival of embryos following injection (Taketo et al. 1991). The histopathologic finding in background FVB/N mice were reported in several works (Hennings et al. 1993; Mahler et al. 1996; Wakefield et al. 2003; Huang et al. 2008; Raafat et al. 2012). However, little is known about of age-related

dynamics of hormonal and metabolic parameters in mice of this strain. At the same time these parameters could serve as biomarkers of aging (Anisimov et al. 2013).

Overexpression of activated HER-2/neu in FVB/N transgenic female mice leads to malignant transformation of mammary epithelial cells followed by development of multiple mammary adenocarcinomas in 100 % of mice (Muller et al. 1988, 1998). These mice usually died before the age of 1 year developing from 1 to 10 mammary adenocarcinomas (Baturin et al. 2001; Anisimov et al. 2005). Transgenic HER-2/neu mice were used in a number of our studies on effect of metformin, melatonin, rapamycin and some other drugs with potentially geroprotective and anticancer activity in mice (Anisimov et al. 2005, 2010a, b). However the comparison of parameters of aging in wild type and transgenic mice was never performed. In this paper we for the first time studied parameters of metabolism and life span, as well as tumor development in wild type FVB/N and transgenic HER-2/neu FVB/N female mice.

Materials and methods

Animals

FVB/N wild type and FVB/N HER-2/neu transgenic female mice for the activated rat *neu* oncogene were originally obtained from Charles River (Hollister, CA) by the Italian National Research Center for Aging (Pierpaoli et al. 2013) were housed and breed in the Department of Carcinogenesis and Oncogerontology, N.N. Petrov Research Institute of Oncology. The mice were kept 5–7 in polypropylene cages (30 × 21 × 10 cm) under standard light/dark regimen (12 h light:12 h darkness) at 22 ± 2 °C and received standard laboratory chow (Anisimov et al. 2013) and tap water *ad libitum*. All studies were planned and performed according to the regulations of European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. CETS No. 123 and were approved by the N.N. Petrov Research Institute of Oncology Ethic Committee. During the study, animals were under veterinarian monitoring for any signs of morbidity and all efforts were taken for alleviation suffering.

Experimental design

One hundred twenty eight female FVB/N wild type and 78 transgenic HER-2/neu mice at the age of 2 months were randomly divided into two groups of each strain. The part of FVB/N wild type mice at the age of 3, 6, 9 and 12 months was sacrificed by decapitation after overnight starvation. Part of transgenic HER-2/neu mice was sacrificed at the age of 3, 6 and 9 months. Samples of blood serum were obtained and stored at −20 °C for subsequent analyses. Other animals of both strains were allowed survive until natural deaths. Once a month all mice were weighted. Once a week all mice were palpated for detection of mammary tumors appearance. The localization and size of tumors were registered on special charts. Progressively growing masses of >3 mm in mean diameter were regarded as tumors The date of each death was registered, and the mean life span, the age of 90 % mortality and maximum life span were estimated.

Biochemical examinations

Metabolic parameters were assessed in blood serum. Glucose concentration was estimated electrochemically by means of express analyzer (i-STAT, Abbot) using CG8+ cartridges. Total cholesterol, triglycerides' and malonic dialdehyde (MDA) concentrations, superoxide-dismutase (SOD) and catalase activities were processed using Stat Fax 3300 analyzer and Spinreact reagent kits according to standard instructions. Vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-1) and nitrogen oxide (NO) concentrations were assessed by means of immune enzyme assay (ELISA) using Cusabio and R&B Systems reagent kits according to standard procedures.

Pathomorphological examination

All animals were autopsied. All tumors, as well as tissues and organs with suspected tumor development were excised and fixed in 10 % neutral formalin. After routine histological processing the tissues were embedded in paraffin. 5–7 μm thin histological sections were stained with hematoxylin and eosine and were microscopically examined. Tumors were

classified according to International Agency for Research on Cancer recommendations (Turusov and Mohr 1994).

Statistics

Experimental results were statistically processed by methods of variation statistics with the use of STATGRAPH statistic program kit. The significance of discrepancies was defined according to Student's t-criterion, Fischer's exact method, χ^2, non-parametric Wilcoxon-Mann–Whitney and Friedman RM Anova on Ranks. Student–Newman–Keuls Method was used for all pairwise multiple comparisons (Goubler 1978).

Results

Age-related body weight dynamics

The mean body weight of female mice of both strains slightly increased with the age. FVB/N wild type mice were heavier than transgenic HER-2/neu females over their life span. Thus, body weight was 28.6 ± 0.45 g in 3-month-old and 29.7 ± 0.69 in 12-month-old female FVB/N wild type mice, whereas it was 23.9 ± 0.28 and 25.0 ± 0.28 g in 3- and 12-month-old transgenic HER-2/neu mice, correspondingly.

Age-related dynamics of food consumption

Food consumption did not change between the 3rd and 12th months of age in both mouse strains. However, transgenic HER-2/neu mice consumed significantly more food than wild type FVB/N females at any age (data are not shown).

Age-related dynamics of metabolic and hormonal parameters in serum of mice

The comparison of age-related dynamics of biochemical parameters showed the differences in the patterns between two strains of mice. Thus, serum level of IGF-1 increased with age in HER-2/neu mice and was higher than that in wild type mice between 3rd and 6th months of life (Fig. 1a). Serum levels of glucose and insulin did not changed with the age in wild type FVB/N mice. However, in HER-2/neu mice its levels was significantly increased with the age and was higher than that in FVB/N wild type mice (Fig. 1b, c). The levels of total cholesterol and triglycerides in the serum of wild type mice did not changed significantly with age, whereas its decreased in transgenic HER-2/neu mice (Fig. 1d, e).

Activity of antioxidant enzymes Cu, Zn-superoxide dismutase (SOD) and catalase slightly decreased with age in wild type mice. In HER-2/neu mice SOD level declined significantly between the 6th and the 9th months of age, whereas catalase activity in this strain increased at the age of 6 months and then decreased (Fig. 1f, g). The level of malonic dialdehyde (MDA) raised with age in FVB/N wild type mice and was significantly higher in these mice as compared with HER-2/neu at the age of 9 months, decreasing after this age (Fig. 1h). The level of nitric oxide (NO) declined with age in wild type mice and did not changed in HER-2/neu mice (Fig. 1i). The level of vascular endothelial growth factor in the serum of mice of both strains did not change between 3th and 9th months of life, however it increased in WT mice at the age of 12 months (Fig. 1j).

Survival and longevity of female FVB/N wild type and HER-2/neu mice

Survival dynamics and parameters of life span of these two mouse strains were significantly different (Tables 1, 2; Fig. 2a). The mean life span was twice longer and maximal life span was 2.9 times longer in FVB/N wild type mice than in transgenic HER-2/neu mice. Forty three percent of wild type mice survived until the age of 2 years and 19 %—survived the age of 800 days whereas no transgenic mice survived longer than 1 year.

Spontaneous tumor development in female FVB/N wild type and HER-2/neu mice

The first tumor-bearing female mouse in wild type mice was detected at the age of 499 days, whereas in transgenic mice—at 150th day. The data on the incidence, localization and type of spontaneous tumors in female FVB/N and transgenic HER-2/neu mice are given in Table 2. Total incidence of tumors in

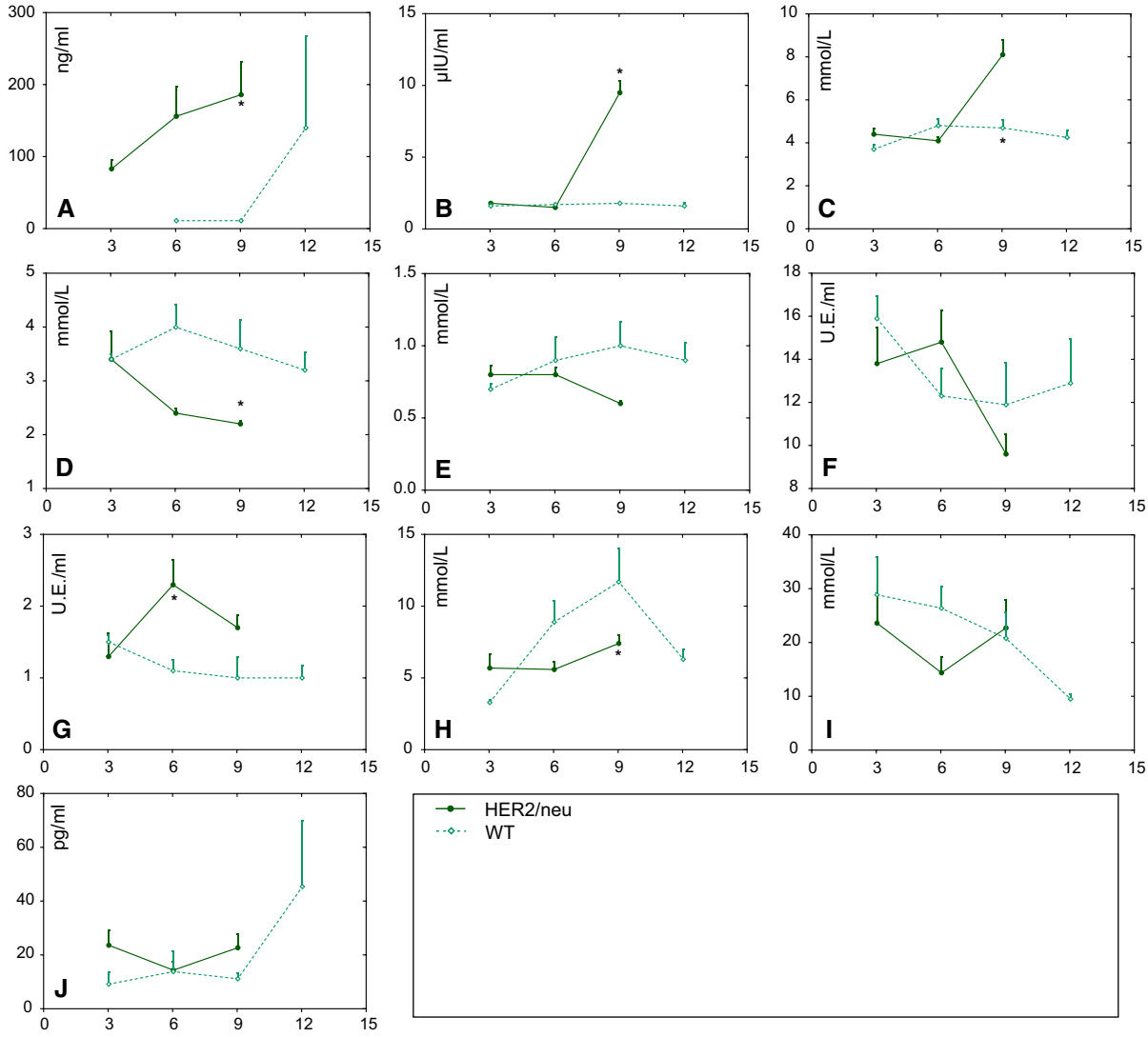

Fig. 1 Age-dependent dynamics of biochemical parameters of the serum in HER-2/neu and wild type FVB/N mice. **a** IGF-1 (ng/ml). **b** Insulin (μlU/ml). **c** Glucose (mmol/l). **d** Total cholesterol (mmol/l). **e** Triglycerides (mmol/l). **f** SOD (U.E./ml). **g** Catalase (U.E./ml). **h** Malonic dialdehyde (mmol/l). **i** Nitric oxide (mmol/l). **j** VEGF-A (pg/ml). The *horizontal line* indicated the age of sampling (months). The difference with the parameter for the same age wild type FVB/N mice is significant *$p < 0.05$, *open circles* FVB/N mice, *closed circles* HER-2/neu mice

wild type mice was 34 %, the incidence of malignant tumors—26 %. The most common neoplasia in FVB/N mice were lung papillary carcinomas (Fig. 3a). These tumors were observed in 18 % of cases, with mean age of death—710 ± 48.5 days and malignant lymphomas (10 %, mean age of death—757 ± 19.1 days, Fig. 3b). Skin papillomas were detected in 2 mice (3 %) who died at the age of 902 and 904 days. Also 2 cases of squamous-cell carcinoma were found in FVB/N mice who died at the age of 780 and 944 days. Solitary Harderian gland cystadenocarcinoma (Fig. 3c) and uterine granules-cell tumors were detected in mice who died at the age of 770 and 793 days, respectively.

One hundred percent of transgenic HER-2/neu mice developed multiple (from 3 to 10) mammary adenocarcinomas (Fig. 3d), the mean number of mammary adenocarcinomas per tumor-bearing mice

Table 1 Parameters of life span and tumor incidence in female wild type and HER-2/neu transgenic FVB/N mice

Parameters	Wild type	HER-2/neu
Number of mice	69	32
Effective number of mice	50	32
Mean life-span of mice, days	600 ± 12.2	278 ± 5[a]
Mean life-span of 10 % long-lived mice, days	921 ± 14.5	333 ± 7.3[a]
Maximal life span, days	993	340
Time of the 1st tumor detection, day	499	150
Number of TBM	17 (34 %)	32 (100 %)[a]
Number of fatal TB-mice	13 (26 %)	32 (100 %)[a]
Total number of tumors	21	212
Localization and type of tumors		
Mammary adenocarcinoma	0	32 (100 %)
Lung papillary adenocarcinoma	9 (18 %)	0
Malignant lymphoma	5 (10 %)	0
Skin papilloma	2	0
Skin squamous-cell carcinoma	2	0
Harderian gland cystadenocarcinoma	1	0
Uterine granules-cell tumor	1	0
Ovarian cystadenoma	1	0

[a] The difference with parameter in wild type mice is significant, $p < 0.001$

Table 2 Survival distribution in female wild type and HER-2/neu transgenic FVB/N mice

Group	Number of mice surviving the age of: (days)											
	150	200	250	300	350	400	500	600	700	800	900	1000
Wild type	69	65	62	59	56	54	50	42	33	14	6	0
HER-2/neu	32	31	29	7[a]	0	0	0	0	0	0	0	0

[a] The difference with the corresponding age in l group of wild type mice is significant: $p < 0.01$ (Fischer's exact test)

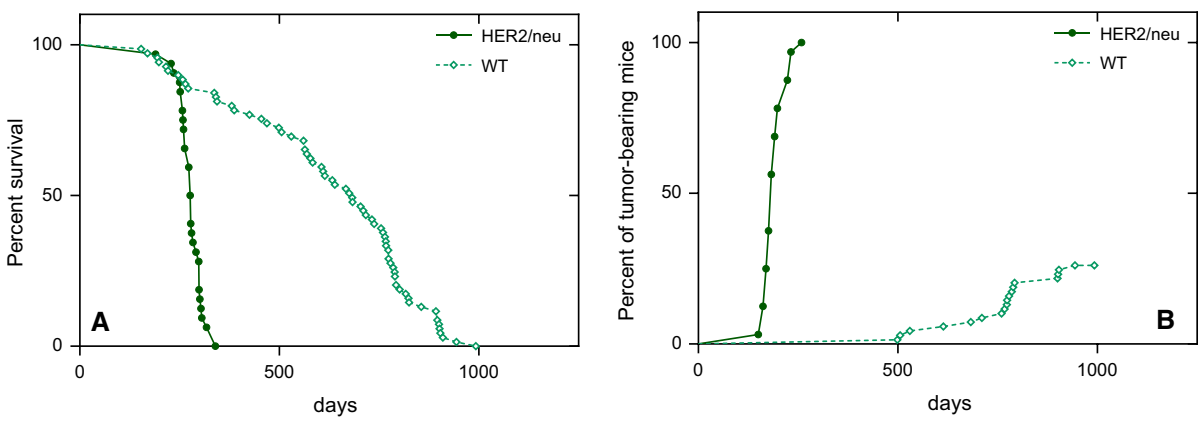

Fig. 2 Survival (**a**) and tumor yield (**b**) curves of wild type (WT) and transgenic HER-2/neu

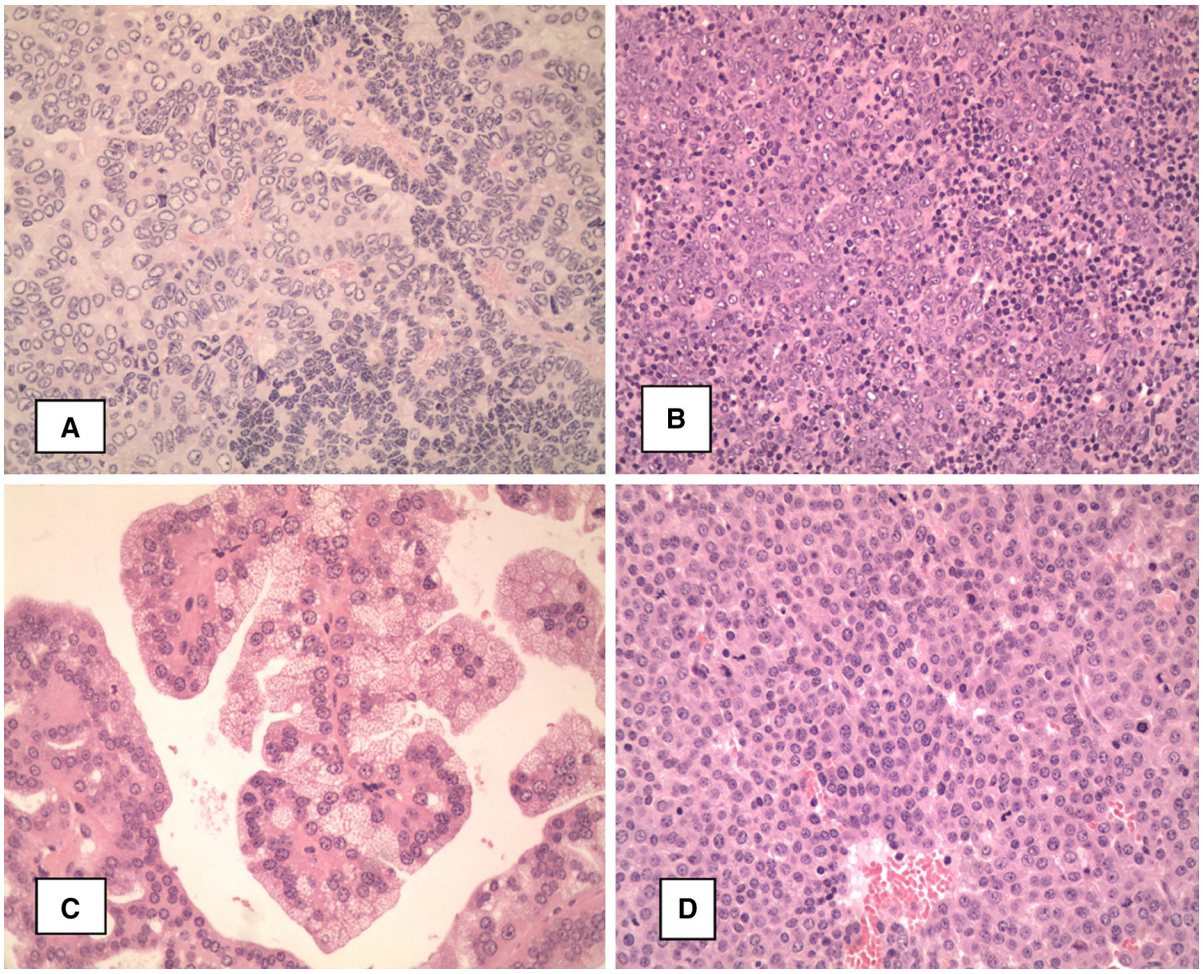

Fig. 3 Microphotographs of spontaneous tumors in FVB/N miceFVB/N female mice. **a** papillary lung adenocarcinoma, H & E, ×400. **b** malignant lymphoma, H & E, ×400. **c** Harderian gland cystadenocarcinoma, H & E, ×400. **d** Mammary adenocarcinoma, H & E, ×400

was 6.25. In 50 % of cases tumor-bearing mice had multiple metastases of mammary adenocarcinoma into the lung. According to the long-rank test there are significant differences in age-related distributions of total tumor occurrence in FVB/N and transgenic HER-2/neu mice ($p < 0.001$; Fig. 2b).

Discussion

Seventy eight percent of wild type FVB/N female mice survived to the age of 14 months, and 43 % survival to the age of 24 months, 14 reported 84 and 61 % surviving female FVB/N mice at the same age, whereas 11 have shown that 50.5 % of female mice of this strain survived the age of 2 years and 11.3 % lived even longer than 800 days. Thus, survival of FVB/N mice of our colony rather similar to reported other authors. Spontaneous tumors in our colony of female FVB/N mice developed in 29 % of all animals. Lung adenocarcinomas were found in 18 % of mice and malignant lymphomas—in 10 %. In Mahler's study (1996), the total tumor incidence in FVB/N mice was 66 % at the age of 2 years, lung tumors (alveolar-bronchiolar adenomas and adenocarcinomas) were observed in 37 % of mice, malignant lymphomas—in 6 % of cases. In retired FVB/N breeder female mice total tumor incidence was 52.3 %, lung tumors—24.7 %, malignant lymphomas—0.5 %, mammary adenocarcinomas and adenoacanthomas—13.9 %;

ovarian tumors 6.7 % (Huang et al. 2008). In our female FVB/N mice as well as in colony of Mahler et al. mammary carcinomas were not observed, whereas there are reports on spontaneous mammary gland carcinomas and pituitary tumor development in breeders FVB/N mice and virgin substrains FVB/NCr and FVB/N-RC female mice (Wakefield et al. 2003; Radaelli et al. 2009; Raafat et al. 2012).

As it was shown in a number of studies, transgenic HER-2/neu FVB/N mice developed mammary carcinomas in 100 % of cases between the 4th and 10th month of age (Muller et al. 1998; Anisimov et al. 2005, 2010a, b). Overexpression of this oncogene plays a causal role in the development and progression of most aggressive types of breast cancer (Gutierrez and Schiff 2011). HER-2 is a member of the human epidermal growth factor receptor (HER/EGFR/ERBB) family. Molecular cloning of the gene showed that HER-2, neu, and ErbB-2 are all encoded by the same orthologs. The HER-2 family is composed of four plasma membrane-bound receptor tyrosine kinases, the other members being epidermal growth factor receptor, erbB-3 (neuregulin-binding; lacks kinase domain), and erbB-4. All four contain an extracellular ligand- binding domain, transmembrane domain, and intracellular domain that can interact with a multitude of signaling molecules and exhibit both ligand-dependent and ligand-independent activity. HER-2 can interrelate with any of the other three receptors, and it is considered to be the preferred dimerisation partner of the other ErbB receptors. Dimerisation results in the autophosphorylation of tyrosine residues within the cytoplasmic domain of the receptors and initiates a variety of signaling pathways. Signaling pathways activated by HER2 include mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K/Akt), phospholipase C, protein kinase C (PKC) and signal transducer and activator of transcription (STAT). These signaling pathways through the ErbB family of receptors promote cell proliferation and oppose apoptosis.

It was shown that the regulation of apoptosis involving p53-dependent cascade is disturbed in the supraoptic and paraventricular nuclei of hypothalamus in transgenic HER2/neu mice (Bazhanova et al. 2007). Authors observed the decrease of caspase-8 and Bax levels, dysregulation of Bcl-2 and Mcl-1 antiapoptotic protein synthesis, decrease and c-Raf-1 expression does not changed in these nuclei of mice.

We investigated the age-related dynamics of some parameters of carbohydrate and lipid metabolism, antioxidant system and angiogenesis in wild type and transgenic HER-2/neu female mice. Transgenic HER-2/neu mice were demonstrated to have much earlier age-related increase in the level of serum IGF-1, insulin, glucose developed more in much younger age than that in wild type FVB/N mice. The critical role of hyperinsulinemia in the development of breast cancer is well known (Gupta et al. 2002). Our data on the age-related dynamics of changes in the system insulin-IGF-1-glucose in transgenic HER-2/neu mice are in agreement with this conclusion. Early switching-off of the estrous function, which we observed in transgenic HER-2/neu female mice (Anisimov et al. 2005, 2010a, b) seems as important biomarker of premature aging of reproductive system in these mice.

The serum level of total cholesterol and triglycerides of transgenic mice was lower in HER-2/neu than that in the wild type mice. Activity of SOD and catalase in the serum of transgenic mice declined between the age 6 and 9 months of age whereas it did not changed with age in wild type mice. There were no sufficient differences in the age-related pattern of the malonic dialdehyde levels in the serum in both mouse strains between the ages of 3 and 9 months, however the level of MDA was higher in wild type mice. The level of VEGF-1 was not changed at the same period but increased in wild type FVB/N mice after the age of 9 months. Raafat et al. (2012) did not find any significant age difference in the level of growth hormone (GH), thyroxin (T4), estrogensor bioactive prolactin in FVB/N-RC mice. The serum level of triiodthironin (T3) was significantly higher in 20-month-old transgenic multiparous and nulliparous FVB/N mice. Data on switching-off of the estrous function at very young age, which we observed in transgenic HER-2/neu female mice (Anisimov et al. 2005, 2010a, b) together with data on hyperinsulinemia, hyperglycemia and decrease of activity of antioxidant system, seems are biomarkers of premature aging in these mice. At the same time these factors providing specific microenvironment for mammary epithelial cells initiated by oncogene promoting development and growth of mammary adenocarcinomas. Observations of geroprotective and anti-carcinogenic effects of antidiabetic biguanide metformin in transgenic HER-2/neu mice (Anisimov et al. 2005, 2010a, b) are in agreement with this suggestion. Our

finding allowed to suggest that transgene HER-2/neu not only initiates malignization of mammary epithelial cells but also induces hormonal-metabolic changes accelerating aging and promoting of mammary carcinogenesis in host animals.

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