Melanoma Cell Lines Role in Obtaining New Drug Candidates for Combating the Malignant Pathology of the Cutaneous Organ

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As prophylactic and therapeutic approaches for melanoma, of great interest and importance are the in vitro studies using cell lines to elucidate several tumoral phenomena. Therefore, the similarities and differences between the different tumor cells must be known and understood in order to obtain a more accurate correlation with processes that occur in vivo. In this study, six cell lines of melanoma, both of mouse and human origin were analyzed from the point of view of cell culture growth, morphology and use in the research of new therapies. In brief, the current paper exhibits a comparison of melanoma cells which can be utilized as a starting point for further in vitro studies and in vivo animal models.

Keywords: melanoma, cells, in vitro, therapy, drugs candidates

People diagnosed with melanoma in the metastatic stage have a drastic prognosis related to survival that is limited to two years. A very aggressive and rare form of melanoma with a reduced survival rate is the sinonasal mucosal melanoma [1]. Despite the significant progress recorded in recent years regarding the prevention, treatment and diagnosis of malignancies from a clinical point of view, it has not yet been possible to find therapies that significantly improve the life expectancy of patients diagnosed with melanoma [2]. A major problem that contributes to the complication of diagnosis and prognosis is related to the diversity of melanoma tissue morphologies, from macroscopic level - associative lesional structures at microscopic level - various cellular forms along with all erroneous changes from molecular and biochemical levels [3, 4]. Studies related to DNA evaluation of lesions and those related to melanoma cell line bases reveal consistent taxonomy of genomic aberrations and transcriptional signatures contributing to highlighting the importance of *in vitro* preliminary studies for finding new therapies to treat melanoma [3, 5].

A possible scenario is based on heterogeneity that combines how melanoma cells behave in different micro-media and the influence manifestation of their own molecular states taking into account that the molecular models currently used for melanoma progression are homogeneous [3]. Molecular studies still represent a challenge for researchers in the field because of the certainty that progression is positively influenced by the constant evolution of molecular changes [6].

Experimental part

Materials and methods

In the current study were selected six different melanoma cell lines, both of murine and human origin, namely: mouse melanoma B16-F0 (ATCC® CRL-6322™), mouse melanoma B16-F10 (ATCC® CRL-6475™), mouse melanoma B164A5 (ECACC 94042254), human melanoma SK-MEL-5 (ATCC® HTB70™), human melanoma SK-MEL-28 (ATCC® HTB72™) and human melanoma SH-4 (ATCC® CRL-7724™) purchased from European Collection of Authenticated Cell Cultures (ECACC) and American Type Culture Collection (ATCC) as frozen vials. Part of the specific characteristics of cells can be seen in table 1.

For cells culture protocols the following specific reagents were needed: culture specific media: Dulbecco's Modified Eagle's Medium for B16-F0, B16-F10, B164A5 and SH-4, and Eagle's Minimum Essential Medium for SK-MEL-5 and SK-MEL-28, supplements: fetal bovine serum (FCS) to a final concentration of 10%, a mixture of antibiotics solution (penicillin + streptomycin – final concentration -1%), and other reagents as : Trypsin/EDTA solution and phosphate saline buffer - PBS. The culture media, supplements and reagents were purchased from ATCC, Sigma Aldrich (Germany) and Thermo Fisher Scientific (USA). All the procedures were realized in standard conditions, cells were preserved at 37°C, 5% CO_2 and have been constantly pursued in culture for macroscopic evolution or microbial contamination.

Table 1
SPECIFIC CHARACTERISTICS OF THE MELANOMA CELLS USED IN THE CURRENT RESEARCH STUDY

Cell type	Organism	Tissue of Origin	Disease
B16-F0	mouse, C57BL/6J	skin	melanoma
B16-F10	mouse, C57BL/6J	skin	melanoma
B164A5	mouse, C57BL/6J	skin	melanoma
SK-MEL-5	human, 24 years, female	skin: derived from metastatic axillary node	malignant melanoma
SK-MEL-28	human, 51 years, male	skin	malignant melanoma
SH-4	human, 60 years, female	skin	melanoma

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Results and discussions

The use of tumor cell lines as models to investigate different molecular processes, like the development, growth and progression of a malignancy, dates since the obtention of the first immortalized cancer cell line (HeLa), in 1951, and still offers multiple advantages, as: an understanding of the molecular pathways involved in diseases tumorigenicity and therapy resistance and the possibility to develop new targeted therapies [7].

The *in vitro* models of melanoma accounts approximatively 5000 cell lines generated, but only for over 200 cell lines exist a genetic profile and a biological characterization [4]. Besides the major role played by cell lines in cancer research, there were also described several limitations, like: 1) loss of stromal, vascular, and immune cellular populations what leads to a different behavior in culture as compared to *in vivo* conditions; 2) possible selection of a subset of clones compliant with culture growth conditions, and 3) lack of *in vivo* microenvironment and the absence of the interactions, thus being difficult or even impossible to recreated these processes *in vivo* [4,7]. There were described disparities in transcriptional and genomic profiles between tumor samples harvested and cancer cell lines for breast, colorectal, ovarian, and also for melanoma [7-10]. Differences between tumor cell lines of the same origin were also stated, and the selection of a cell line for a research study represent a major step in establishing an experimental work plan.

This study aims to highlight the differences between several murine and human melanoma cell lines that are frequently used as models for melanoma studies in the literature.

At a search on PubMed database, using as keywords B16-F10 melanoma cell line, the result obtained consisted of over 2000 publications on this topic, indicating that this cell line is very frequently used, and this kind of model is reproducible.

According to the manufacturer, B16-F10 (ATCC® CRL-6475™) murine melanoma cells present a morphology of spindle-shaped and epithelial-like cells, as it can be observed in figure 1 - 2 a, b. This cell line is described as a subclone of B16 tumor parent line obtained from C57BL/6J mice, by injecting the mice with B16 tumor cells, collecting and culturing secondary growth tumors, procedure that was repeated 10 times. Moreover, it is known that B16-F10 presents a high metastatic potential, leading to lung metastasis after intravenous inoculation [11, 12]. B16-F10 melanoma cells are melanin-producing cells, ability that decreases after multiple passages *in vitro*, phenomenon noticed also in the case of B16-F0 and B164A6 cells. Oxygen deprivation and serum deficiency in culture, determined B16-F10 cells to adapt to the novel conditions by increasing the number of migrating cells and their metastatic potential [13]. This cell line is a suitable transfection host for C57BL/6 and immunosuppressed mice developing subcutaneous tumors (after subcutaneous inoculation) and lung metastasis (after intravenous inoculation in the tail vein) [14,15].

Another melanoma cell line of murine origin presented in this study is B16-F0 (ATCC® CRL-6322™). The morphology of B16-F0 cells is similar to the one described for B16-F10, spindle-shape and epithelial cells (fig. 1-1 a, b). These cells are also capable to produce melanin and were isolated from the skin of C57BL/6 mice but are less metastatic as compared to B16-F10 cells and less applied as *in vitro* model in melanoma studies (environ 130 publications on PubMed database). B16-F0 cells presented a lower migratory capacity as compared to B164A5 cells [16].

B164A5 murine melanoma cell line (ECACC 94042254) was obtained from a melanoma developed in the skin of C57BL/6 mouse and is a pigment-producing cell line [12]. These cells present a fibroblastic-like morphology as it can be seen in figure 1- 3 a, b. B164A5 cells showed a high migratory capacity by filling the gap created for scratch assay after 24h [16]. In addition, it was used to verify the antimelanoma potential of different compounds of chemical (synthetic hormones) [17] and natural origin [18]. Inoculation of B164A5 cells to black mice - C57BL/6 led to the formation of subcutaneous tumors [19], but also lung and spleen metastases [20].

One of the human melanoma cell line widely applied in the melanoma research is SK-MEL-28 (ATCC® HTB72™) (over 400 publications in PubMed database) (7). These cells were generated from a skin melanoma harvested from an adult male and present polygonal shapes (fig. 2 - 2 a, b). Inoculation of SK-MEL-28 cells to nude mice determines the development of malignant melanoma characterized by large round cell type. These cells do not produce melanin, neither *in vitro*, nor *in vivo*. Based on a transcriptional analysis, this cell line was included in the MITF state, that indicates the proliferative and invasive potential of the cells, as well as SK-MEL-5 cells [7].

SK-MEL-5 melanoma cell line (ATCC® HTB70™) is of human origin derived from metastatic axillary node harvested from a female, and a non-melanin producing cell line. The shape of the cells in culture is stellate (fig. 2 - 1 a, b). By inoculation to nude mice, it will be formed a metastatic tumor. The number of studies assigned to SK-MEL-5 cells is lower as compared to SK-MEL-5: environ 120 publications in PubMed database.

Another human melanoma cell line presented here, is SH-4 (ATCC® CRL-7724™). This cell line was harvested from a female that presented skin melanoma and the morphology is composed of a mixture of spindle-shaped and epithelial-like cells (fig. 2 - 3 a, b). In contrast with the

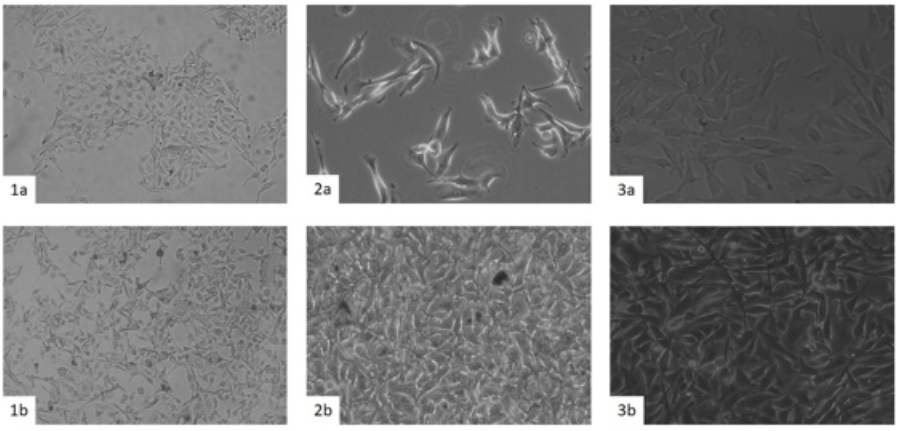

Fig.1. The evolution of culture of murine melanoma cells, a - at the beginning of growth, b - at confluence: 1a,b -B16F0; 2a,b - B16F10 and 3a,b - B164A5

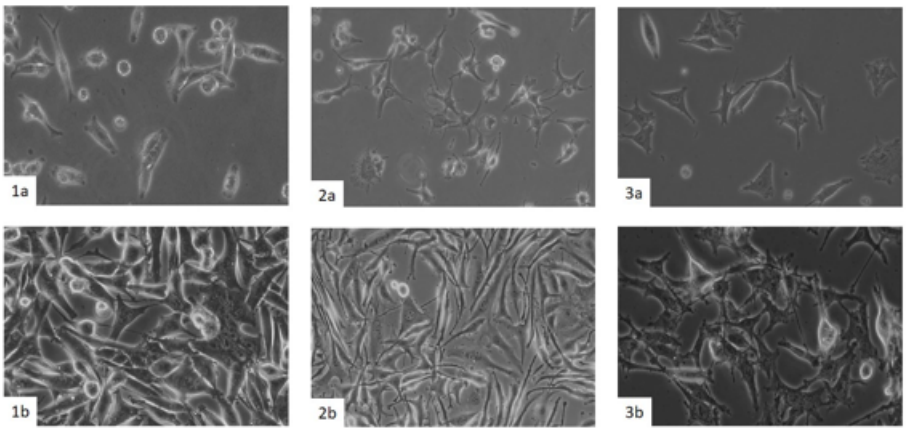

Fig. 2. The evolution of culture of human melanoma cells, a - at the beginning of growth, b – at confluence: 1a,b - SK-MEL-5; 2a,b - SK-MEL-28 and 3a,b - SH-4

other human melanoma cell lines presented in this study, SH-4 cells are capable to produce melanin. SH-4 cell line was established from pleural effusion of a patient with metastatic melanoma, the period required have the final product being of more than 5 months [21].

There are series of different pathological cells including melanoma. These cells could develop different behavior dependent on *in vitro* conditions, origin, treatments and other factors. To obtain a proper result and interpretation the scientists must know particularities regarding these data. Murine metastatic cells (B16F10 and B16 F0) are comparable with human metastatic like SK-MEL-5 and SK-MEL-28.

Conclusions

The significant and rapid progress of *in vitro* technology is extremely useful in elucidating the key mechanisms that are taking place in the evolution of melanoma. The data provided by the behavior of different cell lines of melanoma is a key point in finding new candidates for slowing melanoma progression, reducing the adverse effects of current therapies, and increasing the life expectancy of patients diagnosed with this terrible disease.

References

1. SARAU, C.A., POENARU, M., BALICA, N.C., BADERCA, F. Rom J Morphol Embryol. 58(4), 2017, p.1541.
2. SASSE, A.D., SASSE, E.C., CLARK, L.G., ULLOA, L., CLARK, O.A. Cochrane database of systematic reviews (Online) 2007(1):CD005413.
3. HOEK, K.S., EICHHOFF, O.M., SCHLEGEL, N.C., DOBBELING, U., KOBERT, N., SCHAERER, L., HEMMI S., DUMMER, R. Cancer Res 68(3), 2008, p. 650.
4. CORICOVAC, D., DEHELEAN, C., MOACA, E.A., PINZARU, I., BRATU, T., NAVOLAN, D., BORUGA, O. Int. J. Mol. Sci. 19, 2018, p. 1566.
5. HAQQ, C., NOSRATI, M., SUDILOVSKY, D., et al. Proc Natl Acad Sci USA 102, 2005, p. 6092.
6. MILLER, A.J., MIHM, M.C., Jr. Melanoma. N Engl J Med 355, 2006, p. 51.
7. VINCENT, M.K., POSTOVIT, L.N. Oncotarget 8(6), 2017, p.10498.
8. KLIJN, C., DURINCK, S., STAWISKI, E. W., et al. Nat Biotechnol. 33, 2015, p.306.
9. Cancer Genome Atlas Network Genomic Classification of Cutaneous Melanoma. Cell 161, 2015, p.1681.
10. TIROSH I., IZAR, B., PRAKADAN S. M., et al. Science 352, 2016, p.189.
11. FIDLER IJ. Eur J Cancer. 9, 1973, p.223.
12. DANCIU, C., FALAMAS, A., DEHELEAN, C., et al. Cancer Cell Int. 13, 2013, p.75.
13. MITRUS I, BRYNDZA E, KAZURA M, et al., Acta Biochim Pol. 59(3), 2012, p.363.
14. ZHANG, Z., YU, C., LI, Y., JIANG, L., ZHOU, F. BMC Cancer 15, 2015, p.234.
15. TAKEDA, Y., LI, Q., KAZAROV, A.R., et al. Blood 118, 2015, p.464.
16. SERAFIM, V., SHAH, A., PUIU, M., et al. Int J Mol Med. 40(4), 2017, p.1096.
17. CORICOVAC, D., FARCAS, C., NICA, C., et al., Int J Mol Sci. 19(11), 2018, p. E3600.
18. DANCIU, C., PINZARU, I., CORICOVAC, D., et al., Eur J Pharm Biopharm. 134, 2019, p.1.
19. SOICA, C., DANCIU, C., SAVOIU-BALINT, G., et al., Int J Mol Sci. 15(5), 2014, p.8235.
20. GHEORGHEOSU, D., DEHELEAN, C., CRISTEA, M., MUNTEAN, D. Annals of RSCB XVI (2), 2011, p.148.
21. SEMAN, G., HUNTER, S.J., LUKEMAN, J.M., DMOCHOWSKI, L. In Vitro 11(4), 1975, p.205.

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