Evolution of Murine Melanoma in C57BL/6J Female Mice
A non-invasive evaluation

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One of the most aggressive type of cancer is represented by malignant melanoma, which represents a continuous challenge for researchers in various related fields given that the mechanism is not completely understood, and current treatments do not provide a high survival rate. Animal models have proven to be of great help in the study and understanding of the mechanisms of various cancers including malignant melanoma. Considering those described above, the purpose of this study was to obtain a murine melanoma model using C57BL/6J female mice by subcutaneously inoculation of a B164A5 cell suspension. For the experiments, were used female C57BL/6J mice with the age of 8-10 weeks, 8 mice/group. The day before cells inoculation all the animals were shaved on the back using a razor. B164A5 cell suspension was subcutaneously inoculated to the females and examined daily. All the mice used developed tumors and the values of the noninvasive measurements of the physiological skin parameters were discussed in this material.

Keywords: murine melanoma, C57Bl/6J, female, melanin, erythema

Melanoma is a tumor whose expansion is reflected in the melanocytes, the cells responsible for skin pigmentation, being both aggressive and fatal [1]. The incidence of malignant cutaneous melanoma (MCM) in human is increasing worldwide, from 21.1 to 49.8 per 100,000 people, faster each year than any other type of cancer [2]. The etiology of melanoma is quite complex - environmental factors such as exposure to UV radiation (trigger in about 65% of cases), and various genetic factors contribute to disease appliance. In order to study numerous cancer therapy, animal models have been shown to be highly effective. For the study of melanoma the most used animals are C57BL/6 mice and the murine B16 melanoma cell line, following well established protocols [3-6].

B16 melanoma 4A5 (known in the literature as B16 melanoma cells) is a tumor cell line that shows fibroblast-like features and is able to produce melanin. This cell line was obtained from C57BL/6 mouse skin and it is important to note that these cells may lose their capacity to produce melanin in long term culture. Recent studies used this type of cell line in order to verify the capacity of different agents as inhibitors of melanogenesis [7,8], but also to develop melanoma mouse models for the assessment of potential anticancer agents [9-11].

C57BL/6J is a substrain of C57BL/6 inbred mouse strain developed between 1940s and 1950s by the Jackson Laboratory [12]. The C57BL/6J mice lack mouse nicotinamide nucleotide transhydrogenase (Nnt) gene which plays major roles in the glucose homeostasis and in the insulin secretion [12]. This strain of mice is used very frequently in experiments concerning the development of melanoma animal models, due to its high compatibility with the B16 murine melanoma cells, but this type of mice are used, also, in aging research field [13].

Because the lack of a revolutionary and effective treatment for melanoma, its current study represents a continuing challenge for researchers.

The main purpose of this study was to evaluate certain specific cutaneous parameters, such as erythema and melanin that play major roles in the evolution of melanoma in female mice.

Experimental part

Materials and methods

In the experiment were used B164A5 murine melanoma cells purchased from European Collection of Cell Cultures at passage 3. B164A5 cells were kept in liquid nitrogen and one week before the experiment started, were cultured in high glucose (4.5 g/L) Dulbecco's Modified Eagle Medium (Sigma Aldrich, Germany) supplemented with 15 mM Hepes, 2 mM L-glutamine, 100 U/mL penicillin, 100µg/mL streptomycin, and 10% fetal bovine serum (FBS). After that, cells were kept in a humidified atmosphere with 5% CO_2 at temperature 37°C and were passaged every two days. Using a Neubauer chamber the cells were counted in the presence of Trypan Blue reagent.

The reagents used in the study, such as Dulbecco's Modified Eagle Medium (DMEM), phosphate saline buffer (PBS), penicillin, streptomycin, fetal bovine serum (FBS), Trypan Blue were purchased from Sigma Aldrich. The animals used were female C57BL/6J mice, 8-10 weeks-old, obtained from Charles River Laboratories, Hungary. All experimental procedures which involved animals, were conducted in accordance with the Directive 2010/63/EU and the experimental protocol was approved by the Committee for Ethics Research of the University for Medicine and Pharmacy of Timisoara, Romania. The murine melanoma model was obtained according to the literature [14].

Two main skin parameters (melanin and erythema) were assessed using a Multiprobe Adapter System, MPA5 - Courage-Khazaka (Germany) containing a Mexameter®MX 18 samples.

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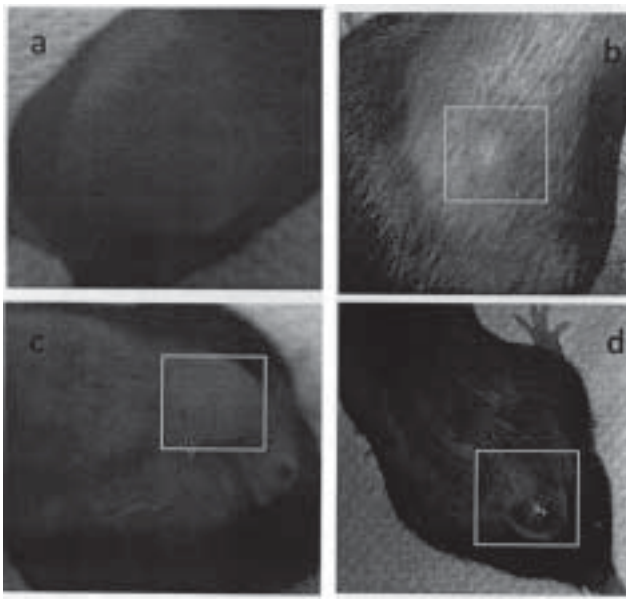

Fig. 1. Evolution of melanoma tumors after tumor cells were subcutaneous inoculated into the C57BL/6J female: a) Control group, b) at day 6 post inoculation, c) at day 13 post inoculation and d) at day 22 post-inoculation

The one-way analysis of variance and Bonferonni–Dunn tests were used to determine the statistical difference between experimental and blank groups; *, ** and *** indicate $p<0.05$, $p<0.01$ and $p<0.001$.

Results and discussion

The main objectives of this study were: a) to obtain a murine melanoma model using B164A5 cells and female mice and b) to evaluate the changes occurred in some skin parameters using noninvasive techniques. To obtain murine melanoma in female mice was used the line of B164A5 murine melanoma cells, prepared as a suspension of $1X10^6$ cells/100μL PBS/mice. The most important step in achieving tumors is the inoculation therefore, subcutaneous injection of the suspension of cells should be done carefully. After injection/inoculation should appear a bubble which disappears the next day. Animals were monitored daily and on the fourth day after inoculation there were seen early signs of cancer appearance. As it can be seen in figure 1, at day 13 the tumors were well-defined. At day 22 post-inoculation (fig. 1d) it can be observed that the shape of the tumor was round as a mole and, also, the color was changed, it became black, and this change can be explained by the amount of melanin secreted by the tumor cells - B164A5.

It is well-known that melanin is a natural substance that gives the color of skin, hair, and eyes. The exposure to UV radiation can cause alterations of DNA in skin cells, including the melanocytes, the cells which secrete melanin; these changes of melanocytes represent a major cause of skin cancer. In the past, scientists believed that melanin protects the skin from harmful effects of UV radiation, but there is evidence that melanin value would actually be associated with damage to skin cells [15].

In this experiment, a slight increase of melanin values was obtained for the studied groups; the most constant trend was obtained in the case of mice from blank group, while some important upward trend was observed in the case of female mice with tumors (fig. 2). These data are in accordance with the color of the tumor - black, due to a high content of melanin secreted by the tumor cells.

Erythema, trans-epidermal water loss (TEWL or TEWA), and the hydration/moisture of *stratum corneum* are three

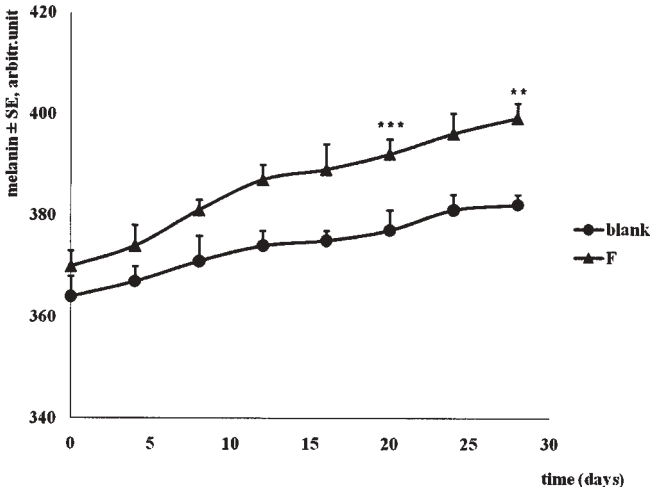

Fig. 2. Melanin evolution over a period of 28 days in the females (F) injected with B164A5 cells compared to the control group

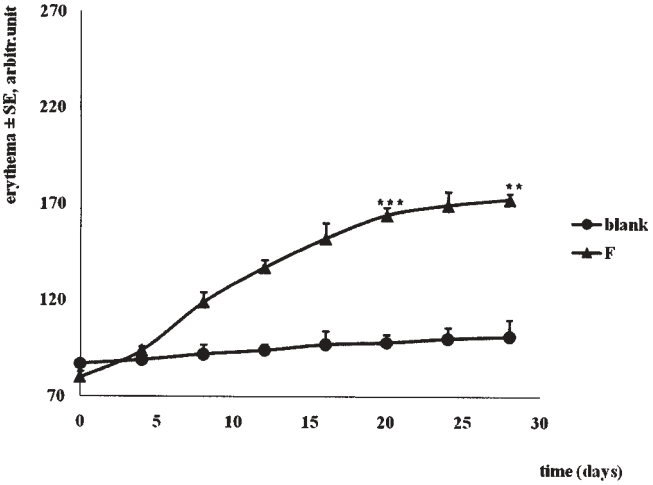

Fig. 3. Erythema evolution over a period of 28 days in the females (F) injected with B164A5 cells compared to the control group

very sensitive skin parameters. The values of these parameters change very much when the skin is altered, alteration that might be caused by a lesion/wound or in the case of contact with any chemical substance which present an irritation potential.

Significant differences between the blank and the other studied groups were obtained. It is important to mention that erythema increase occurred in every experiment on skin, but the ratio between erythema values and experimental period makes the difference between harmful and harmless agents. In this experiment, the following values of the ratio between erythema values and experimental period were obtained: 0.50 units/day (blank group), and 3.29 units/day (F group) values that indicate an important irritation potential as expected.

Conclusions

Thus, it can be concluded that C57BL/6J female mice represent an important tool in the understanding of the melanoma insights. Furthermore, the modifications observed in the values of specific physiological skin parameters indicated a link between the tumor evolution and the changes of skin parameters.

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Manuscript received: 3.08.2015