

Article

Not peer-reviewed version

Differential Expression of Selected Genes Involved in Pheomelanogenesis in Melanotic and Amelanotic Human Melanoma Cells

[Irena Lidia Tam](#)^{*}, [Łukasz Marek](#), [Sławomir Kurkiewicz](#), [Jerzy Stojko](#)

Posted Date: 16 October 2025

doi: 10.20944/preprints202510.1249.v1

Keywords: melanoma; pheomelanin; gene expression; pheomelanogenic genes



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Differential Expression of Selected Genes Involved in Pheomelanogenesis in Melanotic and Amelanotic Human Melanoma Cells

Irena Tam *, Łukasz Marek, Sławomir Kurkiewicz and Jerzy Stojko

Department of Toxicology, Toxicological Analysis and Bioanalysis, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Ostrogórska 30, 41-200 Sosnowiec, Poland

* Correspondence: tami@sum.edu.pl

Abstract

Background/Objectives: Melanin synthesis in normal skin involves numerous genes, and disturbances in their function may increase the risk of malignant transformation of melanocytes. Genes regulating pheomelanin synthesis are of particular interest, as this pigment may actively contribute to cutaneous malignant melanoma pathogenesis. Normal melanocytes differ in pheomelanin content depending on skin phototype, which affects susceptibility to neoplastic transformation. However, the influence of pheomelanin on melanoma cell characteristics has not yet been fully elucidated. **Methods:** To gain further insight, we examined the expression of pheomelanogenesis-related genes in two melanoma cell lines of distinct pigmentation status, amelanotic C32 and melanotic G361. We analysed the expression of 44 genes involved in melanocyte development and pigmentation, classifying them by their direct or indirect role in pheomelanogenesis. Gene expression levels were quantified using real-time reverse transcription polymerase chain reaction (real-time RT-PCR). **Results:** Our results show that melanoma cells with distinct pigmentation phenotypes exhibit heterogeneous expression patterns. For instance, G361 cells expressed higher levels of *POMC*, *MC1R*, *TYRP1*, *TYR*, *SLC45A2*, and *CTNS*, while *SLC7A11* and *DCT/TYRP2* were more abundant in C32 cells. **Conclusions:** These findings suggest that differences in the expression of pheomelanin-related genes may contribute to melanoma cell diversity and could play a role in defining tumour behaviour. Evaluation of melanogenesis-related gene expression and, in particular, the activity of pheomelanogenesis may therefore represent a valuable approach for melanoma classification, prognosis, and therapeutic decision-making.

Keywords: melanoma; pheomelanin; gene expression; pheomelanogenic genes

1. Introduction

Melanogenesis is the biosynthesis of melanin pigments, found in the skin, hair, and eyes, and occurs in melanocytes, whose main role is to produce brown-black eumelanin and yellow-red pheomelanin [1]. The molecular structure of melanin enables the absorption of ultraviolet (UV) and visible light; therefore, these pigments exert protective effects against UVR from sunlight [2]. Melanosomes present in the cytoplasm of melanocytes are responsible for melanin production and storage and require specific structural and enzymatic proteins to function [3,4]. TYR (tyrosinase) and DCT/TYRP2 (*dopachrome tautomerase/tyrosinase related protein 2*) are key enzymes influencing melanin quantity and quality [5,6]. L-tyrosine, derived from L-phenylalanine, serves as the substrate for both melanin types and melanogenesis is tightly regulated by a multienzymatic complex [7,8]. The process begins with tyrosine oxidation to dopaquinone (DQ), catalysed by TYR which then leads to eumelanin or, in the presence of cysteine, pheomelanin via benzothiazine and benzothiazole intermediates [9–11]. Melanogenesis yields mixtures of eumelanin and pheomelanin in varying proportions, largely determined by TYR activity and the concentrations of substrates tyrosine and

cysteine. The key regulatory pathway in melanogenesis is the cAMP-dependent cascade, where cAMP activates PKA, stimulating phenylalanine hydroxylase and transcription of MITF, GTP-CHI, and THI [7,12]. Elevated cAMP may also trigger Ras/ERK, which suppresses melanin synthesis. MITF controls melanosome biogenesis, maturation, and transport [13,14], and hormonal signals via α -MSH/MC1R regulate eumelanin–pheomelanin balance and UV responses [15,16]. PKC β , through RACK1, activates TYR and melanogenesis [17,18]. SOX10 and SOX9 transcription factors regulate MITF and other melanogenic genes; UVB enhances SOX9 activity, boosting melanin production [8,19]. PAX3 supports melanocyte proliferation and, with SOX10, induces MITF expression [20].

Both pheomelanin and eumelanin are capable of protecting the skin against harmful UV radiation [2,21]. However, pheomelanin, also acting in the opposite direction as a strong UV photosensitiser, contributes to the increased susceptibility of fair-skinned individuals to premature ageing, sunburn and malignant transformation [22,23]. It has been suggested that zinc, which is present in increased amounts in red hair, interacts with pheomelanin to produce greater amounts of ROS [24]. Furthermore, the photoreactivity of pheomelanin is higher in the presence of zinc, which induces structural changes within it. The genoprotective function of melanin seems to be of particular importance in skin cancers, including malignant melanoma, which are associated with sunlight exposure [25]. Eumelanin is more genoprotective than pheomelanin; therefore, the balance between the alternative biosynthetic pathways of melanin in the skin has a decisive impact on susceptibility to photocarcinogenesis [25,26]. The beneficial effects of eumelanin result from its ability to absorb UV radiation and to scavenge free radicals generated by this radiation. There is evidence that photolysis products of pheomelanin may be more toxic than those of eumelanin [25,27]. In some fair-skinned individuals, an increased rate of eumelanin turnover occurs in keratinocytes due to enhanced lysosomal degradation, which reduces their level of photoprotection. Pheomelanin may also promote malignant tumour development through indirect effects on the immune system. T lymphocytes require cysteine for proper functioning and activation. In tissues where pheomelanin content is high, cysteine concentrations decline, impairing the ability of local lymphocytes to eliminate mutated cells [28]. In addition, increased levels of ROS generated during pheomelanin synthesis inhibit the activity of CD8⁺ T lymphocytes [29]. While malignant transformation is often associated with the loss of specialised cellular functions, in melanoma the expression of tyrosinase and other melanogenic enzymes is generally elevated [30]. Tyrosinase overexpression in transformed melanocytes has been associated with defective melanosomes [24]. The detection of tyrosinase in blood serves as a diagnostic marker for melanoma; furthermore, tests detecting melanogenic metabolites in blood and urine are used for clinical monitoring [31,32]. Several mechanisms contributing to pheomelanin-induced carcinogenesis are linked to enhanced production of ROS, abundantly generated during its synthesis [27]. Pheomelanin synthesis requires large amounts of antioxidants, which in turn depletes antioxidants such as GSH, rendering melanocytes more vulnerable to damage. Many studies have demonstrated a relationship between GSH deficiency, resulting from its utilisation in pheomelanin synthesis, and cellular oxidative stress [33,34]. Moreover, the presence of sulphur in the aromatic ring of pheomelanin lowers its ionisation potential, making it less stable when generating ROS compared with eumelanin [35]. In recent years, suggestions have emerged that the mechanism of malignant transformation in melanocytes may involve chemiexcitation of melanins under oxidative stress, leading to UVR-independent formation of highly mutagenic cyclobutane pyrimidine dimers (CPDs). Studies have shown that pheomelanin is a much stronger generator of CPDs than eumelanin [35,36].

Tyrosinase, encoded by the *TYR* gene, is the main enzyme responsible for the conversion of tyrosine to melanin [6]. The ratio of eumelanin to pheomelanin depends on TYR activity. It is believed that reduced activity and/or availability of TYR in melanocytes promotes pheomelanin synthesis. TYR, together with two proteins, TYRP1 and TYRP2 (DCT), has been shown to form a multienzyme complex within melanocytes [37]. Mutations in the genes encoding these proteins impair the function of the complex and significantly affect skin pigmentation [38,39]. TYRP1 has been attributed roles in activating and stabilising TYR, shaping melanosomes, and regulating and/or influencing the type of melanin synthesised. DCT, on the other hand, is responsible for the conversion of DOPAchrome into

a carboxylic acid derivative. Its main function is considered to be the regulation of eumelanin and pheomelanin levels in human skin cells. Reports indicate that reduced DCT expression enhances pheomelanogenesis. The absence of promoter activity for all three enzymes (TYR, TYRP1, and DCT) favours the synthesis of pheomelanin [24,40]. This is associated with the inhibition of MITF following the binding of ASP to MC1R, which normally regulates eumelanin synthesis. Binding of ASP to the receptor suppresses eumelanin production, thereby promoting pheomelanin synthesis [41,42]. Conversely, when α -MSH binds to MC1R, melanin synthesis is shifted towards eumelanin. The precursor of α -MSH is POMC. Reduced expression or mutation of the POMC gene prevents α -MSH formation, correlating with increased pheomelanin synthesis [43]. An inactivating mutation in MC1R results in complete inhibition of eumelanin synthesis. This disturbs the balance between the two skin pigments and leads to excessive pheomelanin production, which has been demonstrated to increase the risk of spontaneous malignant transformations towards melanoma [44].

A shift in the eumelanin/pheomelanin ratio towards pheomelanin is also associated with a decrease in intracellular cysteine levels [32,44]. The gene responsible for cysteine transport into melanocytes is *SLC7A11* [34,45]. It encodes the xCT cystine/glutamate antiporter, which supplies cysteine for the synthesis of GSH, the main cellular antioxidant. Reduced GSH, in its active form, acts as a cofactor in ROS detoxification. Increased pheomelanin production results in chronic oxidative stress associated with constant cysteine consumption and, consequently, GSH depletion [24]. GSH deficiency impairs the removal of harmful ROS generated during oxidative stress. This predisposes to melanoma development, as melanocytes become more vulnerable to DNA damage [46]. The cystine/H⁺ symporter encoded by the *CTNS* gene also plays a role in pheomelanin synthesis. It exports cystine from melanosomes. Silencing of *CTNS* due to mutation results in melanosomal acidification, leading to pigmentation defects and a markedly increased risk of melanoma [47–49]. It has been demonstrated that melanosomal pH is a key regulatory mechanism of both eumelanogenesis and pheomelanogenesis [50]. Acidic melanosomal pH promotes pheomelanin synthesis. Individuals with fair skin have relatively more acidic melanosomal interiors compared with those of individuals with darker skin. Numerous signalling cascades regulate melanosomal pH. One is the MC1R-associated cascade, in which activation by α -MSH leads to the production of transmembrane adenylyl cyclases (tmACs), which subsequently induce MITF, crucial for skin pigmentation and UVR protection [13]. Another signalling pathway involves cAMP. It has been shown that soluble adenylyl cyclase (sAC) also regulates melanosomal pH [51]. Loss of sAC activity results in a rapid rise in melanosomal pH and, consequently, reduced pheomelanin synthesis. The *SLC45A2* gene is essential for maintaining melanosomal pH, as it facilitates H⁺ efflux from melanosomes [52]. Loss of *SLC45A2* has been shown to cause melanosomal acidification, leading to reduced pigmentation and increased risk of malignant transformation [53–56].

Each of the above-mentioned genes and their protein products directly or indirectly influences the proper course of pheomelanin synthesis. Changes in the expression of these genes, together with mutations in any of them, may increase the risk of skin cancer development.

In this study, the expression of selected melanogenic genes was assessed in melanoma cell lines with distinct phenotypes, namely the amelanotic C32 and the melanotic G361. It is assumed that the amelanotic line may contain a higher proportion of pheomelanin compared with the melanotic line. This assumption is based on the lighter colour of pheomelanin relative to eumelanin, which hypothetically may be synthesised to a greater extent in lightly pigmented (amelanotic) melanoma than in darkly pigmented (melanotic) melanoma. The inconspicuous appearance of amelanotic melanoma often results in later detection, and delayed diagnosis increases the risk of metastasis. However, to date, no differences in survival have been documented between patients with amelanotic and melanotic melanoma [57,58]. Likewise, there is no evidence for differences in pheomelanin levels between pigmentationally distinct forms of melanoma; nevertheless, some indication supporting this hypothesis arises from observations of normal melanocytes derived from donors with light and dark skin pigmentation [59].

2. Materials and Methods

2.1. Materials

Human melanoma cell lines C32 (CRL-1585TM) and G361 (CRL-1424TM) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). McCoy's 5A Medium (modified with L-glutamine and sodium bicarbonate), penicillin-streptomycin solution (10 000 U penicillin, 10 mg streptomycin/mL) and fetal bovine serum (FBS) were purchased from Merck, Sigma-Aldrich (St. Louis, MO, USA). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1M) was obtained from Gibco, ThermoFisher Scientific (Grand Island, NY, USA). Dulbecco's phosphate buffered saline (DPBS) was from Gibco, Life Technologies (Paisley, PA4, UK).

2.2. Cell Cultures

C32 and G361 human melanoma cells were grown in monolayer in McCoy's 5A Medium, supplemented with HEPES (10 mM), FBS (10%) and antibiotics (penicillin 100 U/mL, streptomycin 100 ug/mL). Cells were seeded into tissue culture flasks at the density of 8×10^4 cells/cm² and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂. When the melanoma cultures reached about 80% confluence, the cells were washed twice with DPBS.

2.3. RNA Isolation and Real-Time PCR

Total cellular RNA was extracted from confluent melanoma cell cultures with TRI Reagent® according to the manufacturer's protocol (Sigma-Aldrich Co, St. Louis, MO, USA). Residual genomic DNA, as well as any traces of melanin were removed using the RNA Clean-up Kit (Syngen Biotech, Wroclaw, Poland). RNA integrity was confirmed by agarose gel electrophoresis. The purity and concentration of the RNA was checked by UV spectrophotometry (*MaestroNano MN-913*; MaestroGen Inc, Hsinchu, Taiwan). Prior to RT-PCR, the RNA samples were treated with DNase 1 Amplification Grade (Sigma-Aldrich Co, St. Louis, MO, USA) according to the manufacturer's instructions. For cDNA synthesis, two micrograms of total RNA from each sample was reverse transcribed using the iScriptTM Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions.

The real-time PCR reactions were performed with the CFX Connect Real-Time System thermal cycler (Bio-Rad Laboratories, Inc., USA). cDNA was amplified using the SsoAdvanced Universal SYBR® Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA); 20 µL of PCR mix was added to each well of PrimePCR plate containing primers for melanocyte development and pigmentation genes (Melanocyte development and pigmentation PrimePCR Plate 96; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Real-time PCR reactions for *SLC7A11* (Hs.390594), *SLC45A2* (Hs.278962), *CTNS* (Hs.187667) and *DCT* gene (Hs.307865) were performed using individual primers designed for these genes (PrimePCRTM SYBR® Green Assay; Bio-Rad Laboratories).

A thermal cycling program used was: activation at 95°C for 30 s, denaturation at 95°C for 5 s (40 cycles), annealing and extension at 60°C for 30 s (40 cycles) and melt curve at 65-95°C (0.5 increments, 5 sec/step). Cycle threshold (Ct) values were used to quantify gene expression.

2.4. Data Analysis

The Ct values (threshold values) of the target genes were normalized against the Ct values of the housekeeping *HPRT1* (hypoxanthine phosphoribosyltransferase 1; Hs.412707) gene, which exhibited a relatively high expression stability in melanoma cell lines [60]. The transcript level of *HPRT1* was comparable between the two lines, confirming the accuracy of normalisation. Each sample was analysed in a minimum of three independent replicates, and the mean value was used in the statistical analysis.

The fold change in gene expression between the melanoma G361 and melanoma C32 pair was calculated using the relative quantification $2^{-\Delta\Delta Ct}$ method. Two-fold or more differences in gene expression between C32 and G361 were considered significant.

Data represent the mean \pm SEM from three independent experiments. The Shapiro–Wilk test was used to assess the normality of the data. One-way analysis of variance followed by post-hoc Tukey's test (TIBCO Software Inc. Statistica version 13.3) were used for group comparisons. P-values < 0.05 were considered statistically significant.

3. Results

Analysis of a panel of 40 genes associated with melanocyte development and pigmentation, performed using a plate containing primers specific for these genes, showed detectable expression of all genes in both melanoma cell lines (Figure 1). Among these, six genes (*KIT*, *KITLG*, *MC1R*, *POMC*, *PRKCB*, and *TYRP1*) exhibited the most pronounced differential expression.

Several genes were consistently expressed at high levels in both cell lines, including *CTNNB1*, *GNAS*, *MITF*, *PRKAR1A*, and *TYR*. By contrast, high expression restricted to the C32 line was observed for *LEF1* and *PRKAR2B*, whereas *TYRP1* was highly expressed only in the G361 line.

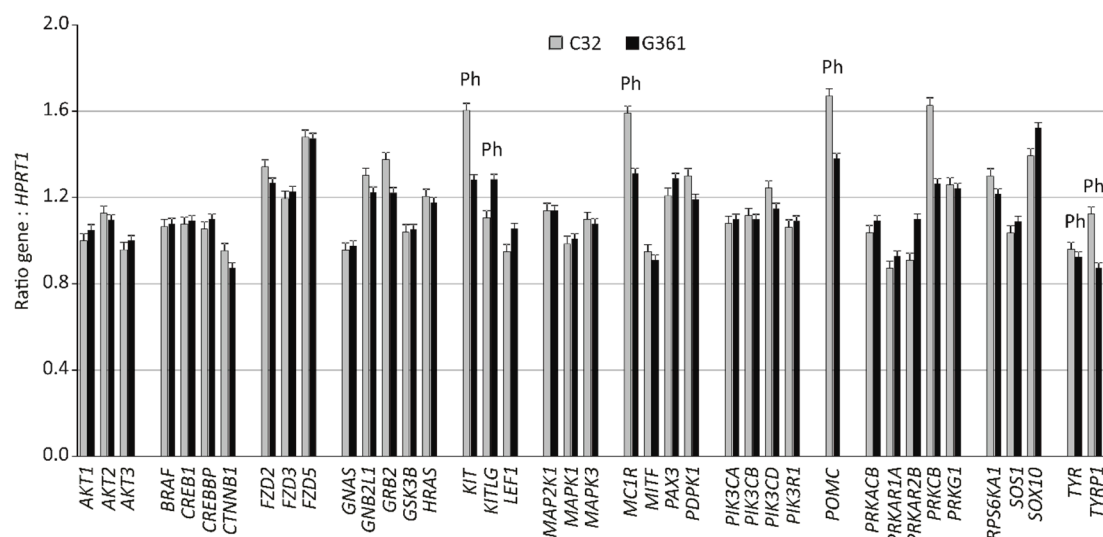


Figure 1. Comparative expression of genes involved in development and pigmentation in amelanotic C32 and melanotic G361 melanoma cells. The bar graphs depict mRNA expression levels (Ct values) normalized to *HPRT1* (mean \pm SD), where higher bars indicate lower gene expression. Ph - pheomelanogenesis-associated genes.

Differential expression was observed for three genes encoding transport proteins involved in pheomelanogenesis (Figure 2). *SLC7A11* and *CTNS* expression differed significantly between the two lines ($p < 0.05$), while *SLC45A2* showed an even greater divergence ($p < 0.01$). Taken together, the melanotic line was characterised by higher expression of *SLC45A2* and *CTNS*, whereas the amelanotic line showed elevated expression of *SLC7A11*. Unexpectedly, *DCT* expression was elevated in the amelanotic melanoma cell line relative to the melanotic counterpart. The observed difference in gene expression was highly statistically significant ($p < 0.001$).

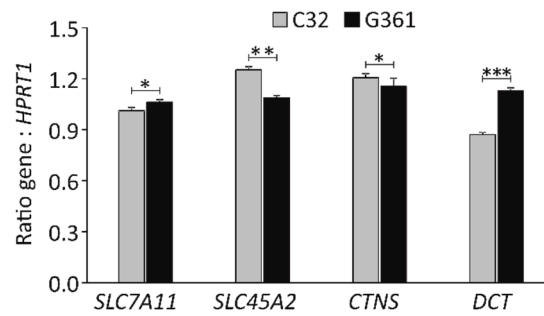


Figure 2. Expression of pheomelanogenesis-related genes in amelanotic (C32) and melanotic (G361) melanoma cells. The bar graphs represent mRNA expression levels normalized to *HPRT1* (mean \pm SD), with bar height inversely correlated with gene expression. Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Significant differences in gene expression between the melanoma cell lines studied were defined using a threshold of a twofold relative ratio ($R \geq 2$). Using this criterion, 13 genes exhibited higher expression in the C32 line (Table 1), while as many as 20 genes were more highly expressed in the G361 line (Table 2).

Table 1. Genes responsible for pigmentation and melanocyte development characterised by higher expression in amelanotic C32 melanoma cells than in melanotic G361 melanoma cells.

Gene name	Gene symbol	UniGene ID	R (fold)	P-value
<i>Dopachrome tautomerase/tyrosinase related protein 2</i> ^(*)	<i>DCT/TYRP2</i>	Hs.307865	37	0.008
Protein kinase cAMP-dependent, type II regulatory subunit beta	<i>PRKAR2B</i>	Hs.433068	16	0.006
KIT ligand	<i>KITLG</i>	Hs.1048	11	0.005
Lymphoid enhancer binding factor 1	<i>LEF1</i>	Hs.555947	5	0.001
SRY-box transcription factor 10	<i>SOX10</i>	Hs.376984	4	0.001
Paired box 3	<i>PAX3</i>	Hs.42146	3	0.015
Protein kinase, cAMP-dependent type I regulatory subunit alpha	<i>PRKAR1A</i>	Hs.280342	2	0.008
Protein kinase, cAMP-dependent, catalytic, beta	<i>PRKACB</i>	Hs.487325	2	0.005
v-akt murine thymoma viral oncogene homolog 1	<i>AKT1</i>	Hs.525622	2	0.011
Son of sevenless homolog 1	<i>SOS1</i>	Hs.709893	2	0.002
v-akt murine thymoma viral oncogene homolog 3	<i>AKT3</i>	Hs.498292	2	0.009
CREB binding protein	<i>CREBBP</i>	Hs.459759	2	0.006
Solute carrier family 7 member 11 ^(*)	<i>SLC7A11</i>	Hs.390594	2	0.010

Template cDNA, prepared from melanoma cells total RNA was characterized using the Melanocyte development and pigmentation PrimePCR Plate or PrimePCR™ SYBR® Green Assay^(*) for a single gene (Bio-Rad Laboratories, Inc.). Two-fold or greater differences in gene expression between C32 and G361 were considered significant.

The most pronounced differences were observed for *PRKCB* and *KIT*, whose expression was increased more than 305-fold and 165-fold, respectively, in G361 cells. Elevated expression of *POMC* and *MC1R* was also characteristic of G361, with *POMC* expressed over 100-fold and *MC1R* more than 80-fold higher than in C32 cells. Significantly increased expression of *TYRP1* was also observed in the non-amelanotic line compared with the amelanotic line, reaching 38-fold higher levels in G361 cells, while *TYR* expression was approximately two-fold higher. Additional genes showing increased

expression in G361 included: *GRB2*, *SLC45A2*, *PDPK1*, *PIK3CD*, *RPS6KA1*, *FZD2*, *GNB2L1*, *CTNNB1*, *MITF*, *CTNS*, *AKT2*, *FZD5*, and *PRKG1* (Table 2).

By contrast, genes with higher expression in the amelanotic C32 line included: *DCT/TYRP2* (37-fold higher), *PRKAR2B* (over 16-fold higher), and *KITLG* (11-fold higher), as well as *LEF1*, *SOX10*, *PAX3*, *PRKAR1A*, *PRKACB*, *AKT1*, *SOS1*, *AKT3*, *CREBBP*, and *SLC7A11* (Table 1). The data also demonstrate that the amelanotic and melanotic melanoma cell lines exhibit distinct expression profiles of genes associated with pheomelanogenesis.

Table 2. Genes responsible for pigmentation and melanocyte development characterised by higher expression in melanotic G361 melanoma cells than in amelanotic C32 melanoma cells.

Gene name	Gene symbol	UniGene ID	R (fold)	P-value
Protein kinase C beta	<i>PRKCB</i>	Hs.460355	305	0.002
KIT-proto-oncogene, receptor tyrosinase kinase	<i>KIT</i>	Hs.479754	165	0.012
Proopiomelanocortin	<i>POMC</i>	Hs.1897	108	0.006
Melanocortin 1 receptor	<i>MC1R</i>	Hs.513829	88	0.018
Tyrosinase-related protein 1	<i>TYRP1</i>	Hs.270279	38	0.005
Growth factor receptor-bound protein 2	<i>GRB2</i>	Hs.444356	12	0.012
Solute carrier family 45 member 2 ^(*)	<i>SLC45A2</i>	Hs.278962	10	0.010
3-phosphoinositide dependent protein kinase 1	<i>PDPK1</i>	Hs.459691	6	0.009
Phosphatidylinositol-4,5-biphosphate 3-kinase catalytic subunit delta	<i>PIK3CD</i>	Hs.518451	5	0.002
Ribosomal protein S6 kinase A1	<i>RPS6KA1</i>	Hs.149957	4	0.010
Frizzled class receptor 2	<i>FZD2</i>	Hs.142912	4	0.006
Guanine nucleotide binding protein subunit-2-like 1	<i>GNB2L1</i>	Hs.5662	4	0.012
Catenin (cadherin-associated protein) beta 1	<i>CTNNB1</i>	Hs.476018	3	0.005
Melanocyte inducing transcription factor	<i>MITF</i>	Hs.166017	3	0.024
<i>Cystinosin</i> , <i>Lysosomal Cystine Transporter</i> ^(*)	<i>CTNS</i>	Hs.187667	2	0.004
HRas proto-oncogene, GTPase	<i>HRAS</i>	Hs.37003	2	0.005
AKT serine/threonine kinase 2	<i>AKT2</i>	Hs.631535	2	0.001
Frizzled class receptor 5	<i>FZD5</i>	Hs.17631	2	0.003
Tyrosinase	<i>TYR</i>	Hs.503555	2	0.015
Protein kinase cGMP-dependent type I	<i>PRKG1</i>	Hs.407535	2	0.009

Template cDNA, prepared from melanoma cells total RNA was characterized using the Melanocyte development and pigmentation PrimePCR Plate or PrimePCR™ SYBR® Green Assay^(*) for a single gene (Bio-Rad Laboratories, Inc.). Two-fold or greater differences in gene expression between C32 and G361 were considered significant.

4. Discussion

The process of melanogenesis in the skin plays a key role in protection against the harmful effects of UVR. Abnormalities that may occur at different stages of pigment synthesis increase the risk of melanoma development [38,39]. Individuals with blonde hair, blue eyes, and fair skin complexion, i.e. with a phenotype suggesting predominance of pheomelanin, are at a higher risk of tumour transformation. However, little is known about a possible association between pheomelanin levels in melanoma cells and clinical features that ultimately affect disease progression and prognosis.

In the present study, the expression of selected genes associated with pheomelanogenesis was evaluated in melanoma cells displaying different pigmentation phenotypes. The research material consisted of two commercial human malignant melanoma cell lines, C32 and G361 (ATCC). The amelanotic melanoma cell line C32 was derived from a 53-year-old Caucasian male, whereas the non-amelanotic cell line G361 originated from a 31-year-old Caucasian male. C32 cells were obtained from depigmented lesions with a weak pigmentation phenotype, while G361 cells originated from strongly pigmented lesions.

Although the term 'amelanotic melanoma' implies a complete absence of tumour pigmentation, these lesions are thought to contain melanin and may be particularly enriched in pheomelanin. Wang *et al.* [61], using the Coherent Raman Scattering (CRS) microscopy, studied unstained sections of three cutaneous tumour lesions. Examination of normal skin surrounding the lesions revealed no pheomelanin, whereas high concentrations were observed within the amelanotic melanoma lesions. Pheomelanin was detected in all three examined samples. The authors emphasised that in order to determine the frequency of pheomelanotic melanoma among patients diagnosed with amelanotic lesions, a larger number of samples should be analysed.

At present, no direct methods are available to allow quantitative determination of pheomelanin in tissue, and mapping its spatial distribution in the skin also remains challenging. Raman spectroscopy therefore, appears to be a highly promising technique for the early detection of amelanotic melanoma - lesions caused by melanocytes that predominantly synthesise pheomelanin [62]. It should be stressed that amelanotic melanoma poses major diagnostic difficulties due to the lack of pigmentation and non-specific clinical presentation [63]. It is frequently misdiagnosed as dermatitis, which further delays proper diagnosis and the initiation of treatment. Consequently, its prognosis is much poorer compared with pigmented melanoma [64].

In this study, expression profiles of 44 genes associated with melanocyte development and pigmentation were analysed and classified according to their direct or indirect roles in pheomelanogenesis. Among them, particular attention was given to *POMC*, *MC1R*, *TYRP1*, and *TYR*.

The obtained results indicate that *TYR* expression was twice as high in G361 cells compared with C32 cells. Literature reports suggest that reduced availability or diminished activity of *TYR* promotes pheomelanin synthesis, with increased levels confirmed in atypical naevi and melanomas [24]. Furthermore, Hu *et al.* [65] demonstrated that in uveal melanoma, total eumelanin content was significantly lower than in normal melanocytes. Considering this, it can be assumed with high probability that decreased eumelanin levels in melanoma cells correlate with increased pheomelanin content. A significant difference between the two cell lines was observed in *TYRP1* expression, which was more than 48-fold higher in G361 cells. One of the most important functions of the *TYRP1* protein is the activation and stabilisation of *TYR*, thereby influencing the type of melanin synthesised. Mutations in *TYRP1* result in the loss of this function, directly affecting *TYR* activity. Insufficient stabilisation of *TYR* causes melanogenesis to shift towards pheomelanin [24]. High relative expression values (G361 vs C32) were also recorded for *MC1R* and *POMC*, with expression more than 80-fold and 100-fold higher, respectively, in G361 cells. Polymorphisms in *MC1R* are associated with common variations in skin colour. Some of these genetic variants occur most frequently in fair-skinned individuals, who are consequently more sensitive to UVR. Such polymorphisms increase pheomelanin production [66]. Mutations in the *MC1R* gene may completely block eumelanin synthesis, directing melanogenesis exclusively towards pheomelanin [24]. Loss of *POMC* function due to mutations in the *POMC* gene directly affects *MC1R* activity, thereby enhancing pheomelanogenesis.

In this study, several other genes with a lesser impact on pheomelanogenesis were also analysed. The greatest difference in expression between the two cell lines was recorded for *PRKCB*, whose expression was 305-fold higher in G361 cells compared with C32. Overexpression of this gene has been shown to correlate with poor prognosis in cutaneous melanoma. Furthermore, *PRKCB* mutations affect *TYR* by inhibiting melanin production, thereby increasing the risk of melanoma transformation [66].

KIT, whose expression was more than 165-fold higher in the non-amelanotic line, is a critical gene in melanogenesis regulation. Hu *et al.* [67] also demonstrated that *KIT* interacts with other genes involved in pheomelanogenesis, such as *TYR*, *MITF*, and *DCT*. Moreover, *KIT* mutations and amplifications are most commonly observed in mucosal and acral melanomas [68,69]. It should be noted that melanoma originates from epidermal melanocytes and shares many molecular similarities with melanocyte precursors. This facilitates melanoma progression. Particularly relevant is the *GRB2/PI3K/AKT* signalling pathway, which is crucial for melanoma cell migration and invasion, and involves genes of central importance in the present study [69]. In this study, the expression of *GRB2* and *AKT2* was elevated by more than 10-fold and approximately 2-fold, respectively, in G361 cell. *GRB2* has been reported to activate *PI3K/AKT* signalling, and its overexpression is directly associated with the promotion of secondary tumour formation. Overexpression of *AKT* induces an invasive melanoma phenotype, contributing to metastasis. Dysregulation of *AKT* pathway activation is observed in up to 70% of melanoma cases [70]. *AKT* activation is mediated by *PDPK1*, whose overexpression due to gene copy number increase occurs in most tumours directly correlating with *PI3K/AKT* pathway mutations [69,71]. Here, we show that *PDPK1* expression was approximately sixfold higher in the non-amelanotic than in the amelanotic cell line.

MITF, which exhibited a threefold higher expression in G361 cells, is a well-characterised transcription factor specific to melanocytes. It coordinates numerous signalling pathways involved in melanoma development. Reduced *MITF* expression is associated with increased activity of a cell cycle inhibitor, correlating with a higher risk of distant metastases. Moderate *MITF* expression, in turn, induces melanoma cell proliferation. *MITF* is activated by *SOX10*, currently used as a highly sensitive and specific marker in the diagnosis of melanocytic tumours, including metastatic melanoma [69]. In this study, *SOX10* expression was four times higher in C32 cells.

Attention should also be drawn to the *Wnt/β-catenin* signalling pathway, which is involved in melanocyte development]. *β-catenin* has been shown to play a dual role in both promoting and inhibiting melanoma progression [56]. Expression of *CTNNB1*, directly engaged in this pathway, was nearly threefold higher in G361 cells. Moreover, *Wnt/β-catenin* signalling mediates the activation of *MITF*. Recent studies have shown that activation of this pathway inhibits *SOX10* expression, thereby blocking melanoma growth [68]. In summary, *MITF*, *SOX10*, and the *Wnt/β-catenin* signalling cascade share molecular similarities with melanocyte precursors, thereby influencing tumour transformation. Due to its role in melanoma pathogenesis and the significant differences in expression observed between the cell lines, the *WNT* signalling pathway associated with the *FZD* family also merits discussion. Deregulation of its components contributes to the metastatic progression of melanoma cells Li *et al.* [72] described the *FZD3* receptor in detail, whose gene, one of three from this family, was also examined in the present study. No significant differences were observed in *FZD3* expression between the two lines; however, differences were found for *FZD2* and *FZD5*. *FZD2* expression was more than fourfold, and *FZD5* was more than twofold higher in G361 cells. Among genes more highly expressed in the amelanotic line compared with the non-amelanotic line were *PRKAR2B*, *KITLG*, *LEF1*, and *PAX3*. Of particular note is *LEF1*, whose expression was almost fivefold higher in C32 cells. *LEF1* is responsible for tumour growth and progression and mediates *MITF* activation *via* the *Wnt/β-catenin* cascade. Kim *et al.* [73] demonstrated that *LEF1* is highly expressed in cultured melanoma cells, and its inhibition delays melanoma cell growth and reduces proliferation.

The paradoxically high expression of *DCT* that we observed in the amelanotic melanoma line C32 reflects a deviation from the expected suppression of melanogenic genes in amelanotic cells. Our results indicate that, in amelanotic melanoma, *DCT* expression may be subject to regulatory cues distinct from those underlying the conventional melanogenic program. Several mechanisms may explain this finding. *DCT* expression can persist independently of functional melanogenesis through *MITF* regulation, even when other enzymes of the pathway are inactive [74]. Moreover, *DCT* may exert unconventional functions, such as antioxidant activity and roles in cell survival and signalling, which could confer an advantage to tumour cells [74,75]. Oncogenic alterations present in C32 (*BRAF*

V600E mutation, *PTEN* loss) may further drive *DCT* transcription via MAPK and PI3K/AKT signalling [76]. Finally, incomplete silencing of the melanocytic programme or defects in protein processing could account for high mRNA levels without pigment production [77].

Transporter genes involved in pheomelanogenesis, such as *SLC7A11*, *SLC45A2*, and *CTNS*, are key regulators of cysteine and melanosomal metabolite availability. We observed that their expression differed across melanoma lines, implying a potential role in the observed variations in pheomelanin synthesis. To date, only limited literature is available regarding the expression of these genes, with most reports focusing on the effects of their silencing [78]. In the present study, we observed clear differences in their expression between the melanoma cell lines analysed. For *SLC7A11* (*xCT*), higher expression was detected in the amelanotic C32 line. This gene encodes a transmembrane exchanger that regulates the uptake of cysteine into the cell and the export of glutamate. In melanocytes, *SLC7A11* is directly involved in pheomelanin synthesis and plays a comparatively smaller role in eumelanin production [45]. Our findings therefore suggest 2-fold greater activity of this exchanger in the amelanotic line, which is very likely to promote increased pheomelanin synthesis in these cells. By contrast, *SLC45A2* expression was substantially higher (10-fold) in the melanotic G361 line. This gene contributes to melanin biosynthesis by encoding a protein localised in the melanosomal membrane, where it regulates melanosomal pH and thereby modulates tyrosinase activity [48,54]. The protein, also known as the H⁺/sugar cotransporter, helps to neutralise melanosomal pH, preventing the reduction of tyrosinase activity (with its optimum pH being 6.8) [52]. Loss of function of *SLC45A2* has been associated with reduced pigmentation and an increased risk of melanocyte carcinogenesis [79]. Furthermore, *in vitro* and *in vivo* studies in mice have demonstrated that deletion of this gene can accelerate melanoma progression and metastasis [55]. A similar pattern was observed for *CTNS*, where expression was higher in the melanotic G361 line. The *CTNS* gene encodes a protein that also participates in the regulation of melanosomal pH and influences tyrosinase activity [80]. Silencing of this gene results in melanosomal acidification (pH 5.8-6.3), which in turn inhibits tyrosinase activity. Under such conditions of reduced tyrosinase function, dopaquinone reacts with cysteine to form cysteinyl-DOPA, leading to increased pheomelanin synthesis [47,81]. Taken together, the higher expression of *SLC45A2* and *CTNS* observed in the G361 line strongly suggests that melanogenesis in these cells is shifted towards eumelanogenesis, which in patients may correspond to the black pigmentation typically observed in such tumours.

5. Conclusions

Our study demonstrates that melanoma cell lines with distinct pigmentation phenotypes exhibit markedly different expression profiles of genes involved in melanogenesis and pheomelanogenesis. The paradoxically high expression of *DCT* in the amelanotic C32 line suggests that melanogenic genes may be regulated independently of visible pigment production, potentially reflecting alternative function of *DCT* in cell survival and signalling. Moreover, differential expression of transporter genes such as *SLC7A11*, *SLC45A2*, and *CTNS* suggests that the balance between cysteine availability and melanosomal environment critically influences the synthesis of pheomelanin versus eumelanin. Specifically, elevated *SLC7A11* expression in amelanotic C32 cells may promote pheomelanin accumulation, whereas higher *SLC45A2* and *CTNS* levels in melanotic G361 cells likely favour eumelanogenesis.

These findings highlight the complex molecular regulation of pigment synthesis in melanoma, and suggest that pheomelanin-related pathways may contribute to tumour phenotype, potentially affecting tumorigenesis, progression, and response to UVR. Understanding the distinct genetic and functional profiles of amelanotic and melanotic melanoma cells may provide insights into their differing clinical behaviour and could inform future diagnostic and therapeutic strategies targeting pigment-related pathways.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1. List of genes assessed for expression with the Melanocyte Development and Pigmentation PrimePCR™ Plate (Bio-Rad Laboratories, Inc.).

Author Contributions: Conceptualisation, I.T., Ł.M. and S.K.; methodology, I.T.; formal analysis, I.T.; investigation, I.T., Ł.M., S.K. and J.S.; writing-original draft, I.T., Ł.M. and S.K.; writing-review and editing, I.T., Ł.M., S.K. and J.S.; visualisation, I.T. and S.K.; supervision, J.S.; project administration, I.T. and J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Medical University of Silesia in Katowice, Poland (grant no. PCN-1-046/K/1/F and BNW-1-054/K/3/F).

Data Availability Statement: The original contributions presented in this study are included in the article material. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

cAMP	Cyclic adenosine monophosphate
CPDs	Cyclobutane pyrimidine dimers
CREB	cAMP response element binding protein
CTNS	Lysosomal cysteine transporter
DCT	Dopachrome tautomerase (alias Tyrosinase-related protein 2)
GSH	Glutathione
MC1R	Melanocortin 1 receptor
MITF	Microphthalmia-associated transcription factor
MSH	Melanocyte stimulating hormone
PKA	Protein kinase A
PKC	Protein kinase C
POMC	Proopiomelanocortin
ROS	Reactive oxygen species
sAC	Soluble adenylyl cyclase
SOX	SRY-Box transcription factor
TYR	Tyrosinase
TYRP1	Tyrosinase-related protein 1
TYRP2	Tyrosinase-related protein 2
UVR	Ultraviolet radiation

References

1. Yamaguchi, Y.; Brenner, M.; Hearing, V.J. The Regulation of Skin Pigmentation *. *J. Biol. Chem.* **2007**, *282*, 27557–27561. <https://doi.org/10.1074/jbc.R700026200>.
2. Brenner, M.; Hearing, V.J. The Protective Role of Melanin Against UV Damage in Human Skin†. *Photochem. Photobiol.* **2008**, *84*, 539–549. <https://doi.org/10.1111/j.1751-1097.2007.00226.x>.
3. Cichorek, M.; Wachulska, M.; Stasiewicz, A.; Tymieńska, A. Review Paper
Skin Melanocytes: Biology and Development. *Adv. Dermatol. Allergol. Dermatol. Alergol.* **2013**, *30*, 30–41. <https://doi.org/10.5114/pdia.2013.33376>.
4. Borovanský, J.; Riley, P.A. Physiological and Pathological Functions of Melanosomes. In *Melanins and Melanosomes*; Borovanský, J., Riley, P.A., Eds.; Wiley-Blackwell: Weinheim, Germany, **2011**; Vol. 12, pp. 343–381 ISBN 978-3-527-32892-5.
5. Park, H.Y.; Kosmadaki, M.; Yaar, M.; Gilchrest, B.A. Cellular Mechanisms Regulating Human Melanogenesis. *Cell. Mol. Life Sci.* **2009**, *66*, 1493–1506. <https://doi.org/10.1007/s00018-009-8703-8>.
6. Bae-Harboe, Y.-S.C.; Park, H.-Y. Tyrosinase: A Central Regulatory Protein for Cutaneous Pigmentation. *J. Invest. Dermatol.* **2012**, *132*, 2678–2680. <https://doi.org/10.1038/jid.2012.324>.

7. Videira, I.F. dos S.; Moura, D.F.L.; Magina, S. Mechanisms Regulating Melanogenesis*. *An. Bras. Dermatol.* **2013**, *88*, 76–83. <https://doi.org/10.1590/S0365-05962013000100009>.
8. D’Mello, S.; Finlay, G.; Baguley, B.; Askarian-Amiri, M. Signaling Pathways in Melanogenesis. *Int. J. Mol. Sci.* **2016**, *17*, 1144. <https://doi.org/10.3390/ijms17071144>.
9. Ito, S.; Wakamatsu, K. Chemistry of Mixed Melanogenesis—Pivotal Roles of Dopaquinone. *Photochem. Photobiol.* **2008**, *84*, 582–592. <https://doi.org/10.1111/j.1751-1097.2007.00238.x>.
10. Simon, J.D.; Peles, D.; Wakamatsu, K.; Ito, S. Current Challenges in Understanding Melanogenesis: Bridging Chemistry, Biological Control, Morphology, and Function. *Pigment Cell Melanoma Res.* **2009**, *22*, 563–579. <https://doi.org/10.1111/j.1755-148X.2009.00610.x>.
11. Kondo, T.; Hearing, V.J. Update on the Regulation of Mammalian Melanocyte Function and Skin Pigmentation. *Expert Rev. Dermatol.* **2011**, *6*, 97–108. <https://doi.org/10.1586/edm.10.70>.
12. Buscà, R.; Ballotti, R. Cyclic AMP a Key Messenger in the Regulation of Skin Pigmentation. *Pigment Cell Res.* **2000**, *13*, 60–69. <https://doi.org/10.1034/j.1600-0749.2000.130203.x>.
13. Aoki, H.; Moro, O. Involvement of Microphthalmia-Associated Transcription Factor (MITF) in Expression of Human Melanocortin-1 Receptor (MC1R). *Life Sci.* **2002**, *71*, 2171–2179. [https://doi.org/10.1016/S0024-3205\(02\)01996-3](https://doi.org/10.1016/S0024-3205(02)01996-3).
14. Vachtenheim, J.; Borovanský, J. “Transcription Physiology” of Pigment Formation in Melanocytes: Central Role of MITF. *Exp. Dermatol.* **2010**, *19*, 617–627. <https://doi.org/10.1111/j.1600-0625.2009.01053.x>.
15. Slominski, A.; Wortsman, J. Neuroendocrinology of the Skin1. *Endocr. Rev.* **2000**, *21*, 457–487. <https://doi.org/10.1210/edrv.21.5.0410>.
16. Abdel-Malek, Z.A.; Swope, V.B. Epidermal Melanocytes: Regulation of Their Survival, Proliferation, and Function in Human Skin. In *Melanoma Development: Molecular Biology, Genetics and Clinical Application*; Bosserhoff, A., Ed.; Springer: Vienna, Austria, **2011**; pp. 7–33 ISBN 978-3-7091-0371-5.
17. Park, H.-Y.; Wu, C.; Yonemoto, L.; Murphy-Smith, M.; Wu, H.; Stachur, C.M.; Gilchrist, B.A. MITF Mediates CAMP-Induced Protein Kinase C- β Expression in Human Melanocytes. *Biochem. J.* **2006**, *395*, 571–578. <https://doi.org/10.1042/BJ20051388>.
18. Denning, M.F. Specifying Protein Kinase C Functions in Melanoma. *Pigment Cell Melanoma Res.* **2012**, *25*, 466–476. <https://doi.org/10.1111/j.1755-148X.2012.01015.x>.
19. Wegner, M. All Purpose Sox: The Many Roles of Sox Proteins in Gene Expression. *SOX Transcr. Factors* **2010**, *42*, 381–390. <https://doi.org/10.1016/j.biocel.2009.07.006>.
20. Kubic, J.D.; Young, K.P.; Plummer, R.S.; Ludvik, A.E.; Lang, D. Pigmentation PAX-ways: The Role of Pax3 in Melanogenesis, Melanocyte Stem Cell Maintenance, and Disease. *Pigment Cell Melanoma Res.* **2008**, *21*, 627–645. <https://doi.org/10.1111/j.1755-148X.2008.00514.x>.
21. Solano, F. Melanins: Skin Pigments and Much More—Types, Structural Models, Biological Functions, and Formation Routes. *New J. Sci.* **2014**, *2014*, C. <https://doi.org/10.1155/2014/498276>.
22. Takeuchi, S.; Zhang, W.; Wakamatsu, K.; Ito, S.; Hearing, V.J.; Kraemer, K.H.; Brash, D.E. Melanin Acts as a Potent UVB Photosensitizer to Cause an Atypical Mode of Cell Death in Murine Skin. *Proc. Natl. Acad. Sci.* **2004**, *101*, 15076–15081. <https://doi.org/10.1073/pnas.0403994101>.
23. Mariano, A.; Bigioni, I.; Scotto d’Abusco, A.; Baseggio Conrado, A.; Maina, S.; Francioso, A.; Mosca, L.; Fontana, M. Pheomelanin Effect on UVB Radiation-Induced Oxidation/Nitration of L-Tyrosine. *Int. J. Mol. Sci.* **2021**, *23*, 267. <https://doi.org/10.3390/ijms23010267>.
24. Nasti, T.H.; Timares, L. MC1R, Eumelanin and Pheomelanin: Their Role in Determining the Susceptibility to Skin Cancer. *Photochem. Photobiol.* **2015**, *91*, 188–200. <https://doi.org/10.1111/php.12335>.
25. Riley, P.A. Melanogenesis and Melanoma. *Pigment Cell Res.* **2003**, *16*, 548–552. <https://doi.org/10.1034/j.1600-0749.2003.00069.x>.
26. Zanetti, R.; Prota, G.; Napolitano, A.; Martinez, C.; Sancho-Garnier, H.; Østerlind, A.; Sacerdote, C.; Rosso, S. Development of an Integrated Method of Skin Phenotype Measurement Using the Melanins. *Melanoma Res.* **2001**, *11*, 551–557. <https://doi.org/10.1097/00008390-200112000-00001>.
27. Morgan, A.M.; Lo, J.; Fisher, D.E. How Does Pheomelanin Synthesis Contribute to Melanomagenesis?: Two Distinct Mechanisms Could Explain the Carcinogenicity of Pheomelanin Synthesis. *BioEssays* **2013**, *35*, 672–676. <https://doi.org/10.1002/bies.201300020>.

28. Srivastava, M.K.; Sinha, P.; Clements, V.K.; Rodriguez, P.; Ostrand-Rosenberg, S. Myeloid-Derived Suppressor Cells Inhibit T-Cell Activation by Depleting Cystine and Cysteine. *Cancer Res.* **2010**, *70*, 68–77. <https://doi.org/10.1158/0008-5472.CAN-09-2587>.
29. Kusmartsev, S.; Nefedova, Y.; Yoder, D.; Gabilovich, D.I. Antigen-Specific Inhibition of CD8+ T Cell Response by Immature Myeloid Cells in Cancer Is Mediated by Reactive Oxygen Species. *J. Immunol.* **2004**, *172*, 989–999. <https://doi.org/10.4049/jimmunol.172.2.989>.
30. Boyle, J.L.; Haupt, H.M.; Stern, J.B.; Multhaupt, H.A.B. Tyrosinase Expression in Malignant Melanoma, Desmoplastic Melanoma, and Peripheral Nerve Tumors. *Arch. Pathol. Lab. Med.* **2002**, *126*, 816–822. <https://doi.org/10.5858/2002-126-0816-TEIMMD>.
31. Stevens, G.L.; Scheer, W.D.; Levine, E.A. Detection of Tyrosinase mRNA from the Blood of Melanoma Patients. *Cancer Epidemiol. Biomarkers Prev.* **1996**, *5*, 293–296.
32. Wakamatsu, K.; Fukushima, S.; Minagawa, A.; Omodaka, T.; Hida, T.; Hatta, N.; Takata, M.; Uhara, H.; Okuyama, R.; Ihn, H. Significance of 5-S-Cysteinyldopa as a Marker for Melanoma. *Int. J. Mol. Sci.* **2020**, *21*, 432. <https://doi.org/10.3390/ijms21020432>.
33. Ranadive, N.S.; Shirwadkar, S.; Persad, S.; Menon, I.A. Effects of Melanin-Induced Free Radicals on the Isolated Rat Peritoneal Mast Cells. *J. Invest. Dermatol.* **1986**, *86*, 303–307. <https://doi.org/10.1111/1523-1747.ep12285496>.
34. Galván, I.; Inácio, Â.; Romero-Haro, A.A.; Alonso-Alvarez, C. Adaptive Downregulation of Pheomelanin-related *Slc7a11* Gene Expression by Environmentally Induced Oxidative Stress. *Mol Ecol* **2017**, *26*, 849–858. <https://doi.org/10.1111/mec.13952>.
35. Premi, S. Role of Melanin Chemiexcitation in Melanoma Progression and Drug Resistance. *Front. Oncol.* **2020**, *10*, 1305. <https://doi.org/10.3389/fonc.2020.01305>.
36. Premi, S.; Wallisch, S.; Mano, C.M.; Weiner, A.B.; Bacchiocchi, A.; Wakamatsu, K.; Bechara, E.J.H.; Halaban, R.; Douki, T.; Brash, D.E. Chemiexcitation of Melanin Derivatives Induces DNA Photoproducts Long after UV Exposure. *Science* **2015**, *347*, 842–847. <https://doi.org/10.1126/science.1256022>.
37. Kobayashi, T.; Hearing, V.J. Direct Interaction of Tyrosinase with Tyrp1 to Form Heterodimeric Complexes in Vivo. *J. Cell Sci.* **2007**, *120*, 4261–4268. <https://doi.org/10.1242/jcs.017913>.
38. Slominski, R.M.; Sarna, T.; Płonka, P.M.; Raman, C.; Brożyna, A.A.; Slominski, A.T. Melanoma, Melanin, and Melanogenesis: The Yin and Yang Relationship. *Front. Oncol.* **2022**, *12*, 842496. <https://doi.org/10.3389/fonc.2022.842496>.
39. Snyman, M.; Walsdorf, R.E.; Wix, S.N.; Gill, J.G. The Metabolism of Melanin Synthesis—From Melanocytes to Melanoma. *Pigment Cell Melanoma Res.* **2024**, *37*, 438–452. <https://doi.org/10.1111/pcmr.13165>.
40. Costin, G.-E.; Hearing, V.J. Human Skin Pigmentation: Melanocytes Modulate Skin Color in Response to Stress. *FASEB J.* **2007**, *21*, 976–994. <https://doi.org/10.1096/fj.06-6649rev>.
41. Aberdam, E.; Bertolotto, C.; Sviderskaya, E.V.; Thillot, V. de; Hemesath, T.J.; Fisher, D.E.; Bennett, D.C.; Ortonne, J.-P.; Ballotti, R. Involvement of Microphthalmia in the Inhibition of Melanocyte Lineage Differentiation and of Melanogenesis by Agouti Signal Protein *. *J. Biol. Chem.* **1998**, *273*, 19560–19565. <https://doi.org/10.1074/jbc.273.31.19560>.
42. Vachtenheim, J.; Novotna, H.; Ghanem, G. Transcriptional Repression of the Microphthalmia Gene in Melanoma Cells Correlates with the Unresponsiveness of Target Genes to Ectopic Microphthalmia-Associated Transcription Factor. *J. Invest. Dermatol.* **2001**, *117*, 1505–1511. <https://doi.org/10.1046/j.0022-202x.2001.01563.x>.
43. Harno, E.; Gali Ramamoorthy, T.; Coll, A.P.; White, A. POMC: The Physiological Power of Hormone Processing. *Physiol. Rev.* **2018**, *98*, 2381–2430. <https://doi.org/10.1152/physrev.00024.2017>.
44. Wakamatsu, K.; Zippin, J.H.; Ito, S. Chemical and Biochemical Control of Skin Pigmentation with Special Emphasis on Mixed Melanogenesis. *Pigment Cell Melanoma Res.* **2021**, *34*, 730–747. <https://doi.org/10.1111/pcmr.12970>.
45. Galván, I.; Inácio, Â.; Dañino, M.; Corbí-Llopis, R.; Monserrat, M.T.; Bernabeu-Wittel, J. High SLC7A11 Expression in Normal Skin of Melanoma Patients. *Cancer Epidemiol.* **2019**, *62*, 101582. <https://doi.org/10.1016/j.canep.2019.101582>.

46. Chintala, S.; Li, W.; Lamoreux, M.L.; Ito, S.; Wakamatsu, K.; Sviderskaya, E.V.; Bennett, D.C.; Park, Y.-M.; Gahl, W.A.; Huizing, M.; et al. *Slc7a11* Gene Controls Production of Pheomelanin Pigment and Proliferation of Cultured Cells. *Proc. Natl. Acad. Sci.* **2005**, *102*, 10964–10969. <https://doi.org/10.1073/pnas.0502856102>.
47. Chiaverini, C.; Sillard, L.; Flori, E.; Ito, S.; Briganti, S.; Wakamatsu, K.; Fontas, E.; Berard, E.; Cailliez, M.; Cochat, P.; et al. Cystinosin Is a Melanosomal Protein That Regulates Melanin Synthesis. *FASEB J.* **2012**, *26*, 3779–3789. <https://doi.org/10.1096/fj.11-201376>.
48. Koppula, P.; Zhang, Y.; Zhuang, L.; Gan, B. Amino Acid Transporter SLC7A11/XCT at the Crossroads of Regulating Redox Homeostasis and Nutrient Dependency of Cancer. *Cancer Commun.* **2018**, *38*, 1–13. <https://doi.org/10.1186/s40880-018-0288-x>.
49. Ohbayashi, N.; Fukuda, M. Recent Advances in Understanding the Molecular Basis of Melanogenesis in Melanocytes. *F1000Research* **2020**, *9*, F1000 Faculty Rev-608. <https://doi.org/10.12688/f1000research.24625.1>.
50. Ancans, J.; Tobin, D.J.; Hoogduijn, M.J.; Smit, N.P.; Wakamatsu, K.; Thody, A.J. Melanosomal PH Controls Rate of Melanogenesis, Eumelanin/Phaeomelanin Ratio and Melanosome Maturation in Melanocytes and Melanoma Cells. *Exp. Cell Res.* **2001**, *268*, 26–35. <https://doi.org/10.1006/excr.2001.5251>.
51. Ota, K.; Zhou, D.; Zippin, J. 2321: Soluble Adenylyl Cyclase (SAC) Regulates Melanogenesis and Melanocyte Response to UVB. *J. Clin. Transl. Sci.* **2017**, *1*, 7–7. <https://doi.org/10.1017/cts.2017.40>.
52. Le, L.; Escobar, I.E.; Ho, T.; Lefkovith, A.J.; Latteri, E.; Haltaufderhyde, K.D.; Dennis, M.K.; Plowright, L.; Sviderskaya, E.V.; Bennett, D.C.; et al. SLC45A2 Protein Stability and Regulation of Melanosome PH Determine Melanocyte Pigmentation. *Mol. Biol. Cell* **2020**, *31*, 2687–2702. <https://doi.org/10.1091/mbc.E20-03-0200>.
53. Ibarrola-Villava, M.; Fernandez, L.P.; Alonso, S.; Boyano, M.D.; Peña-Chilet, M.; Pita, G.; Aviles, J.A.; Mayor, M.; Gomez-Fernandez, C.; Casado, B.; et al. A Customized Pigmentation SNP Array Identifies a Novel SNP Associated with Melanoma Predisposition in the SLC45A2 Gene. *PLoS ONE* **2011**, *6*, e19271. <https://doi.org/10.1371/journal.pone.0019271>.
54. Park, J.; Talukder, A.H.; Lim, S.A.; Kim, K.; Pan, K.; Melendez, B.; Bradley, S.D.; Jackson, K.R.; Khalili, J.S.; Wang, J.; et al. SLC45A2: A Melanoma Antigen with High Tumor Selectivity and Reduced Potential for Autoimmune Toxicity. *Cancer Immunol. Res.* **2017**, *5*, 618–629. <https://doi.org/10.1158/2326-6066.CIR-17-0051>.
55. Liu, Y.; Chi, W.; Tao, L.; Wang, G.; Deepak, R.N.V.K.; Sheng, L.; Chen, T.; Feng, Y.; Cao, X.; Cheng, L.; et al. Ablation of Proton/Glucose Exporter *SLC45A2* Enhances Melanosomal Glycolysis to Inhibit Melanin Biosynthesis and Promote Melanoma Metastasis. *J. Invest. Dermatol.* **2022**, *142*, 2744–2755.e9. <https://doi.org/10.1016/j.jid.2022.04.008>.
56. Liu, J.; Fukunaga-Kalabis, M.; Li, L.; Herlyn, M. Developmental Pathways Activated in Melanocytes and Melanoma. *Adv. Melanocyte Melanoma Biol.* **2014**, *563*, 13–21. <https://doi.org/10.1016/j.abb.2014.07.023>.
57. Muinonen-Martin, A.J.; O’Shea, S.J.; Newton-Bishop, J. Amelanotic Melanoma. *BMJ* **2018**, *360*, k826. <https://doi.org/10.1136/bmj.k826>.
58. Gong, H.-Z.; Zheng, H.-Y.; Li, J. Amelanotic Melanoma. *Melanoma Res.* **2019**, *29*, 221–230. <https://doi.org/10.1097/CMR.0000000000000571>.
59. Dzierżęga-Lęcznar, A.; Kurkiewicz, S.; Tam, I.; Marek, Ł.; Stępień, K. Pheomelanin Content of Cultured Human Melanocytes from Lightly and Darkly Pigmented Skin: A Pyrolysis-Gas Chromatography/Tandem Mass Spectrometry Study. *J. Anal. Appl. Pyrolysis* **2017**, *124*, 349–354. <https://doi.org/10.1016/j.jaap.2017.01.009>.
60. Christensen, J.N.; Schmidt, H.; Steiniche, T.; Madsen, M. Identification of Robust Reference Genes for Studies of Gene Expression in FFPE Melanoma Samples and Melanoma Cell Lines. *Melanoma Res.* **2020**, *30*, 26–38. <https://doi.org/10.1097/CMR.0000000000000644>.
61. Wang, H.; Osseiran, S.; Igras, V.; Nichols, A.J.; Roider, E.M.; Pruessner, J.; Tsao, H.; Fisher, D.E.; Evans, C.L. In Vivo Coherent Raman Imaging of the Melanomagenesis-Associated Pigment Pheomelanin. *Sci. Rep.* **2016**, *6*, 37986. <https://doi.org/10.1038/srep37986>.
62. Neves, A.C. de O.; Zougagh, M.; Ríos, Á.; Tauler, R.; Wakamatsu, K.; Galván, I. Pheomelanin Subunit Non-Destructive Quantification by Raman Spectroscopy and Multivariate Curve Resolution-Alternating Least

- Squares (MCR-ALS). *Chemom. Intell. Lab. Syst.* **2021**, *217*, 104406. <https://doi.org/10.1016/j.chemolab.2021.104406>.
63. Adler, M.; White, C. Amelanotic Malignant Melanoma. *Semin Cutan Med Surg* **1997**, *16*, 122–130. [https://doi.org/10.1016/s1085-5629\(97\)80006-5](https://doi.org/10.1016/s1085-5629(97)80006-5).
64. Cheung, W.L.; Patel, R.R.; Leonard, A.; Firoz, B.; Meehan, S.A. Amelanotic Melanoma: A Detailed Morphologic Analysis with Clinicopathologic Correlation of 75 Cases. *J. Cutan. Pathol.* **2012**, *39*, 33–39. <https://doi.org/10.1111/j.1600-0560.2011.01808.x>.
65. Hu, D.-N.; Wakamatsu, K.; Ito, S.; McCormick, S.A. Comparison of Eumelanin and Pheomelanin Content between Cultured Uveal Melanoma Cells and Normal Uveal Melanocytes. *Melanoma Res.* **2009**, *19*, 75–79. <https://doi.org/10.1097/CMR.0b013e328329ae49>.
66. Wang, J.; Yang, J. Identification of Significant Genes with a Poor Prognosis in Skin Cutaneous Malignant Melanoma Based on a Bioinformatics Analysis. *Ann. Transl. Med.* **2022**, *10*, 448–448. <https://doi.org/10.21037/atm-22-1163>.
67. Hu, S.; Chen, Y.; Zhao, B.; Yang, N.; Chen, S.; Shen, J.; Bao, G.; Wu, X. KIT Is Involved in Melanocyte Proliferation, Apoptosis and Melanogenesis in the Rex Rabbit. *PeerJ* **2020**, *8*, e9402. <https://doi.org/10.7717/peerj.9402>.
68. Guo, W.; Wang, H.; Li, C. Signal Pathways of Melanoma and Targeted Therapy. *Signal Transduct. Target. Ther.* **2021**, *6*, 424. <https://doi.org/10.1038/s41392-021-00827-6>.
69. Pu, Y.; Lei, M.; Chen, Y.; Huang, Y.; Zhang, L.; Chen, J.; Zhang, Y.; Shao, X.; Liu, L.; Chen, J. Hey1 Promotes Migration and Invasion of Melanoma Cells via GRB2/PI3K/AKT Signaling Cascade. *J. Cancer* **2021**, *12*, 6979–6988. <https://doi.org/10.7150/jca.60974>.
70. Robertson, G.P. Functional and Therapeutic Significance of Akt Deregulation in Malignant Melanoma. *Cancer Metastasis Rev.* **2005**, *24*, 273–285. <https://doi.org/10.1007/s10555-005-1577-9>.
71. Gagliardi, P.A.; Puliafito, A.; Primo, L. PDK1: At the Crossroad of Cancer Signaling Pathways. *AGC Kinases Cancer Metastasis Immunecheckpoint Regul. Drug Resist.* **2018**, *48*, 27–35. <https://doi.org/10.1016/j.semcan.2017.04.014>.
72. Li, C.; Nguyen, V.; Clark, K.N.; Zahed, T.; Sharkas, S.; Filipp, F.V.; Boiko, A.D. Down-Regulation of FZD3 Receptor Suppresses Growth and Metastasis of Human Melanoma Independently of Canonical WNT Signaling. *Proc. Natl. Acad. Sci.* **2019**, *116*, 4548–4557. <https://doi.org/10.1073/pnas.1813802116>.
73. Kim, G.-H.; Fang, X.-Q.; Lim, W.-J.; Park, J.; Kang, T.-B.; Kim, J.H.; Lim, J.-H. Cinobufagin Suppresses Melanoma Cell Growth by Inhibiting LEF1. *Int. J. Mol. Sci.* **2020**, *21*, 6706. <https://doi.org/10.3390/ijms21186706>.
74. Ainger, S.A.; Yong, X.L.; Wong, S.S.; Skalamera, D.; Gabrielli, B.; Leonard, J.H.; Sturm, R.A. DCT Protects Human Melanocytic Cells from UVR and ROS Damage and Increases Cell Viability. *Exp. Dermatol.* **2014**, *23*, 916–921. <https://doi.org/10.1111/exd.12574>.
75. Roider, E.; Lakatos, A.I.T.; McConnell, A.M.; Wang, P.; Mueller, A.; Kawakami, A.; Tsoi, J.; Szabolcs, B.L.; Ascsillán, A.A.; Suita, Y.; et al. MITF Regulates IDH1, NNT, and a Transcriptional Program Protecting Melanoma from Reactive Oxygen Species. *Sci. Rep.* **2024**, *14*, 21527. <https://doi.org/10.1038/s41598-024-72031-9>.
76. Dankort, D.; Curley, D.P.; Cartlidge, R.A.; Nelson, B.; Karnezis, A.N.; Damsky Jr, W.E.; You, M.J.; DePinho, R.A.; McMahon, M.; Bosenberg, M. BrafV600E Cooperates with Pten Loss to Induce Metastatic Melanoma. *Nat. Genet.* **2009**, *41*, 544–552. <https://doi.org/10.1038/ng.356>.
77. Laurette, P.; Coassolo, S.; Davidson, G.; Michel, I.; Gambi, G.; Yao, W.; Sohler, P.; Li, M.; Mengus, G.; Larue, L.; et al. Chromatin Remodellers Brg1 and Bptf Are Required for Normal Gene Expression and Progression of Oncogenic Braf-Driven Mouse Melanoma. *Cell Death Differ.* **2020**, *27*, 29–43. <https://doi.org/10.1038/s41418-019-0333-6>.
78. Li, P.; Yu, J.; Huang, F.; Zhu, Y.-Y.; Chen, D.-D.; Zhang, Z.-X.; Xie, Z.-C.; Liu, Z.-Y.; Hou, Q.; Xie, N.; et al. SLC7A11-Associated Ferroptosis in Acute Injury Diseases: Mechanisms and Strategies. *Eur. Rev. Med. Pharmacol. Sci.* **2023**, *27*, 4386–4398. https://doi.org/10.26355/eurrev_202305_32444.

79. Hopkins, Z.H.; Carlisle, R.P.; Frost, Z.E.; Curtis, J.A.; Ferris, L.K.; Secrest, A.M. Risk Factors and Predictors of Survival Among Patients with Amelanotic Melanoma Compared to Melanotic Melanoma in the National Cancer Database. *J. Clin. Aesthetic Dermatol.* **2021**, *14*, 36–43.
80. Bin, B.-H.; Bhin, J.; Yang, S.H.; Shin, M.; Nam, Y.-J.; Choi, D.-H.; Shin, D.W.; Lee, A.-Y.; Hwang, D.; Cho, E.-G.; et al. Membrane-Associated Transporter Protein (MATP) Regulates Melanosomal PH and Influences Tyrosinase Activity. *PLOS ONE* **2015**, *10*, e0129273. <https://doi.org/10.1371/journal.pone.0129273>.
81. Ito, S.; Wakamatsu, K. Human Hair Melanins: What We Have Learned and Have Not Learned from Mouse Coat Color Pigmentation. *Pigment Cell Melanoma Res.* **2011**, *24*, 63–74. <https://doi.org/10.1111/j.1755-148X.2010.00755.x>.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.