SMGroup

Human Skin Melanoma Cell Lines Collection

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INTRODUCTION

Melanoma Cell Collection of 19 human skin melanoma cell lines derived from patients' metastases was created in N. N. Blokhin Russian Cancer Research Center. The cell lines were encoded as *mel Kor, mel Mtp, mel Il, mel Is, mel Si, mel Me, mel Gus, mel Z, mel Ksen, mel Hn, mel Gi, mel Ibr, mel R, mel Rac, mel Ch, mel Bgf, mel H* and *mel Cher*, except for *mel P*. All the lines are stored in the biobank of cryopreserved biological materials in liquid nitrogen at - 196 ˚C as working bank and seeding bank in N. N. Blokhin Russian Cancer Research Center. 16 of these cell lines are stored in Russian Cell Culture Collection of Vertebrates (**RCCC V**, St. Petersburg).

Melanoma cell lines are specified for the following characteristics:

- Expression of genes immune and cancer-testis antigens;
- Regulation of anti-tumor immune response involving non-classical human Major Histocompatibility Complex **(MHC)** molecule HLA-E;
- Potential for gene recombination by tag-7 and GM-CSF for secretion of corresponding proteins (*short Peptidoglycan Recognition Protein*, **PGRP-S** and **GM-CSF),** which are chemotaxic towards peripheral blood monocytes and activation of dendritic cell maturation;
- Status of vasculogenic mimicry;
- Effect of WNT-ligands on phenotype;
- Concentration of mutant KFP with spectral fluorescent features for the prognosis of sensor development based on induction resonance energy.
- Potential for developing subcutaneous xenografts in immune-deficient mice.

A panel of methods including flow cytometry, immune fluorescence, immuno cytochemistry, PCR and experimental chemotherapy, proved the variety of the cell lines according to their cultural, cyto-morphological, molecular-genetic and pathological-physiological characteristics.

Melanoma cell suspensions were obtained from the surgically removed metastases of patient's skin melanoma and cultivated *in vitro* with 20 passages. By passage 15 stable cell lines were established, which mostly presented a monolayer with colonies as the center of cell monolayer growth. The studies have shown that certain cell lines grow as semi-suspensions - some part of these cells grows in adhesion to the plastic surface; another part grows in suspension (Figure 1).

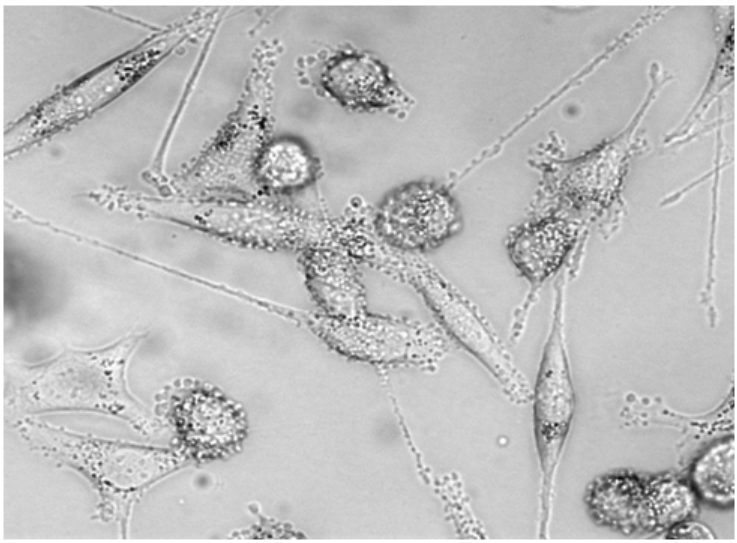

Figure 1: Cell line *mel Gi* (photo of the culture, ×100).

Cytograms with no cells of lymphocyte pathological signs revealed cellular polymorphism, which is characteristic for melanoma cells. The cytological examination showed that the cell lines consist of epithelioid, spindle-like and nevus-like cells or their combination. Cellular variety also refers to the grade of polymorphism and atypical forms, pigment concentration, mitosis, and number of giant multinuclear and single-nuclear cells. The epithelioid cell lines include a large number of giant multinuclear cells, chaotically distributed among single-nuclear elements. Cell line *mel R* includes mostly cricoid-like cells of different size, partly cells with oval or round shape with 1-3 mitosis in the visual field. Cytograms of spindle-like melanoma lines include cells of this shape (elongated or spindle-like) with short or long cytoplasmic extensions, which may interweave with other cytoplasmic filaments. Multinuclear cells with 3-5 nuclei in a small number differ by their shape from giant multinuclear cells of epithelioid melanoma. Cell lines of nevus-like cells include medium-size and small cells of oval, elongated and multi-filament shapes; 4 cultures develop pigment in the cytoplasm.

Cytomorphological characteristics of the cell lines are markedly different by the grade of differentiation. Cell lines *mel Si* and *mel Me*, are highly differentiated and include rather monomorphological and giant multinuclear cells with practically no mitosis (Figure 2).

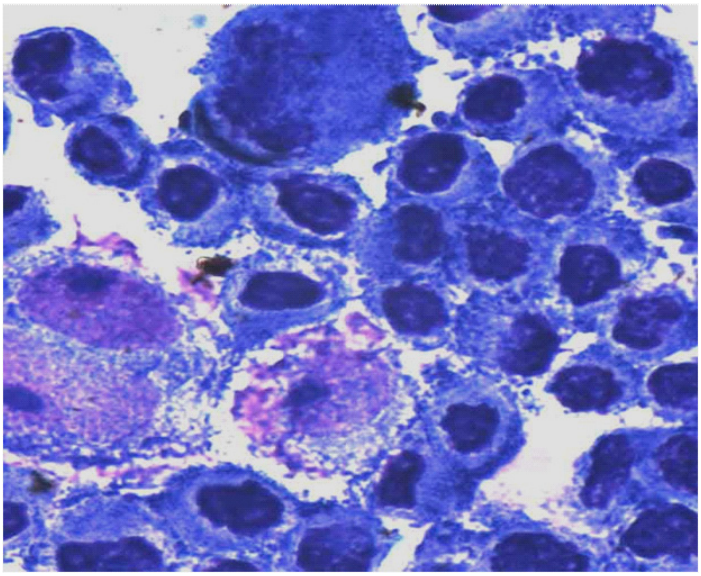

Figure 2: Cell line *mel Si* (photo of the culture, ×100).

Melanoma lines *mel BgF, mel Il, mel P, mel R, mel Ksen, mel Z, mel Hn* are moderately differentiated since they include small, medium or large cells with extensive basophilic cytoplasm with no clear contours or with fringe margins and eccentrically or centrally located nuclei, which have rough-lumped hyper chromic chromatin with one, rarely 2-3 nucleolus and low differentiated cells as well as giant multinuclear cells with a small number of mitosis (Figure 3).

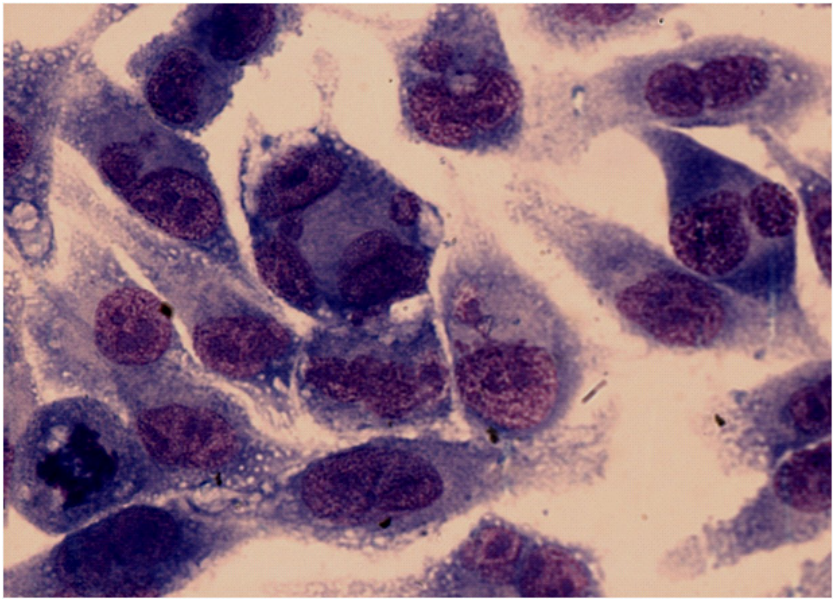

Figure 3: Cell line *mel Il* (photo of the culture, ×100).

Low differentiated cell lines *mel Kor, mel Gus, mel Mtp, mel Is, mel Gi, mel Ibr, mel Cher, mel Ch, mel H,* and *mel Rac* with multiple atypical mitosis include a large number of clearly polymorphic ugly giant cells with hyper chromic nuclei, irregular contours and membrane, 1-6 nucleolus and rough-lumped round, oval, elongated or irregular shaped chromatin (Figure 4).

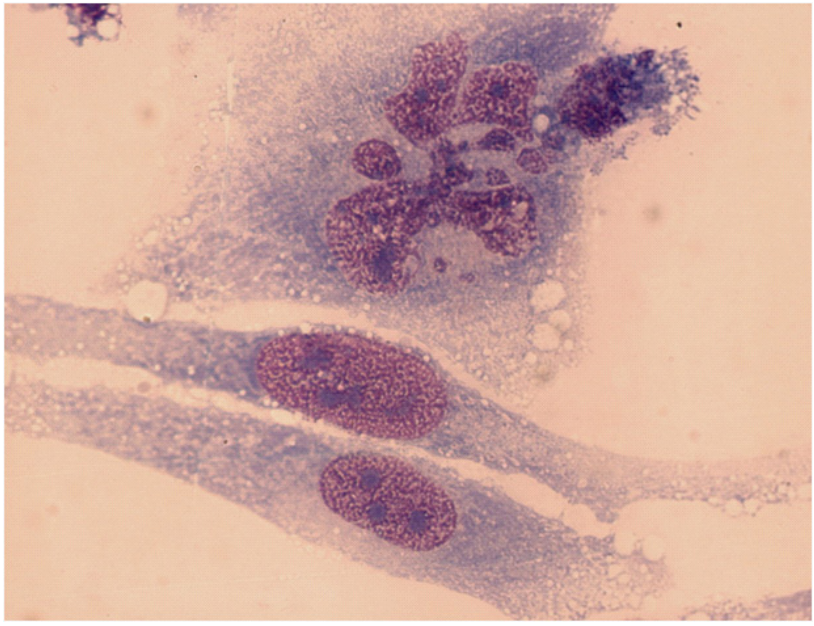

Figure 4: Cell line *mel Mtp* (photo of the culture, ×100).

Cytogenetic analysis has demonstrated that all the lines have altered in chromosome number and structure chromotype. Modal number of chromosomes corresponds to diploid-like and triploid-like number. Some cultures have cells with double number of chromosemes (tetraploid and hexaploid). These cultures have nuclei with different diameters while the staining density and chromatin status are the same, which are typical for interphase nuclei. Taking into account doubled structural chromosome abnormalities (e.g., circular chromosome) it is possible to suggest that there is an increased ploidy in the cell culture rather than two different cell lines in one culture. Karyotyping showed that modal number of chromosomes in highly differentiated cell lines corresponds to diploid-like cromotype and is 46-49 in lines *mel Me* and *mel Si*, and 47-50 in *mel Me*. Both cultures have 4/1 cell of total number of identified chromosomal damages including structural and in number (aneusomia). Moderately differentiated cell lines *mel IL, mel P* and *mel BGF* have 5-7/1 cell of revealed chromosome abnormalities; the lines are of different ploidy - di-, tri-, and tetraploid. Low differentiated cells have modal number of chromosomes corresponding to triploid chromotype - lines *mel Kor, mel Gus, mel Mtp, mel Is, mel Gi* and *mel Ibr*, tetraploid - lines *mel Cher*, *mel Chu* and *mel H* and diploid-like - line *mel Rac*. Most low differentiated cell lines have >5 chromosome abnormalities identified in structure and number, such as diploid culture *mel Gus*, where it reached 11 alterations.

Antigens of the cell lines include cancer-testis antigens [1], MHC antigens, and melanoma-associated antigens (melanoma differentiation markers). Expression of CD63 is registered in all melanoma lines, Melan A/MART1- in lines *mel Kor, mel IL, mel Is, mel Me, mel Gus, mel Z,* and *mel Hn*; tyrosinase - in *mel Cher, mel Kor, mel IL, mel Is, mel Si,* and *mel Z*; HMB45 - in *mel P, mel Kor, mel IL, mel Is, mel Si, mel Me, mel Gus, mel Z, mel Ksen,* and *mel Hn*.

Immunophenotype of all cell lines (markers of transplantation of immunity) is characterized by the lack of CD3 and CD20 - lymphoid markers of T- and B-lymphocytes. Expression of HLA-ABC antigen is registered in all cell lines except for *mel Kor*, while HLA class II - only in lines *mel Mtp, mel Is, mel Si, mel Me, mel Gus, mel Ksen, mel Hn, mel Gi, mel Ibr, mel Rac, mel Ch*, and *mel Cher*, with 14.3-97.8% of antigen-positive cells. Cell line *mel Kor* has neither MHC antigens of class I (HLA-A,B,C) nor II (HLA-DR). Most frequent genes are: A26(10) - in *mel P, mel Kor, mel Mtp, mel Il, mel Z, mel Rac*; HLA3 - in *mel Kor, mel Mtp, mel Si, mel Gus, mel Ch, mel H*; HLA2 and HLA23 in *mel P, mel Il, mel Me, mel Hn, mel Ibr, mel R, mel Rac, mel Bgf, mel Cher*. CD86 was revealed in *mel P* only; CD80 - 10-24.4% of antigen-positive cells-in lines *mel P, mel IL, mel Is, mel Me, mel Rac, mel Cher*. Cell line *mel P* expresses both molecules CD86 and CD80. All cell lines express surface antigen CD54, adhesion molecule ICAM-1, ligand for LFA-1 and MAC-1. Antigen HMW-positive (by monoclonal antibody ICO218 staining) were 71.4% of cell lines *mel P, mel Kor, mel Mtp, mel Il, mel Si, mel Gi, mel Ibr, mel R, mel Rac, mel Ch* [2].

Tumor stem cell markers (CD133, CD117, CD90, CD34, CD44 and CD24) of different expression rate and various combinations were registered in all cell lines [3].

The most frequent gene which is observed in 95% cell lines isS100b, a little less frequent - in 84% of cell lines - gene SILV, in 79% - gene TYR and in 74% -gene MLAN.

Cancer-testis genes are expressed at different rates: MAGE A2 is revealed in 95% of cell lines, while MAGE B2 - in 37% only [1].

Analysis of the therapeutically significant genes *BRAF* and *NRAS* as well as their mutations showed that *BRAF* was expressed in melanoma lines *mel P, mel Il, mel Is, mel Si, mel Z, mel Hn, mel Gi, mel Ibr, mel R, mel Ch, mel BGF, mel H*, and *mel Cher*, with substitution of *V600E* in *mel P, mel Is, mel Si, mel Z, mel Hn, mel Gi, mel Ibr, mel R, mel Ch, mel H, and mel Cher*; substitution of *V600K* - in *mel Il*, and *mel BGF*; substitution of *L597Q* - in *mel Si*. Mutation *Q61K* of *NRAS* was registered in *mel Kor, mel Mtp, mel Me, mel Gus,* and *mel Rac*. Cell line *mel Ksen* had no mutations *BRAF* and *NRAS*.

Melanoma lines *mel Cher/BRAF*$^{+}$*, mel Rac/NRAS*$^{+}$ and *mel Ibr* demonstrated potential for adaptation *in vivo* to form stable subcutaneous xenografts in immune-deficient mice *Balb/c nude*; the first two lines were highly sensitive to specific target therapy by Vemurafenib and Trametinib, respectively [4].

Thus, the characteristics of the cell lines included in the Collection of human skin melanoma cell lines present an important foundation for using these lines in basic cancer research to study progression of disseminated melanoma and new prognostic factors of the course of disease; for creating new in vivo models of melanoma; and for developing new anti-melanoma drugs for practical oncology including whole-cell vaccines, target agents and new drug combinations. Practical importance of the Collection is based on the usage of cell lines and subcutaneous xenografts *mel Cher/BRAF*$^{+}$ with proved potential for vasculogenic mimicry [5] in pre-clinical studies of a novel agent targeting integrin alpha-V-beta-3; fluorescent cell line mel Kor-Turbo RFP - used for visualization of metastatic cells in PDT [6]; and whole-cell vaccine “Allogen” on the basis of tag7 transfected cell line *mel P* [7] in clinical studies in patients with disseminated melanoma.

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