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Role of geneticin in isolation and culturing of skin melanocytes and melanoma cells

Original Study

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Abstract

Introduction. Development of an effective, suitable, and reliable method for both the isolation and culturing of melanocytes is crucial for studies on pathomechanisms of skin diseases originating from melanocyte disorders. In this study, we have investigated the influence of geneticin (G418), a substance used for melanocyte selection, in the view of the frequency of presence of cells such as keratinocytes and fibroblasts, widely known as contaminators of melanocyte-originating cell cultures.
Materials and Methods. Study was conducted on primary, freshly isolated melanocytes, keratinocytes, fibroblasts, and melanoma cells as well as on commercially available melanoma cell lines MeWo, G-361, and A375. Cells were cultured in different culture media supplemented with various concentrations of geneticin ranging from 0.05 to 1 mg/mL. Cell viability, proliferation rate and detection of apoptotic/necrotic cells was assessed.
Results. Choice of culture media supplemented with various concentrations of geneticin (0.05 mg/mL, 0.1 mg/mL, 0.5 mg/mL and 1 mg/mL) strongly affect viability of melanocytes, fibroblasts, and keratinocytes. Selective culture media without FBS facilitate the process of melanocytes and melanoma pure cell culture, yet without geneticin supplementation are insufficient for complete eradication of fibroblast contamination from cell culture.
Conclusions. In this study we provide, for the first time, the dose-response action of keratinocytes and fibroblasts upon geneticin stimulation in different culture media and show that a low concentration (0.05 mg/mL) of geneticin added to the selective culture media may be safely implemented to facilitate the production of melanocyte and melanoma cell cultures that are free from frequent cell contaminants.

Keywords

melanocytes • fibroblasts • keratinocytes • melanoma • geneticin • cell culture contamination

1. Introduction

Human skin melanocytes, consisting of ca. 3–7% of all epidermal cells, are indispensable for the synthesis of melanin during a multistep process called melanogenesis, determining skin and hair coloration [1]. Every dysfunction of melanocytes may lead to the development of various disorders in skin pigmentation, such as hypo- or hyperpigmented spots. Moreover, some of these changes may progress into malignant melanoma, a tumor with very high mortality rate [2]. Although novel treatments of melanoma (e.g., oncogene-targeted therapies and immune checkpoint blockade approaches) have been introduced in recent years, a very high proportion of poorly responding patients have been recognized, making melanoma one of the most challenging and studied disorders nowadays.

Research on melanoma and melanocytes are commonly conducted on well-established human melanoma cell lines, such as MeWo, A-375 and SK-MEL-1 [3]. However, for translational studies and specific drug testing for melanoma patients' personalized treatment, development of novel methods of primary melanocyte and melanoma cell cultures is highly recommended [4]. These primary cultures may be also very useful for studies concerning other melanocyte disorders, such as vitiligo [5].

Even though novel protocols aimed at the effortless culturing of melanocytes and melanoma cells were implemented in some centers [3], methods based on the enzymatic release of epidermal cells are commonly used in laboratory practice [6], [7]. The major limitation of these methods, besides the necessity of obtaining large skin samples to allow the study of a sufficient number of melanocytes, is a high frequency of contamination of melanocytes with fibroblasts and keratinocytes. Keratinocytes may be separated from the cell culture by their different times of trypsinization. Also, in order to eliminate keratinocytes, selective culture media enriched with substances that induce melanocytic proliferation, such as cholera toxin (CT), 12-O-Tetradecanoylphorbol-13-acetate (TPA),

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phorbol-12-myristate-13-acetate (PMA), are still commonly used. Unfortunately, those substances are potent to affect the physiology of melanocytes during a long period of cell culturing, which limits their wider usefulness in subsequent experimental and clinical approaches [8]. Additionally, the effectiveness of selective culture media dedicated to melanocyte culturing depends on the initial rate of co-isolated contaminants, which reduce sufficient and complete elimination of quickly dividing fibroblasts. In a few studies, a selective compound called geneticin was successfully applied for the separation of the melanocytes or melanoma-originating cells from contaminated fibroblasts during culturing. Geneticin, the aminoglycoside antibiotic, widely known as G418, is known to decrease the translation accuracy of 16S ribosomal RNA [9]. Even though geneticin was used as an agent suitable for the removal of fibroblast contamination in a few studies, knowledge regarding the influence of the higher concentrations of G418 on different types of skin cells, such as melanocytes or keratinocytes, is still limited, insufficient, and unvalidated. Thus, data on G418 as a possibly toxic substance not only for bacteria but also for protozoans, plants, and worms as well as some mammalian cells are still limited [10]. On the other hand, the implementation of G418 substance, which may be specific for fibroblast elimination and might not have a potential effect on melanocyte physiology, would be highly beneficial for the elaboration of the wide-scale studies on melanocytes and melanoma behavior in the future. In the present study, we provide a complex analysis regarding the influence of geneticin dosage schedule on the viability of melanocytes, melanoma cells, and other cells originating from the skin.

2. Materials and methods

2.1. Collection of tissue samples

Samples of normal human skin were obtained from four females aged 35–50 years who underwent esthetic abdominoplasty at the Department of Plastic Surgery, Medical Centre of Postgraduate Education, Orlowski Memorial Hospital in Warsaw. Bioptates of recurrent metastatic malignant melanoma were obtained during surgical excision at the Maria Skłodowska-Curie Institute of Oncology, Warsaw, Poland from two lymph nodes of a 76-year-old male patient. .

2.2. Primary cells and cell lines

Primary cells of melanocytes, keratinocytes, fibroblasts, and melanoma were isolated from explants as described previously [3]. The commercial cell line of MeWo (lymph node–derived metastasis, HTB-65), G-361 (skin-derived metastasis, CRL-1424) and A375 (amelanotic metastasis, CRL-1619) were obtained from the American Tissue Culture Collection (ATCC, Manassas, VA, USA) and cultivated in accordance with the supplier's instructions.

2.3. Cell stimulation with geneticin

Melanocytes, melanoma cells, keratinocytes, and fibroblasts were seeded at the density of $1*10^4$ cells/well onto 96-well plates (Nest Scientific Biotechnology, Wuxi, Jiangsu, China) in culture media commonly used for culture of mentioned cells such as DMEM, RPMI-1640, M254 culture media (all from ThermoFisher Scientific Inc., Waltham, MA, USA) or KGM2 culture medium (PromoCell, Heidelberg, Germany). DMEM and RPMI-1640 culture media (without phenol red) were supplemented with 10% fetal bovine serum (FBS, ThermoFisher Scientific Inc.). After 24 hours, various concentrations of geneticin (G418, Thermo Fisher Scientific Inc.; dissolved in Milli-Q water) ranging from 0.05 to 1 mg/mL were added to the cultured cells for 24 hours. The metabolic activity of cells was measured after subsequent short (24 h) (Figure 1A) and long (72 h) (Figure 1B) periods of cell culture after stimulation with geneticin. For the long period of cell culture (Figure 1B) culture medium was changed after 24-hour post-stimulation for cells' regeneration in fresh medium that was used before geneticin stimulation.

2.4. The MTT viability assay

For MTT assays cells were seeded on the 96-well plates at the density 1×10^4 cells/well according to Sobiepanek et al. 2022 [11]. The working dilution of 0.5 mg/mL MTT salt (Sigma-Aldrich, St. Louis, MO, USA) in DMEM or RPMI-1640 media (ThermoFisher Scientific Inc.) was used and further incubation was carried out for 2 hours at 37°C in a humidified atmosphere of 5% CO_2. Formazan was released from the cells after the addition of DMSO (Roth) and the absorbance was measured at 570 nm with a microplate reader (Synergy H4 Hybrid, Biotek; VT, USA). The results were expressed as percents of the untreated cells (control).

2.5. The BrdU proliferation assay

The influence of geneticin on the primary fibroblast proliferation was studied during the BrdU Cell Proliferation Assay (Merck) performed according to the producer's protocol. To the cells seeded on the 96-well plates at the density 1×10^4 cells/well, a BrdU reagent was added 6 hours before the end of the 72-hour incubation with geneticin. Then, cells were fixed, washed and the incubation with the primary anti-BrdU monoclonal antibody was carried out for 1 hour. Next, the cells were washed, and the secondary goat anti-mouse IgG peroxidase conjugate antibody was added and incubated for 30 minutes. After washing, the TMB peroxidase substrate was added to the cells and incubated for 30 minutes. After the application of the stop buffer, the absorbance was measured at 450 and 540 nm (background) with a microplate reader (Synergy H4 Hybrid, Biotek; VT, USA). The results were presented as percents of the untreated cells (control).

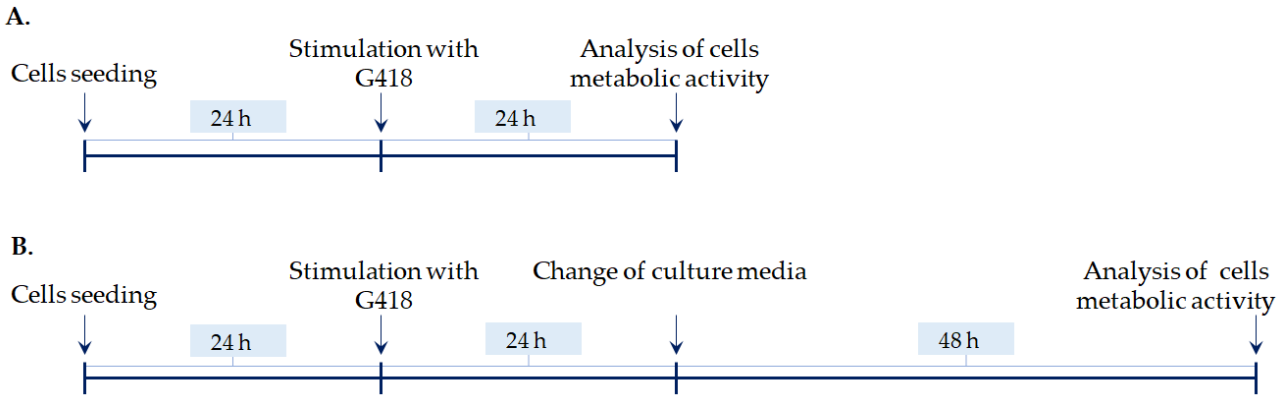

Figure 1. Schematic presentation of the analysis of G418 influence on cells' metabolic activity

2.6. The quantitative detection of apoptotic/necrotic cells

The quantitative detection of apoptotic/necrotic cells was performed with the Annexin-V-FLUOS Staining Kit (Sigma Aldrich). Fibroblasts were seeded onto the 96-well plates at the density 1×10^4 cells/well and the geneticin solutions (0.05, 0.1, 0.5 or 1 mg/mL) and positive controls were added to the cells for a 24-hour incubation (apoptosis - 2 µM staurosporine, necrosis - 50 µg/ml etoposide) as well. Next, the solutions were replaced by a fresh medium without the compounds and the cells were allowed to recover for 48 hours. The assay was performed according to the producer's protocol. The cells were washed and incubated for a further 15 minutes with annexin and propidium iodide in the binding buffer. Before the analysis, cells were washed once. The fluorescence was read at 490/525 nm for annexin and 540/617 nm for propidium iodide on the microplate reader (Synergy H4 Hybrid, Biotek; VT, USA).

2.7. Immunofluorescence staining

Immunofluorescent labeling was used to examine cell culture homogeneity and the general condition of cultured cells. In vitro growing melanocytes, keratinocytes, and fibroblasts were seeded 1:1:1 onto cell culture slides and cultivated in various concentrations of geneticin for short-term and long-term investigation mentioned in Figure 1. After fixation in 4% paraformaldehyde for 20 minutes, permeabilization in 0.1% Triton X-100 for 3 minutes at room temperature, washing in phosphate-buffered saline (PBS, ThermoFisher Scientific Inc.), and blocking with 3% BSA in PBS for 30 minutes at room temperature, specimens were treated for 1 hour at room temperature with rabbit anti-TYR antibody (melanocyte marker; 1:100, cat. no. GTX16389, GeneTex), mouse anti-NG2 antibody (melanocyte marker; 1:50, cat. no. sc-166179 Santa Cruz Biotechnology, Inc.), mouse anti-KRT14 antibody (keratinocyte marker; 1:500, cat. no. MA5-11599, ThermoFisher Inc.), or rabbit anti-COL1 antibody (fibroblast marker, 1:1000, cat. no. PA5-95137, ThermoFisher Scientific Inc.). FITC goat anti-rabbit secondary antibody (cat. no. NB7159, Novus Biologicals) and Alexa Fluor 555 goat anti-mouse secondary antibody (cat. no. A32727, ThermoFisher Scientific Inc.) were used to detect the primary antibodies. For secondary antibodies, dilution of 1:250 was applied. In order to assess homogeneity of cell culture, for each culture condition, 100 cells (fibroblasts, keratinocytes or melanocytes) were counted according to their specific staining.

2.8. Literature search

The National Center for Biotechnology Information Database (https://www.ncbi.nlm.nih.gov/, accessed 19 January 2023) was used for acquiring selected literature (n = 171). Search terms were: "Geneticin" combined with "Melanocytes" or "Keratinocytes" or "Fibroblasts". Hand searches of relevant publications that were not discovered in the PubMed database provided additional sources of information (n=4).

2.9. Statistical analysis

Data was processed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). The statistical significance was determined using a two-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Geneticin acts differently on normal human skin cells

Melanocytes treated with four concentrations of geneticin (0.05 mg/mL, 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL) in M254 medium (medium dedicated for melanocyte culture) showed high viability observed after 24 and 72 hours after stimulation (Figure 2A). Viability of the metabolically active melanocytes reached 90% when stimulated with a low dosage of geneticin (0.05 mg/mL), and nearly 80% when high dosages of geneticin (0.5 mg/mL or 1 mg/mL) were used. Even though no significant changes in fibroblast viability were observed after 24 hours of incubation

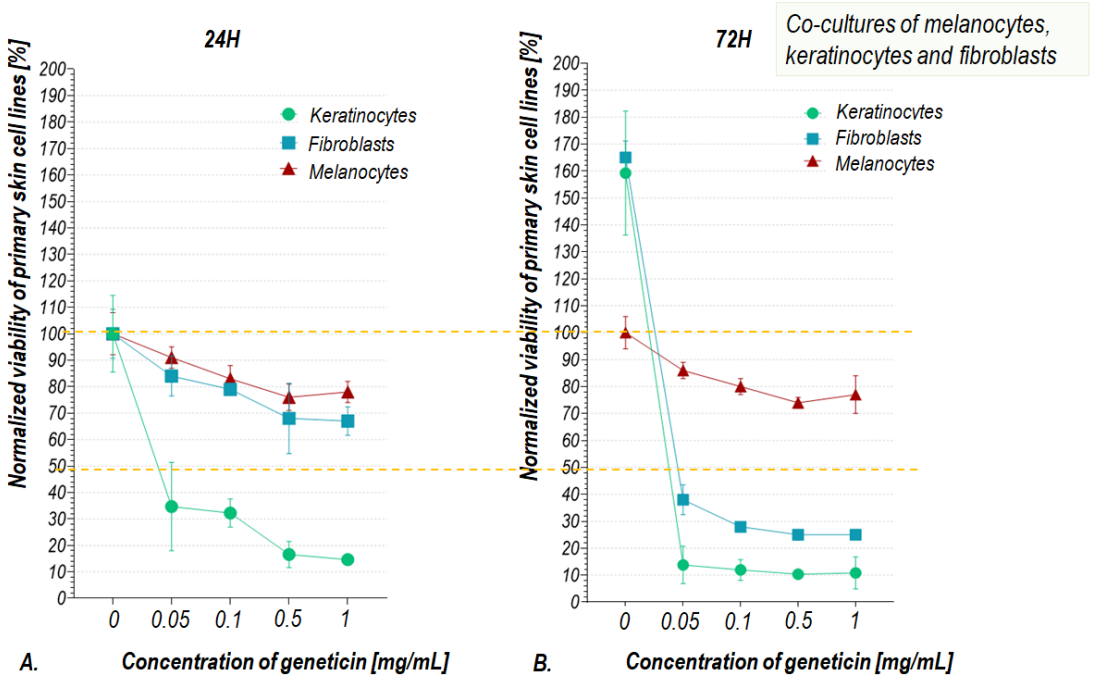

Figure 2. Viability of normal human skin cells, cultured in M254 culture medium, after cell culture stimulation with different concentrations of geneticin for 24 hours (A) and 72 hours (B)

with geneticin (compared to melanocytes), a significant dose-dependent reduction in viability was observed after 72 hours of culture (p-value <0.0001) (Figure 2B). All concentrations of geneticin significantly reduced proliferation rates of keratinocytes, as observed just after 24 hours of cell culturing (p-value <0.0001). Even a concentration as low as 0.05 mg/mL reduced the amount of metabolically active keratinocytes to 30% after 24 hours, and even greater values were observed after 72 hours of culturing (Figure 2A,B).

To analyze whether the decrease of keratinocyte viability was caused by the components of Medium 254, rather than possible geneticin toxic effect, keratinocytes in various media supplemented with 10% FBS (DMEM and RPMI) or without FBS supplementation (KGM), were used (Figure 3) [12]. After 72 hours of cell culturing, the proliferation rate of the viable keratinocytes (not stimulated with geneticin) was similar for all culturing media applied and the total cell number was nearly two times higher when compared to values obtained after 24 hours of cell culturing (Figure 3A,B). Contrarily, even as small a concentration of geneticin as 0.05 mg/mL was able to lead to the substantial reduction of keratinocyte viability observed even after 24 hours of cell stimulation cultured in media without FBS (like KGM and M254).

Then, we investigated whether geneticin will be toxic to fibroblasts cultured in media recommended for their propagation (including DMEM and RPMI, both with 10% FBS supplementation) (Figure 4). Incubation with higher doses of geneticin (0.5 - 1 mg/mL) has decreased the viability of fibroblasts in all cases (Figure 4) after 24 and 72 hours of cell stimulation. Lower doses of geneticin were not sufficient to reduce the viability of fibroblasts in this FBS-supplemented culture medium. On the other hand, the viability of fibroblasts was significantly lowered for all tested geneticin concentrations (0.05 - 1 mg/mL), when cultured in M254 medium (Figure 4A,B). Interestingly, even low concentrations of geneticin (0.05 mg/mL) has led to a significant reduction in cell viability observed after 72 hours of cell stimulation (p-value <0.0001) (Figure 4B).

As differences in fibroblast viability were observed between DMEM culture medium supplemented with 10% FBS and Medium 254, both were selected for further evaluation of fibroblast proliferation rate by means of the BrdU assay (Figure 5A). As pictured in Figure 5A, even as low a concentration of geneticin as 0.05 mg/mL has led to a significant reduction in fibroblast proliferation rate (15%), as observed after 72 hours of cell stimulation (p-value <0.0001).

Quantitative analysis of the apoptotic and necrotic cell capacities showed that fibroblasts stimulated with geneticin underwent apoptosis rather than necrosis, regardless of the geneticin concentration (Figure 5B). When a low concentration of geneticin (0.05 mg/mL) was used, fibroblasts in the necrosis state were nearly undetected, while the death of fibroblasts was caused by apoptotic events (p-value <0.0001).

When cultivated in M254 culture medium, immunofluorescence labeling demonstrated that treatment with

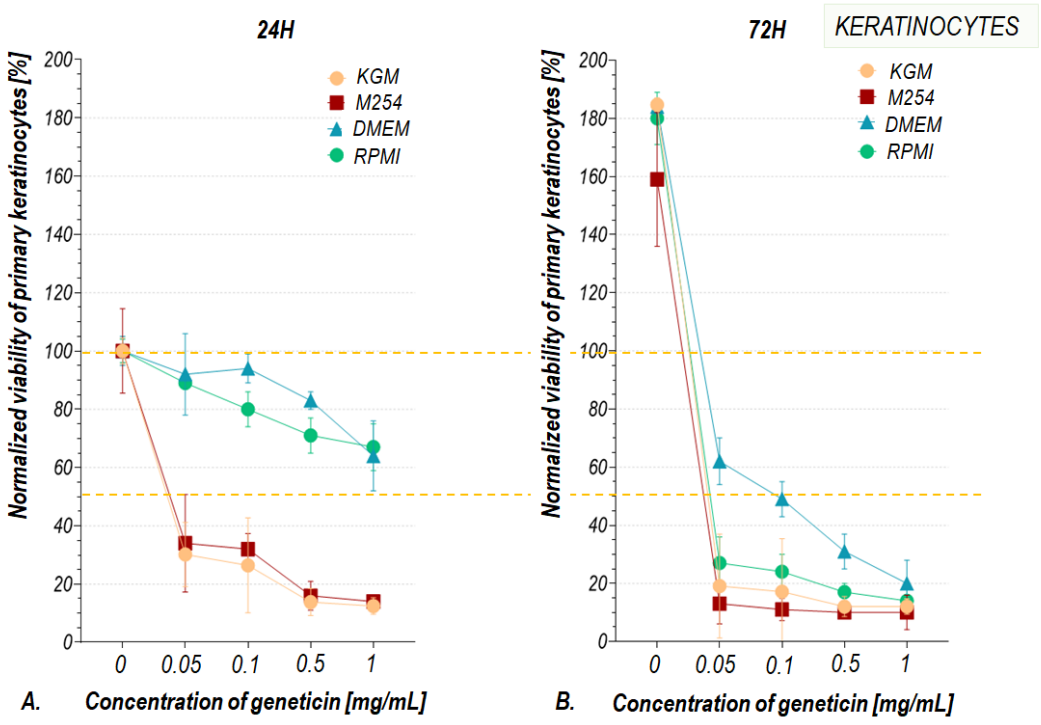

Figure 3. The viability of primary keratinocytes measured 24 h (A) and 72 h (B) after cell culture stimulation with increasing concentrations of geneticin, measured by the means of MTT assay. All absorbance values were normalized versus control keratinocytes obtained after 24 hours of cell culture

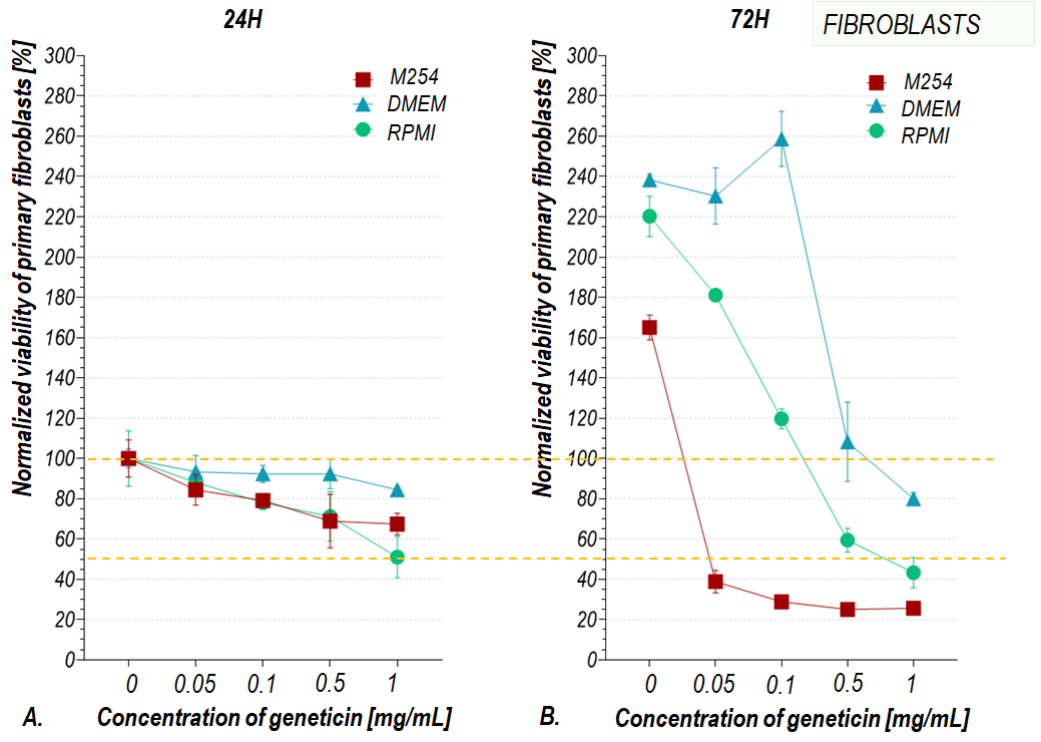

Figure 4. The viability of primary fibroblasts measured 24 h (A) and 72 h (B) after cell culture stimulation with increasing concentrations of geneticin measured by the means of MTT assay. All absorbance values were normalized versus control fibroblasts obtained after 24 hours of cell culture

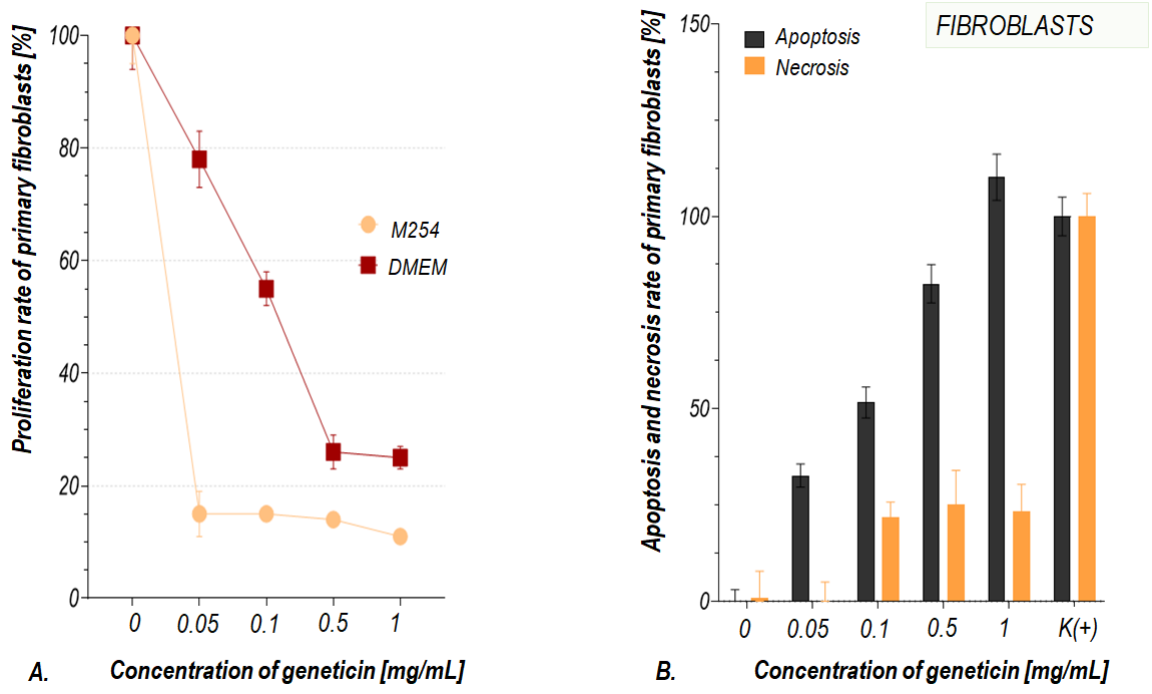

Figure 5. The proliferation rates of primary fibroblasts cultured in different media supplemented with geneticin (0.05-1 mg/ml) measured using the BrdU assay (A). The quantitative analysis of apoptosis and necrosis of fibroblasts treated with geneticin with staurosporine/etoposide as positive control samples (K(+)), respectively (B)

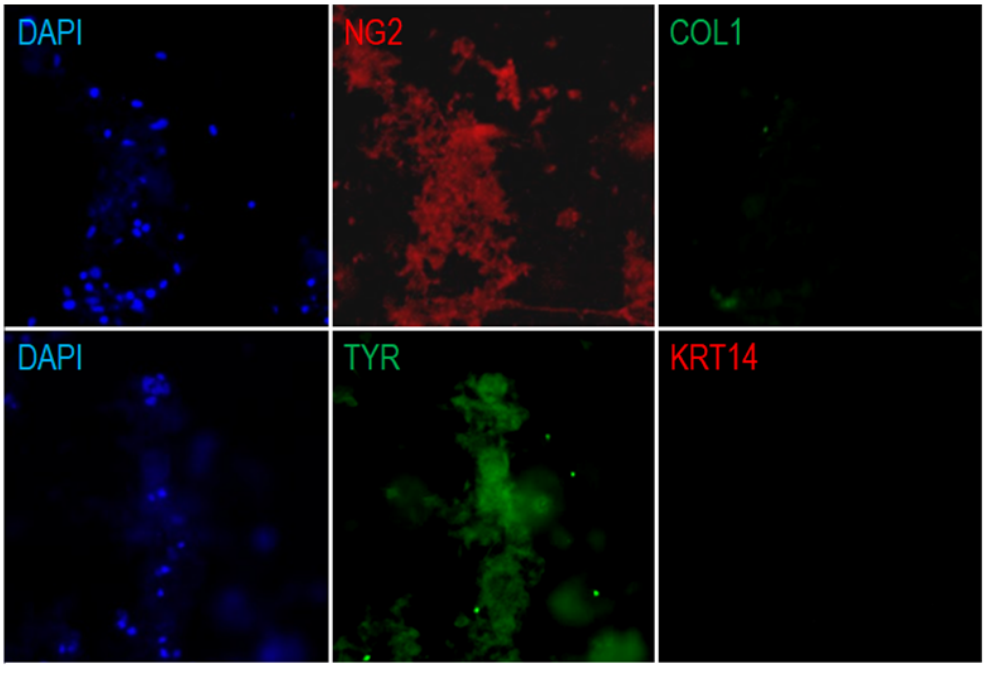

Figure 6. Immunofluorescence staining of melanocytes, keratinocytes and fibroblasts cultured in medium M254 for 72 hours after 0.05 mg/mL geneticin treatment. The nuclei of the cells were stained blue with DAPI. TYR and NG2 are markers of melanocytes, while COL1 and KRT14 are markers of fibroblasts and keratinocytes, respectively

Table 1. Percentage of particular cells in co-cultures of melanocytes, fibroblasts, and keratinocytes in different culture media for 72 hours following treatment with 0.05 or 0.1 mg/mL geneticin

Culture media	Cell type	Control	Geneticin (0.05 mg/mL)	Geneticin (0.1 mg/mL)
DMEM + 10% FBS	Melanocytes	15%	29%	37%
	Fibroblasts	67%	66%	60%
	Keratinocytes	18%	5%	3%
RPMI + 10% FBS	Melanocytes	26%	40%	66%
	Fibroblasts	60%	55%	30%
	Keratinocytes	14%	5%	4%
M254	Melanocytes	27%	97%	98%
	Fibroblasts	40%	2%	1%
	Keratinocytes	33%	1%	1%

geneticin at concentrations of 0.05 mg/mL and 0.1 mg/mL results in cell culture homogeneously consisted of melanocytes (Table 1). Figure 6 shows an example of immunofluorescence staining for co-culture of melanocytes, keratinocytes, and fibroblasts grown in medium M254 for 72 hours following treatment with 0.05 mg/mL geneticin. Even though geneticin at 0.1 mg/mL results in higher purity of melanocyte cell culture (Table 1), the overall condition of cells is poorer, and total amount of cells is less frequent when compared to 0.05 mg/mL. State of melanocytes cultured in higher concentrations of geneticin is even worse (data not shown).

3.2. Melanoma primary cells and commercial cell lines are differently affected by geneticin

Treatment of melanoma cell lines with different concentrations of geneticin (0.05 mg/mL, 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL) in M254 culture medium for 24 h showed a dose-dependent influence on cell viability (Figure 7). The highest decrease in metabolic activity after 24 hours of cell culturing was observed after cell stimulation with a high concentration of geneticin (above 0.5 mg/mL). After 72 hours of cell culturing, even higher reduction in cell viability was observed for the majority of melanoma cells. Incubation of the primary metastatic melanoma cells with geneticin resulted in a decrease in the cells' viability and metabolic activity only after the application of the highest concentrations of geneticin. Interestingly, even though most melanoma cell lines (primary metastatic melanoma cells and G-361 cell line) were sensitive only to high concentrations of geneticin (above 0.5 mg/mL), some melanoma cell lines (MeWo, A375) were resistant to the highest tested concentrations of geneticin (1 mg/mL) tested. On the other hand, in the low concentration of geneticin (0.05 mg/mL) viability of the primary melanoma cells and cell lines was greater than 80%.

4. Discussion

Treatment of cells with geneticin may significantly influence their properties: their metabolic activity, proliferation rate, or apoptosis. According to the available literature, geneticin is mainly used for studies aimed at the selection of genetically engineered cells, or translational *read-through* at some disease-relevant stop codons experiments (Figure 8). Unfortunately, among 12 publications available that met the criteria for further analysis, only 2 studies showed geneticin dose-dependent effect on fibroblast mortality. In one study, the viability of fibroblasts was assessed during cell counting [13], while in the second one, fibroblast mortality rate was assessed via microscopy [14]. Unfortunately, no further studies aimed at determining mortality rates of fibroblasts and keratinocytes co-cultured with melanocytes were conducted, however, 0.1 mg/mL concentration of geneticin was used in 10 other studies. Therefore, this study provides, for the first time, dose-response action of keratinocyte and fibroblast behaviors upon geneticin stimulation in different culture media. Moreover, it shows the influence of different geneticin concentrations on various primary and commercially available melanoma cell lines. Our results might facilitate processes of melanocyte and melanoma cell culturing and allow the elimination of the most frequent melanocyte cell culture hindrance, that is, fibroblasts.

In our previous study, we showed that melanocytes growing from skin explants for longer than six days are contaminated with fibroblasts, which rapidly became the dominating cell type [3]. Therefore, in this study we analyzed various concentrations of geneticin (0.05 - 1 mg/mL) in order to select the proper concentration useful for fibroblast elimination from the 7–10th day of skin explant culturing which at the same time will not show toxicity features for melanocytes or melanoma cell cultures. As geneticin affects cells with high turnover (such as keratinocytes) and melanocytes are known to be slowly proliferating cells, their behavior was observed in our study for 72 hours after cell stimulation. Application of 0.05 mg/mL of geneticin generally reduced the viability of melanocytes (to 85%) as compared to control, but this value did not significantly decrease in comparison with cells 24 hours after cell stimulation (Figure 2). Also, the viability of melanoma cells was not heavily impacted by the concentration of 0.05 mg/mL of geneticin (Figure 7). On

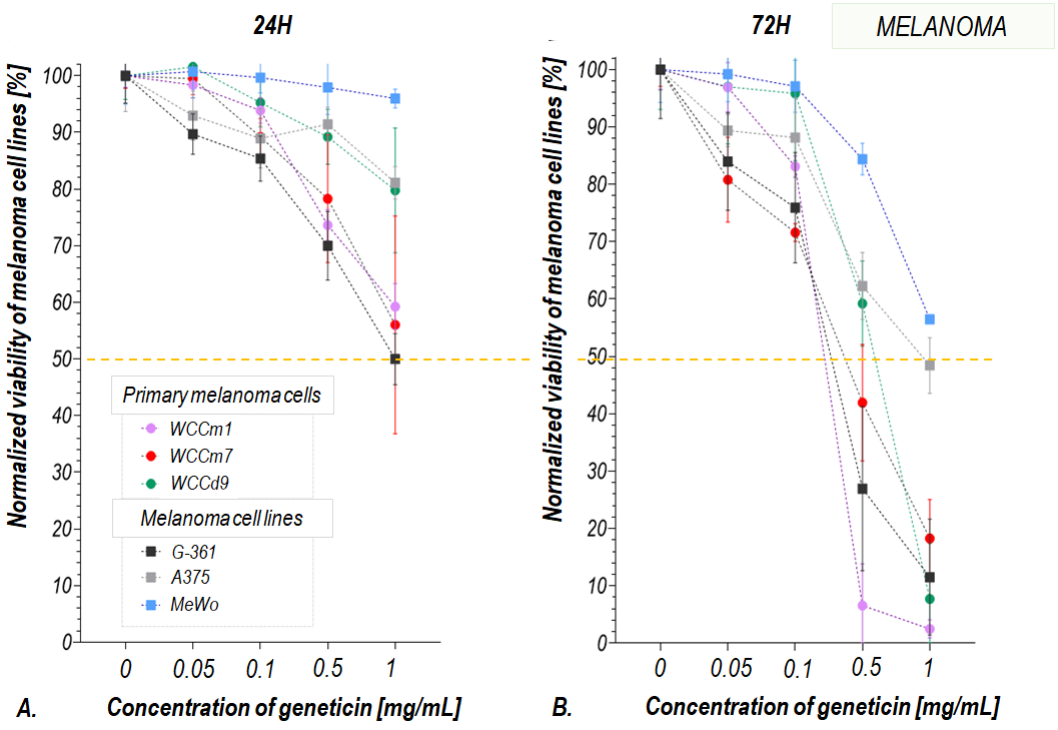

Figure 7. The MTT results of the influence of geneticin on the primary human metastatic melanoma cell lines (WCCm1, WCCm7, WCCd9) and commercial cell lines of metastatic melanoma (A375, G-361 and MeWo) observed 24 hours (A) and 72 hours (B) after cell stimulation in M254 culture medium

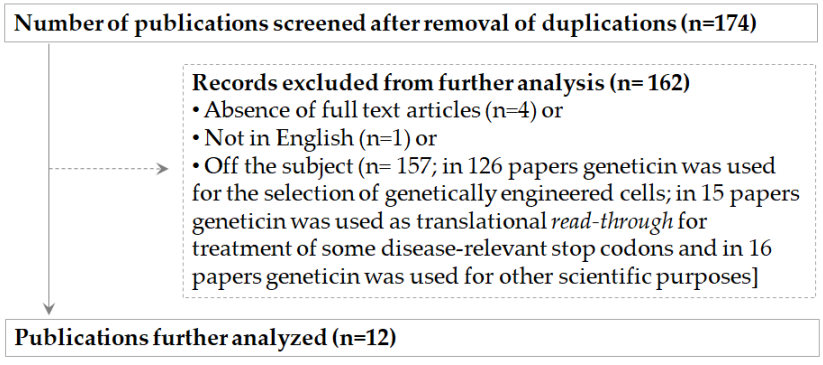

Figure 8. Flow of information through the selected literature about concentrations of geneticin used for culture of pure melanocytes

the contrary, the usage of concentrations greater than 0.1 mg/mL strongly reduced the viability of melanocytes and some of the melanoma cell lines. This observation stays in agreement with the results of Shen et al. [15] showing that concentrations of geneticin higher than 0.5 mg/mL might significantly reduce the viability of melanocytes. Taking the results altogether, a concentration of 0.05 mg/mL of geneticin was found to be suitable to maintain high viabilities of melanocytes and melanoma cell lines. Thus, our further studies aimed at determining whether a given concentration might be also effective in the elimination of fibroblasts and keratinocytes from melanocyte co-culture.

Even though melanocytes were cultured in a medium dedicated to melanocyte culturing (Medium 254), fibroblasts and keratinocytes showed very high viability and proliferation rates in this medium, as shown in Figure 2. Rapidly growing fibroblasts may easily overgrow melanocyte-originating cells, making further studies on melanocytes or melanoma cells impossible without costly cell immunosorting methodology.

Geneticin in all concentrations tested was found to be toxic to keratinocytes regardless of the medium used for cell culture (Figure 3). Therefore, subsequent studies should concentrate on the selection of keratinocytes before stimulation with geneticin

and different times of trypsinization [3]. Interestingly, the effectiveness of geneticin on the primary fibroblasts depended on the medium used for the cell culture; we found that geneticin-based elimination of fibroblasts from cells culture depends strictly on the implemented culture media. Indeed, depending on the culture medium composition, geneticin may have a variable effect on cell growth and proliferation rate. In some cell cultures treated with geneticin increased utilization of glutamine was observed, while in some other cell cultures, the flux of glucose instead of lactate production was common, towards pathways leading to provide energy or biosynthesis [16]. Fibroblasts cultured in DMEM medium supplemented with 10% FBS were found to be more resistant to various geneticin concentrations as compared to those cultured in Medium 254, even though without geneticin they were able to proliferate rapidly in this medium (Figure 2, 4). This result seems to be in line with other analyses published in 10 out of 12 publications that met the final search criteria. Stimulation with higher concentrations of geneticin, dissolved in culture media supplemented with various concentrations of FBS, had to be used, or treatment had to be repeated. Our results showed that fibroblast culturing conducted in medium M254 allowed for the reduction of the needed geneticin concentration in culture media up to 0.05 mg/mL (Figure 2,4).

G418 is a known factor able to inhibit cell culture proliferation, which is why we have evaluated the influence of geneticin concentrations on fibroblast proliferation. The addition of geneticin to fibroblasts in medium 254 caused a significant decrease in the cells' proliferation for all concentrations under study (Figure 5) and confirmed geneticin potential in selective elimination of fibroblasts from the co-culture in medium 254. Indeed, geneticin strongly influenced cells on rate of proliferation. Geneticin binds to the ribosomes and hinders translation in both prokaryotic and eukaryotic cells; thus it interferes with protein synthesis, especially in rapidly proliferating cells [17].

Finally, we analyzed what type of cell death was activated in fibroblasts upon geneticin stimulation. Obtained results showed that implementation of 0.05 mg/mL of geneticin did not activate necrosis but rather apoptosis in fibroblasts. This is in line with the observation of Jin et al. showing that geneticin activates caspase-3-dependent apoptosis as initiated by the release of cytochrome c from mitochondria or stress regulators of the endoplasmic reticulum (ER) [18]. Therefore, cultured melanocytes will not be harmed by post-necrotic events such as subsequent release of lysosomal enzymes from fibroblasts.

5. Conclusions

Low concentration (0.05 mg/mL) of geneticin in M254 culture medium did not significantly influence melanocytes' and melanoma cell lines' viability, but significantly reduced the viability and proliferation rates of frequent contaminants of cell culture such as fibroblasts and keratinocytes. As geneticin leads to fibroblast death via a non-inflammatory, programmed cell death (apoptosis) pathway, a low concentration of geneticin (0.05 mg/mL) can be successfully used as a factor able to eliminate contamination of fibroblasts from melanocytes and melanoma cell cultures. These results may facilitate the process of culturing pure melanocytes, which is indispensable for future studies focused on cell therapies in hypopigmentation disorders, such as vitiligo, and those aimed at the development of novel therapeutic approaches for the treatment of melanoma as well.

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Authors' contribution

AŚ and AS designed the study. AŚ, AS, and MS performed the literature search. NK provided normal human tissues for melanocyte isolation. AŚ, AS, MS, KŁ, MR, and IL performed the experiments. AŚ, AL, BO, JM, and AS analyzed the data. AŚ, AL, JM, and AS wrote the article.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Ethics approval

All participants provided written informed consent. The study was accepted and carried out in accordance with the rigorous regulations of the relevant local Ethical Committees at Medical University of Warsaw (KB/216/2017) and at the Medical Centre of Postgraduate Education, Orłowski Memorial Hospital in Warsaw (63/PB/2016, and 87/PB/2020).

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