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Growth and metastasis of human malignant melanoma SK-MEL-2 cell line in SCID mice

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Abstract An *in vivo* model for human melanoma was established with the growth and metastasis of SK-MEL-2 cells. The tumor was introduced into C.B-17 SCID(severe combined immunodeficiency) mice intraperitoneally, subcutaneously and intravenous inoculations. Tumors developed in 100% of mice inoculated subcutaneously and intraperitoneally, both at site of inoculation and as metastatic tumor in the liver, lungs and diaphragm. With intravenous inoculation, 50% of mice showed metastasis in the spleen. Additionally, metastatic foci that were not detected either by gross and/or standard histopathologic examination, were demonstrated in the spleen and lungs by immunohistochemistry with HMB-45 monoclonal antibody. We conclude that the SCID mouse supports growth and metastasis of human malignant melanoma SK-MEL-2 cells.

Key words: immunohistochemistry, melanoma, metastasis, SCID, SK-MEL-2

Introduction

The growth of human tumors in nude mice might be limited by their residual immunity.[1] Young or the immunosuppressed nude mice are much better hosts for human tumor growth than adult mice, which suggests that some immune resistance may develop with age.[2] Thus a mouse with broad immune deficiencies might serve as a suitable host for human tumor grafts. The C.B-17 SCID(severe combined immunodeficiency) mouse lacks both functional B- and T-cells due to nonfunctional rearrangements of immunoglobulin and T-cell receptor genes.[3,4] Natural killer cells, macrophages, and other hematopoietic cell lineages do not appear to be affected by the autosomal recessive SCID mutation.[5-7] The SCID mice have been found to support the growth and metastasis of several human tumor cell lines as well as fresh human tumors.[8-10]

Current therapy of melanoma in human has limited efficacy. The most active standard cytotoxic chemotherapeutic agents result in response rates of only 10-20% with no survival benefit.[11] Several human melanoma cell lines were recently reported to spontaneously metastasize in athymic nude mice,[12-14] and SCID mice[15,16] providing useful animal models for studying the fatal clinical disease. While, *in vivo* models in SCID mice showing tumor growth of 70% with human malignant melanoma cells using two cell lines (A375P, C8161), and metastasis ranging from 20 to 30% with subcutaneous and intraperitoneal exposure respectively[15] have been demonstra-

ted, new models with the higher tumor take rate and metastasis are needed.

The objectives of the present study were to determine the incidence of growth and the extent of metastasis of human melanoma cells in the SCID mouse. The experiment demonstrated that the SCID mouse was capable of supporting growth and metastasis of human malignant mela -noma.

Materials and Methods

Animals

C.B-17 *scid/scid* were bred and maintained in the specific pathogen-free animal facility at Korea Research Institute of Bioscience and Biotechnology. Animals were given autoclaved food and water *ad libitum*. Mice of either sex were used at 6-8 weeks of age. All manipulations were carried out in a Clean Rack(positive system, Dae Han Laboratory Animal Research Center Co., Korea).

Cell line and inoculations

The human malignant melanoma cell line, SK-MEL-2, cultured at 37℃, 5% CO_2 in RPMI 1640 tissue culture medium and supplemented with 10% fetal bovine serum(FBS) and penicillinstreptomycin. The cells were detached from tissue culture plastic with 0.05% trypsin -0.02% EDTA, washed twice and resuspended as a single cell suspension in Ca^{2+}, Mg^{2+}-free Hanks' balanced salt solution. SK-MEL-2 cells(2×10^6 cells/200$\mu\ell$) were inoculated subcutaneously into the flank, intraperitoneally, and intraveneously through the lateral tail vein. Tumor growth at the inoculation sites was measured three times weekly.

Histology and immunohistochemistry

Tissues were fixed in 10% buffered neutral formalin, embedded in paraffin, cut at approximately 4 microns, stained with H-E and examined microscopically. Immunohistochemical studies using mouse monoclonal antibodies against the HMB-45 (Zymed Laboratories Inc., CA)[17]melanoma-related antigens confirmed the presence of human melanoma cells in mouse tissues.

Results

Table 1 shows the incidence of tumors both at inoculation site and metastasis. Mice injected subcutaneously developed local tumor growth. The time to development of a palpable tumor varied from 7.5 to 11 weeks(median 9.5 weeks).

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Fig. 2. Abdominal cavity; mouse. Variably sized tumor nodules(arrows) were detected at the mesentery and in ascitic fluid.

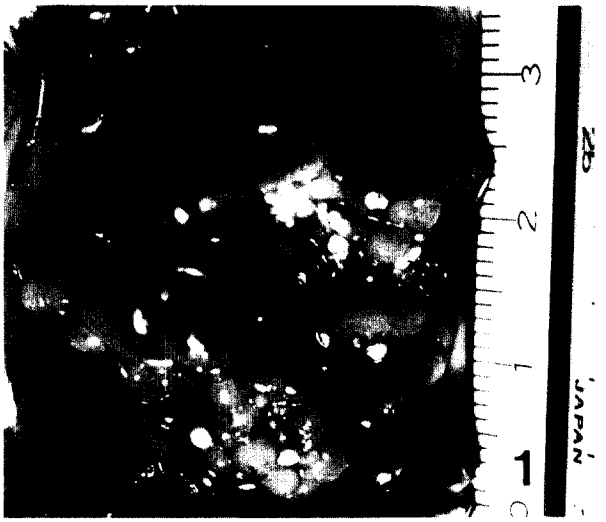
Fig. 3. Mass; mouse. Note the large pigmented melanoma cells with pleomorphic nuclei and prominent nucleoli(arrows). H-E. Bar=40μm.

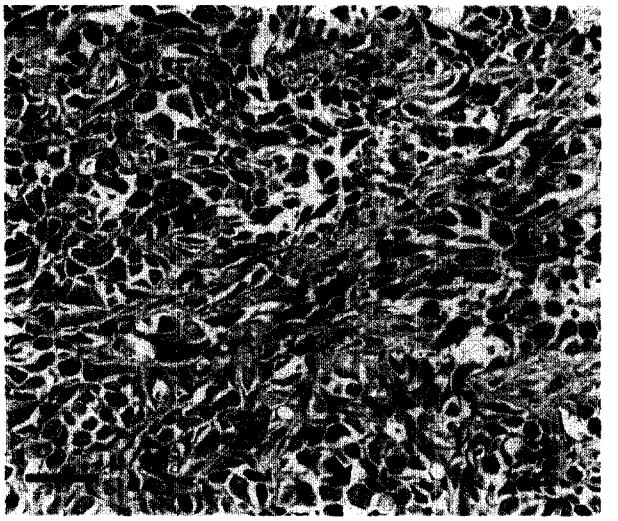
Fig. 4. Mass; mouse. Note the HMB-45 melanoma antigen(arrow) in the cytoplasm of the melanoma cells. ABC methods. Mayer's hematoxylin counterstain. Bar=20μm.

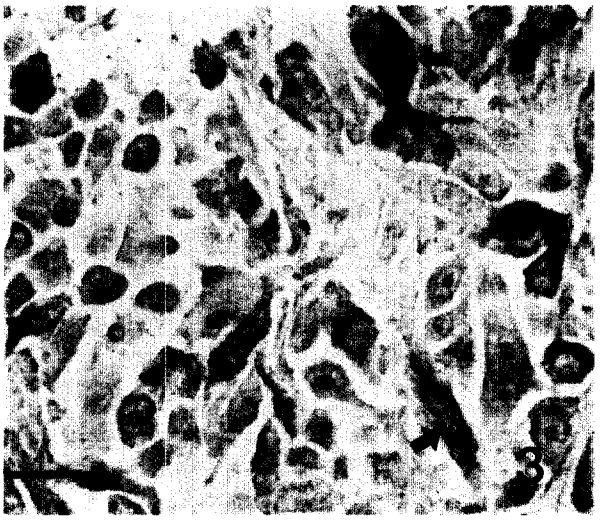
Fig. 5. Liver; mouse. Note the HMB-45 melanoma antigen(arrows) in the metastatic tumor in portal area. ABC methods. Mayer's hematoxylin counterstain. Bar=40μm.

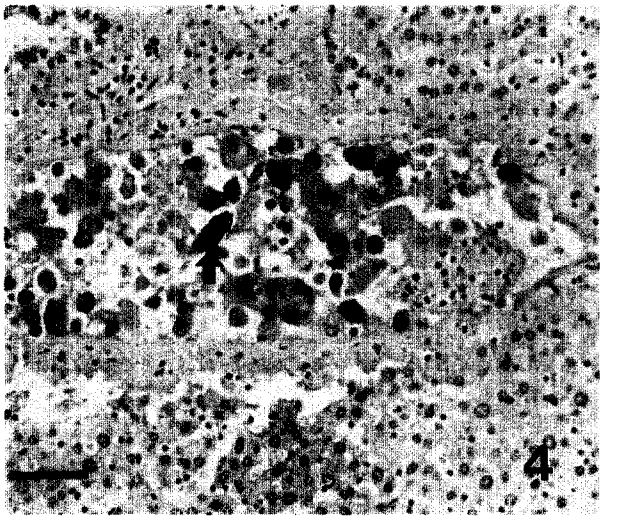
Fig. 6. Spleen; mouse. Note the HMB-45 melanoma antigen(arrows) in the melanoma cells infiltrated in the spleen. ABC methods. Mayer's hematoxylin counterstain. Bar=40μm.

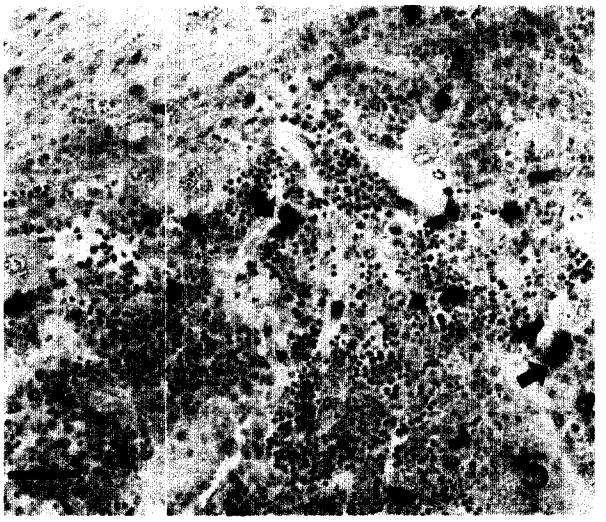
Fig. 7. Lung; mouse. Note the HMB-45 melanoma antigen(arrows) in metastatic tumor cells in spleen. ABC methods. Mayer's hematoxylin counterstain. Bar=40μm.

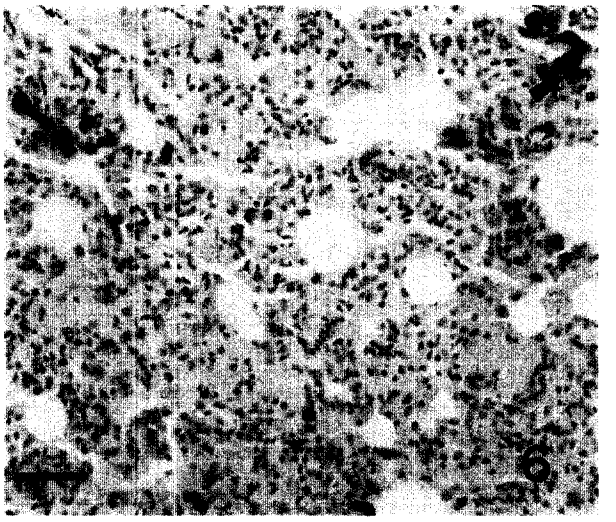












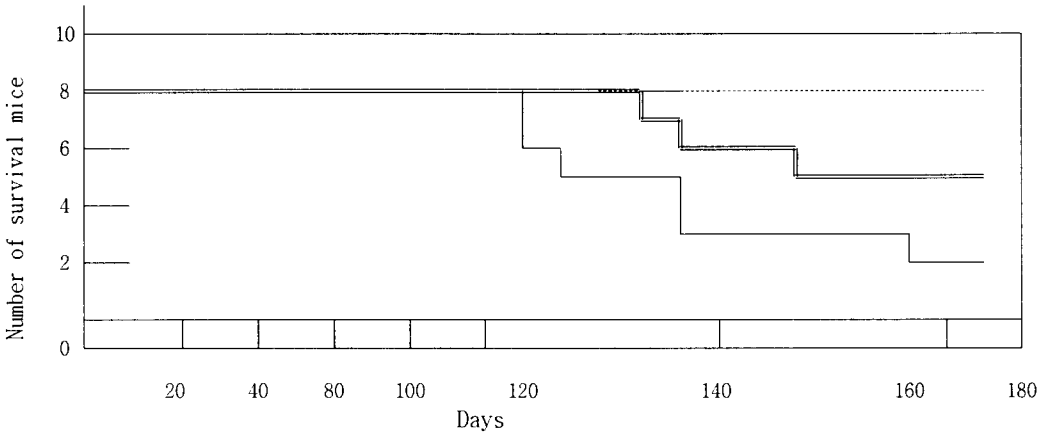


Fig. 1. Survival of SCID mice with SK-MEL-2 melanoma metastases. SK-MEL-2 was inoculated subcutaneously (═), intraperitoneally (—) and intravenously (---).

Table 1. Growth and metastasis of SK-MEL-2 cell line in SCID mice

Route of tumor inoculation*	Incidence of the primary tumors	Incidence of the metastasis	Sites of metastasis (Number of animals)
i.p.	8/8	5/8	liver(5), diaphragm(4), lung(1)
s.c.	8/8	1/8	lung(1)
i.v.	0/8	4/8	spleen(4), lung(2)

* : i.p.; intraperitoneal inoculation, s.c.; subcutaneous inoculation, i.v.; intravenous inoculation

All eight melanomas inoculated subcutaneously were successfully transplanted into SCID mice. After initial detection, all subcutaneous tumors grew progressively and did not regress. 63% of mice inoculated subcutaneously survived beyond 23 weeks after tumor inoculation(Fig. 1).

After intraperitoneal inoculation, tumors were commonly observed in the liver, diaphragm and on peritoneal surfaces of abdominal organs, and a rare lung metastasis in 1 mouse(Table 1). All eight melanomas inoculated intraperitoneally were successfully transplanted into SCID mice. Variable size (1-5mm in diameter) tumor nodules were readily detected in the mesentery and on peritoneal surfaces of the liver and spleen. Ascitic fluid also contained viable tumor cells(Fig. 2). 25% of mice inoculated intraperitoneally survived beyond 23 weeks after tumor inoculation(Fig. 1). Tumor growth were not observed in the body cavities and organs by 23 weeks.

Both subcutaneous and intraperitoneal inoculation resulted in parenchymal invasion of the liver, lung and diaphragm. Primary and metastatic tumors were composed of pigmented cells with pleomorphic nuclei and prominent nucleoli (Fig. 3). Subcutaneous and intraperitoneal tumors were composed of tumor cells supported by few intratumor blood vessels and extensive zones of central necrosis. Immunohistochemical studies with monoclonal antibody against human melanoma-related antigen, HMB-45, confirmed the presence of human malignant melanoma cells in the mouse tissues. HMB-45 positive cells

were detected not only in primary tumors(Fig. 4), but also in metastatic foci in liver, lung and diaphragm. The metastatic tumor cells were observed in the portal vein of the liver following intraperitoneal inoculation(Fig. 5). Additionally, metastatic foci that were not detected either during gross and histopathologic examination using conventional H-E stain, were later demonstrated in the spleen(Fig. 6) and lung(Fig. 7) by immunohistochemical stainings.

Discussion

In this report, we described that the SCID mouse supports growth and metastasis of human malignant melanoma cell. In this study, 8 of 8 human melanoma SK-MEL-2 cell lines grew progressively after subcutaneous and intraperitoneal inoculation into C.B-17 SCID mice. A noteworthy event was that 5 of 8 melanomas spontaneously metastasized in the liver, diaphragm and lung following intraperitoneal inoculation. The metastatic process involves distinct phases, including the release of tumor cells from the primary lesion into blood or lymph vessels, the ability of tumor cells to arrest in distant sites and to invade the extracellular matrix.[18,19] We observed similar event of vascular invasion in the portal vein of the liver after intraperitoneal inoculation which suggested hematogeneous dissemination. During the last several years, the growth and dissemination of a human melanoma with established cell lines and fresh tumor cells have been described.[15,20] Taylor et al.[15] reported that the highest take rate occurred with established melanoma cell line (70%) and that tumor incidence in SCID mice varied from 77% after intraperitoneal inoculation to about 45% with subcutaneous and intravenous inoculation. We observed that tumor incidence after intraperitoneal and subcutaneous inoculation in SCID mice was 100%. The high rate of tumor growth and spontaneous metastasis in our study may be an indication that melanoma propagates and disseminates in SCID mice more readily than in other congenitally immunodeficient strains. Previous studies reported that the SCID mouse gave superior growth and metastatic spread of transplanted human germinal cell tumors when compared with nude mice.[21] In our SCID mouse model, detailed pathologic examinations were required to detect all sites of tumor spread, and immunohistochemical studies using HMB-45 monoclonal antibodies facilitated demonstration of tumor foci in the spleen and lung that were not initially detected during gross and conventional histopathologic examinations. Studies with the SCID mouse model described herein will allow *in vivo* investigations of new cytotoxic drugs, immunomodulatory cytokines and monoclonal antibody therapies with relevance to human malignant melanoma.

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