Abstract. Two-year-old mice of the long-living transgenic mice of the aMUPA strain were previously found to show higher tumor resistance than the their initial wild-type (WT) strain (Tirosh, 2003). To better understand the mechanism underlying the differences in tumorigenesis rates between the two mouse lines, the rate of tumorigenesis and survival effects were studied in aMUPA mice and parental WT mice exposed to dimethylbenz(a)anthracene (DMBA). Each animal received three intragastric feedings of DMBA, each one week apart, at doses of 2, 1, and 1 mg dissolved in 0.2 ml corn oil; thus, the total amount of the carcinogen was 4 mg/mouse. Control mice received corn oil. The aMUPA mice exhibited distinctly higher survival rates in experimental chemically-induced tumorigenesis compared to their WT counterparts: 93% vs. 67%, p=2.7. The rate of tumorigenesis differed between the mouse lines (yield was 1.5 and 2.1), owing to a distinct tendency toward decreased tumor frequency in the skin and forestomach in the aMUPA mice. The experimental duration was also significantly higher for transgenic mice: 35.9±1.2 weeks compared to 30.5±1.3 weeks in WT mice, p<0.01. The lungs, forestomach and skin were target organs for the carcinogenic effect of DMBA. Our observations suggest that aging promotes the rate of spontaneous and induced tumorigenesis.

The increased incidence of cancer with age has long been interpreted as suggesting that multiple genetic changes are required for carcinogenesis. In recent years, various transgenic and knockout rodent models with extended life span (Ames dwarf mutant mice, p66-/- knockout mice, MUPA and MGMT transgenic mice) have been shown to resemble controls with the respect to incidence of spontaneous tumors, whereas in the former the latent period of tumor development increases (1). Animals with accelerated aging have shown increased incidence and shorter latency of tumors. The differences in longevity and pathology incidence, including cancer incidence, among rodent strains, provide strong evidence of a genetic influence on these parameters (2). Genetically modified animal models characterized by shortened or extended life spans therefore provide a unique opportunity to evaluate the role of aging genes in the mechanisms of carcinogenesis (3). Transgenic and null-mutant animal models also offer an important means of identifying and studying both carcinogens and chemopreventive agents.

In our previous study, 2-year-old mice of the transgenic aMUPA strain, characterized by significantly reduced body weight and size, lower body temperature and decreased plasma corticosterone in old age relative to their initial wild-type (WT) strain (4, 5), were found to have higher resistance in spontaneous tumorigenesis than the WT strain (6). In the present report, the rate of chemically-induced tumorigenesis, tumor target organs and survival effects were analyzed in long-living aMUPA-transgenic mice and parental WT mice exposed to the well-known carcinogen dimethylbenz(a)anthracene (DMBA).

Materials and Methods

Mice. Twelve-week-old homozygous transgenic long-living aMUPA female mice and parental WT mice of the same gender and age (the NIH inbred mouse line FVB/N) were propagated and maintained at the Weizmann Institute’s Transgenic Mouse

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Facilities according to NIH Guidelines for the Care and Use of Laboratory Animals (4, 5). The mice were housed in small cages, five per cage, at 23°C, with a 12-h light/12-h dark cycle and free access to water and food (Experimental Animal Center Mice and Rat Breeding Diet).

**Statistical analysis.** Data were analyzed using one-way ANOVA and quantified using the immunoperoxidase technique (NeoMarkers, Fremont, CA, USA). All markers were visualized and for CD8+ lymphocytes (Talrom, Rehovot, Israel) and CD79a/mb-1 commercial markers TCA-MR5100 for CD4+ and TCA-MR5200 and eosin (H&E). To more accurately determine the type of tumors, conditions of fixation and staining of 3-μm sections with hematoxylin studies were performed using standard procedures, with uniform pathological examination. The following tumorigenic indicators were evaluated in each mouse: the number and location of the tumors, their histological type and the yield coefficient, namely, the ratio of total number of tumors to the number of tumor-bearing mice. The survival time of tumor-bearing mice was also calculated to evaluate duration of the latent period in the development of internal tumors.

**Morphometric and immunohistochemical studies.** All histopathological studies were performed using standard procedures, with uniform conditions of fixation and staining of 3-μm sections with hematoxylin and eosin (H&E). To more accurately determine the type of tumors, the number and type of immune cells were evaluated with the commercial markers TCA-MR5100 for CD4+ and TCA-MR5200 for CD8+ lymphocytes (Talrom, Rehovot, Israel) and CD79a/mb-1 (NeoMarkers, Fremont, CA, USA). All markers were visualized and quantified using the immunoperoxidase technique.

**Statistical analysis.** Data were analyzed using one-way ANOVA and \( \chi^2 \). Pairs of means were compared using the Tukey HSD test.

### Results

**Tumorigenic experiment.** The αMUPA mice exhibited distinctly higher survival rates in experimental DMBA-induced tumorigenesis than their WT counterparts \( (p=2.7; \) Table I). An increase in the number of tumor-bearing mice and in the yield of tumor knots compared to their spontaneous appearance \( (6) \) were observed in both groups, but the duration of involvement in the experiment was significantly higher in the αMUPA mice (Table II).

The rate of tumorigenesis differed between mouse strains (yields of 1.5 and 2.1 for the αMUPA and WT strains, respectively) owing to a distinct tendency toward decreased tumor frequency in the skin and forestomach in the αMUPA mice (Table II). The length of time the tumor-bearing mice remained in the experiment was also significantly higher in the αMUPA mice (Table II).

### Table I. Survival rate of αMUPA and wild-type (WT) mice.

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Initial number</th>
<th>Type of treatment</th>
<th>Terminal number</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>30</td>
<td>DMBA</td>
<td>20</td>
<td>67</td>
</tr>
<tr>
<td>WT</td>
<td>32</td>
<td>Corn oil</td>
<td>21</td>
<td>66</td>
</tr>
<tr>
<td>αMUPA</td>
<td>30</td>
<td>DMBA</td>
<td>28</td>
<td>93*</td>
</tr>
<tr>
<td>αMUPA</td>
<td>26</td>
<td>Corn oil</td>
<td>25</td>
<td>96*</td>
</tr>
</tbody>
</table>

*Significantly different from wild-type value, \( p<0.05 \).

**Morphological analysis.** Lung tumors, the most widespread spontaneous tumors in mice, are located sub-pleural and are recognized as single or multiple clusters from alveolar-bronchiolar cells. They are usually adenomas of various structures. Malignant zones were found in large adenomas. A high number of lung tumors was observed in both mouse groups, but the survival rate of the lung tumor-bearing mice was significantly higher in the αMUPA mice (Table III).

The mouse stomach is composed of approximately equal-sized forestomach and glandular portions. Spontaneous squamous-cell carcinoma of the forestomach is unknown, but may be induced chemically: treatment with DMBA resulted in a high number of mice with tumors in the forestomach (Table IV). Benign tumors were squamous-cell papillomas, sometimes of epithelial cysts. The malignant tumors were squamous-cell carcinomas.

Spontaneous skin tumors in mice are very rare and in general, do not exceed 1% (7). The high number of skin tumors that appeared in our experiment in relatively young mice (Table V) was therefore quite unexpected. This may be related to the effect of DMBA as a representative of the carcinogenic polycyclic aromatic hydrocarbons (PAHs). These components are fat-soluble and their local and systemic effects are limited by the conditions of their deposition. Application of PAH to the skin causes squamous-cell papillomas, keratoacanthomas and squamous-cell carcinomas (8). DMBA caused a wide range of different types of tumors in the skin of the appendages and in the connective tissue: squamous-cell carcinoma, papillomas, keratoacanthomas, trichoepitheliomas, trichofolliculomas, sebaceous adenomas with cystic formations, angioleiomyoma and liposarcoma (Table VI, Figure 1).

Our observations indicate that the lungs, forestomach and skin are target organs for the carcinogenic effect of DMBA in long-living mice. The morphological characteristics of the tumors were similar in the compared mouse lines, *i.e.* transgenic αMUPA and their WT counterparts.

### Discussion

The αMUPA mice exhibited a distinctly higher survival rate in experimental, chemically-induced tumorigenesis than their
WT counterparts. Our experiment also verified that, despite their different rates of aging (4, 5), both mouse strains showed a similar response to the effect of DMBA when this response was evaluated in terms of number of tumor-bearing mice. Differences were found in tumor yield and in the duration of each mouse strain’s involvement in the experiment. The percentage of lung tumors was similar in both lines, whereas the number of tumors of the forestomach and skin was lower in αMUPA mice. All of these differences may be related to the correlation between a high rate of DNA repair and life span. A similar effect was described for the long-living mouse line C57Bl/6 vs. the short-lived line BALB/c (9).

Transgenic αMUPA mice spontaneously eat less (approximately 20%) and live longer (also approximately 20%) than their WT counterparts, thus resembling dietary-restricted mice (4, 5). Calorically restricted diets are known to be accompanied by a decrease in the number of spontaneous tumors (10). Two-year-old female mice of the αMUPA and especially MUPA-15 strains have been shown to exhibit higher resistance to the appearance of spontaneous tumors compared to the initial parental WT: there, the number of tumor-bearing mice was 16% and 62%, respectively (6). In WT FVB/N mice, the initial strain for the transgenic MUPA strains, survival to 24 months of age was approximately 60% in both sexes, and the incidence of mice with tumors at this time was 55% in males and 66% in females (11). The survival rate of the αMUPA mice after exposure to DMBA was 93%.

The well-known accumulation of DNA damage with age has prompted some researchers to suggest that the risk of spontaneous tumors should increase in long-living murine strains relative to short-lived ones (1, 11-13). However, no
Figure 1. Tumors of the skin and soft connective tissues. H&E. A, C, E x80. B,D,F x200. (A) Keratoacanthoma, (B) Sebaceous adenoma with cystic formation, (C) Trichoepithelioma, (D) Liposarcoma, (E) Angioleiomyoma, (F) Fragment of (E) Note abundance of small blood vessels (arrows) inside interlaced bundles of muscle fibers.
significant positive correlation between life span and tumor incidence has been found in the different strains of inbred mice: in some long-living and short-lived mouse strains, the incidence of spontaneous tumors is low, whereas in others, characterized by different life spans, spontaneous tumor incidence is high (from 80 to 100% of cases) (1).

Another relationship was found in homozygous transgenic long-living αMUPA mice and their parental WT mice. In our previous study, we found that the number of spontaneous tumors differed in the studied strains (αMUPA, MUPA-15 and WT); a significantly lower number of tumor-bearing mice was seen in both MUPA strains (6). Proliferative lesions were found in different tissues, mainly in the lungs.

The results of the present study indicate that αMUPA mice react to chemically-induced tumorigenesis with a significant increase, relative to the control WT, in survival rate and in the duration of the tumor-bearing mice’s involvement in the experiment, and by a distinct tendency toward a decrease in the number of tumors in the skin and stomach.

Aging promotes the accumulation of mutations in cells which are important for the initiation of carcinogenesis in target organs and tissues (3, 14, 15). Our observations, both published previously (6) and presented here, show that it is not the age itself but the rate of aging that promotes spontaneous and induced tumorigenesis.

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References


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