DEVELOPMENT OF THE B16 MURINE MELANOMA MODEL

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Summary
Cutaneous malignant melanoma (CMM) is an aggressive type of skin cancer with significantly high morbidity and mortality and an increased incidence in the last years. The aim of the present study was to develop a spontaneously - arising murine melanoma model employing the B16 murine melanoma cell line using C57BL/6J mice in order to observe the process of solid-tumor formation and the following metastatic process. In the day 20 after inoculation of B16 cells at C57BL/6J mice, histological studies revealed the presence of malignant melanoma and metastasis in liver, lungs and spleen. Our data show that the B16 mouse melanoma model is an easy to reproduce in vivo model of carcinogenesis that will be further used in order to test therapeutical agents against skin carcinoma.

Keywords: melanoma, B16 cell line, mouse, metastasis

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Introduction
Cutaneous malignant melanoma (CMM) is the most lethal form of skin cancer and its incidence has been steadily increasing worldwide, resulting in a major public health problem (Markovic et al., 2007). Melanoma is a malignant tumor derived from epidermal melanocytes and can occur in any tissue that contains these cells, including noncutaneous sites such as the oral mucosa, nasopharynx, paranasal sinuses, tracheobronchial tree, vulva, vagina, anus, urinary tract, central nervous system, and eye (Cummins et al., 2006).

The first description of melanoma as a disease entity was provided in 1806 by Rene Laennec, the inventor of the stethoscope, in his presentation to the Faculté de Médecine in Paris and the first published use of the word “melanoma” appeared in 1812 in Laennec manuscript, but references to “black cancer” and “fatal black tumors with metastasis” can be traced back to the writings of the legendary Greek physician Hippocrates in the fifth century B.C (Bennet and Hall, 1994; Chin et al., 1998).

A large body of evidence with respect to animal models of melanoma was provided in the last decade due to the increased incidence and high mortality of melanoma. Historically, the animals models studied in most detail have been the Xiphophorus hybrid fish and the South American opossum (Kusewitt and Ley, 1996; Dlugosz et al., 2002, Ley, 2002). Other melanoma models were developed on hamster (Della Porta et al., 1995), guinea pig (Pawlowski et al., 1980) and angora goat (Green et al., 1996). The problem with all such models, however, was that arising melanocytic tumors do not resemble human CMM from histopathological point of view, and extensive genetic manipulation is not possible. The mouse represents an especially attractive system because of the availability of an extensive genetic base.
upon which to build (Dlugosz et al., 2002). The Sinclair swine and dogs also developed melanoma tumors, but the murine models were by far the most widely employed animal model of melanoma and included the induction of tumors via application of physical agents or transgenic manipulations as well as the inoculation of mice with tumor-cell line such as the spontaneously occurring Harding-Passey, Cloudman and B16 cell lines (Carson and Walker, 2002).

The aim of the present study was to develop a spontaneously - arising murine melanoma model employing the B16 murine melanoma cell line using C57BL/6J mice in order to observe the process of solid-tumor formation and the following metastatic process.

Material and methods

Cell preparation. B16 melanoma 4A5 (ECACC and Sigma Aldrich, origin Japan stored UK ) cells were grown in DMEM media, supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 mg/ml) in specific cells culture plates. Cells were incubated with 1 ml of media at 37°C in an incubator for cell culture with 95% air and 5% CO₂.

Animals. C57BL/6J mice of eight weeks were purchased from Charles River (Sulzfeld, Germany). The experimental protocol followed all NIAH (National Institute of Animal Health) rules: animals were kept on a 12h/12h light/dark cycle, at a normal (24 °C) animal house temperature, humidity above 55%, were fed ad libitum and had free access to water. The experiments were approved by University of Medicine and Pharmacy Timisoara Bioethical Committee.

Experimental design. Melanoma model obtained by inoculation of B16 murine melanoma cell line. C57BL/6J mice (n=4/group) were shaved dorsally once a week with an electric razor, or as needed to remove hair. B16 cells (1x10⁶ cells in 0.5 ml in saline) were administered IP and in day 20, mice were killed.

Histology. Histological samples were prepared from skin samples and tumors from each group of mice and for each treatment. Samples tissue were fixed in 4% formalin solution which was improved with a silver colloidal solution (1/1 v/v). The histologically prepared samples were stained with hematoxylin and eosin (H&E) and examined by electron microscopy.

Results

Melanoma model obtained by inoculation of B16 murine melanoma cell line. The mice treated with B16 murine melanoma cell line suspension developed tumors rapidly in different organs, such as: skin, lung, liver and spleen. Lungs were the first organs affected by metastasis (Figure 1).
Histopathology of the skin lesions and tumors. Histological analysis of skin samples revealed dermic tumoral proliferation consisting of round and oval tumor cells with nuclei at the periphery (Figure 2).

![Figure 2](image1.png)

**Figure 2.** A. Wide area of skin ulceration in the surface (HE x 200); B. Dermic tumor proliferation (HE x 400); C. Round and oval tumor cells with nuclei at the periphery (HE x 400).

The histological lung samples showed pulmonary stasis, tumor proliferation with a large number of blood vessels and inflammation of bronchial wall. Tumor proliferation consisted in beaches and lobules of round and oval cells with vesicular nuclei and a small number of pleomorphic nuclei (Figure 3).

![Figure 3](image2.png)

**Figure 3.** A. Lung tumor proliferation with blood vessels (HE x 400); B. Pulmonary stasis combined with tumor proliferation (HE X 200).

Histological analysis of spleen samples indicated a tumor proliferation similar with the lungs one characterized by oval tumor cells with pale eosinophilic cytoplasm and vesicular nuclei (Figure 4).

![Figure 4](image3.png)

**Figure 4.** A. Splenic tumor proliferation (HE x 400); B. Oval tumor cells with pale eosinophilic cytoplasm and vesicular nuclei (HE x 400).
Another organ affected by metastasis was the liver. The liver presented a moderate stasis and a spot-type tumor proliferation, characterized by tumor cells with vesicular nuclei and blood vessels - similar with lung tumor proliferation (Figure 5).

**Figure 5.** A. Liver tumor proliferation with blood vessels (HE x 400); B. Tumor cells with vesicular nuclei (HE x 400).

**Discussion**

The use of animal models in the study of malignant melanoma represented an important source of information about the disease mechanisms, the molecular events in cells and their progress towards malignancy. Inoculation of B16 cells determined the rapid development of melanoma, followed by metastasis in lung, liver and spleen, in less than a month, thus confirming the results obtained by Waheed Roomi M. (2008).

The initial neoplastic lesion arose in the skin at the base of the ear and the tumor is described as metastatic to lung, liver and spleen (Green, 1968; Alvarez, 2002).

In 1970, Dr. Isaiah Fidler carefully documented the final disposition of the melanoma cells after IV injection in mice. In order to develop this study B16 cells were labeled with $^{125}$I-5-iodo-2-deoxyuridine. At 14 days after injection only the lungs contained labeled cells that could be observed as tumor nodules in that moment.

Liver, spleen, kidneys and blood all showed the early presence of the labeled cells, but none of these tissues show the establishment of tumors at 14 days postinjection. Intravenously-injected B16 melanoma cells in mice could lead to rapid accumulation of cells in the pulmonary tissue, lead to early high levels of tumor cells in circulation or ultimately produce a very low rate of tumor formation in the animals (Fidler, 1970, Alvarez 2002). Starting from his initial studies, Fidler obtained new subtypes of B16 cells, some of them with a higher metastatic potential (B16-F1 and B16-F10) (Fidler, 1973).

**Conclusion**

In vivo melanoma models represent a great step in oncology research due to their great similarity to clinical metastasis and we could easily reproduce the B16 mouse melanoma model in our Labs. View the relative short delay of time needed to reproduce it, we hope to get further insights not only in the steps of carcinogenesis but also in assessing the anti-melanoma effects of different natural or chemical agents.

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References
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