High *SLC7A11* expression in normal skin of melanoma patients

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26 **Abstract**

27 **Background**

28 Melanoma is one of the highest metastatic cancers and its incidence is rapidly increasing.
29 A great effort has been devoted to determine gene mutations and expression profiles in
30 melanoma cells, but less attention has been given to the possible influence of melanin
31 synthesis in melanocytes on melanomagenesis. *SLC7A11* encodes the cystine/glutamate
32 antiporter xCT and its expression increases the antioxidant capacity of cells by providing
33 cysteine that may be used for glutathione (GSH) synthesis. Melanocytes, however, can
34 also use cysteine for pheomelanin synthesis and pigmentation. Therefore, pheomelanin
35 synthesis may lead to chronic oxidative stress. Possible consequences of this for
36 melanomagenesis have never been explored.

37 **Methods**

38 We quantified the expression of *SLC7A11* and other genes that are involved in the
39 synthesis of pheomelanin but do not regulate the transport of cysteine from the
40 extracellular medium to the cytosol (*CTNS*, *MC1R*, *ASIP* and *SLC45A2*) in non-tumorous
41 skin of 45 patients of cutaneous melanoma and 50 healthy individuals. We controlled for
42 the effects of Fitzpatrick skin type, age, gender, body mass, frequency of sun exposure
43 and sunburns and number of melanocytic nevi, as well as for the intrinsic antioxidant
44 capacity as given by the expression of the gene *NFE2L2*.

45 **Results**

46 The expression of *SLC7A11*, but not of the other genes, was significantly higher in
47 melanoma patients than in healthy individuals. This was independent of phenotypic factors
48 and antioxidant capacity, thus supporting an effect of pheomelanin-induced oxidative
49 stress on melanomagenesis.

50 **Conclusion**

Our findings indicate that *SLC7A11* downregulation in normal epidermal melanocytes may represent a preventive treatment against melanoma.

Keywords gene expression; melanogenesis; melanoma; Pheomelanin; oxidative stress; skin pigmentation.

1. Introduction

Melanoma cells are one of the highest metastatic cancer cells [1], and the incidence of invasive melanoma has risen steadily in the last two decades worldwide [2]. A significant effort is being devoted to the identification of mutations in genes [3-5] and gene expression profiles [6, 7] associated to melanoma progression. Most of these studies have focused on tumor cells either *in vitro* or *in vivo*, but less attention has been given to the physiology of normal melanocytes that can potentially become melanoma cells. The fact that melanocytes synthesize melanin pigments has been treated with relative little consideration, though the amount of melanins synthesized has been shown to affect the mechanical properties of melanocytes and their capacity to metastasize [8]. The melanogenic activity of melanocytes can hardly be inhibited, but the chemistry of the melanins that are synthesized may be partly modified, although genes regulating melanin synthesis have barely been investigated in relation to melanoma beyond the melanocortin 1 receptor (*MC1R*) gene [9]. Recent findings, however, indicate that melanin chemistry fulfills an essential role in melanomagenesis, opening new avenues for melanoma treatment. In particular, the synthesis pathway of the sulphurated form of melanin, termed pheomelanin, has been shown to be involved in melanoma progression by a mechanism of reactive oxygen species (ROS)-mediated stress causing DNA damage that is independent of exposure to ultraviolet (UV) light [10]. In the past few decades, Prota *et al.* had already highlighted the association between pheomelanin and melanomagenesis [11].

Oxidative stress is probably generated during the pheomelanin synthesis pathway in melanocytes because the sulfhydryl group of cysteine is incorporated into the pigment structure, which can reduce the availability of cysteine to form the antioxidant glutathione (GSH) and thus produce a situation of chronic oxidative stress when pheomelanin synthesis is abundant and constant [12, 13]. Thus, intramelanocytic cysteine can enter the pheomelanin synthesis pathway instead of the GSH synthesis pathway, and as

101 pheomelanin is transferred to keratinocytes and associated epidermal structures such as
102 hair [14], cysteine used for pheomelanin synthesis cannot be recovered for physiological
103 use as an antioxidant.

104 Pheomelanin-mediated oxidative stress may be key for understanding
105 melanomagenesis because it leads to a novel perspective of melanocytes: these are the
106 only cells that synthesize pheomelanin, making that part of cellular cysteine is used for
107 pheomelanin synthesis instead of GSH synthesis. This may limit the antioxidant capacity
108 of melanocytes. The recognition of this characteristic feature of melanocytes may help to
109 understand the outcome of melanoma. In this regard, the gene *SLC7A11* (solute carrier
110 family 7 member 11), which encodes the cystine/glutamate antiporter xCT (system x_c⁻)
111 [15], is highly expressed in cancer cells because of its regulatory role of cystine uptake
112 and incorporation into GSH [16]. *SLC7A11* thus enhances the antioxidant capacity of cells,
113 and its inhibition, which can be mediated by p53, induces ferroptosis upon oxidative stress
114 [17]. This explains why *SLC7A11* is highly expressed in several cancer types such as
115 breast [18], bladder [19] and glioma [20]. If *SLC7A11* is expressed in normal melanocytes,
116 the synthesis of pheomelanin by these cells may cause chronic oxidative stress, however.
117 Thus, melanoma progression may not only be affected by *SLC7A11* expression in
118 melanoma cells, but also by *SLC7A11* expression in normal melanocytes. This may cause
119 oxidative stress at the level of organism that makes melanocytes prone to DNA damage
120 and to become, therefore, melanoma cells [7]. This possibility has never been explored,
121 but pheomelanin is synthesized by epidermal melanocytes in all skin types [21], meaning
122 that pheomelanin-mediated oxidative stress can potentially occur in all pigmentation
123 phenotypes.

124 Here we show that *SLC7A11* expression in normal skin (i.e., not affected by
125 melanoma lesions) is significantly higher in cutaneous melanoma patients than in healthy
126 individuals, pointing to a previously unsuspected marker of melanoma risk. This is

independent of several phenotypic and behavioral factors known to affect the risk of melanoma, notably Fitzpatrick skin type, but also age, gender, body mass, number of melanocytic nevi, frequency of sun exposure and sunburn frequency [22-27]. This is also independent of the intrinsic antioxidant capacity as given by the expression of the gene *NFE2L2*, which encodes the transcription factor NRF2, the master regulator of the cellular antioxidant response [28]. Additionally, we investigated other genes involved in pheomelanin synthesis, but no differential expression between melanoma patients and controls was found. These genes are *CTNS*, which codes for a cystine/H⁺ symporter that exports cystine out of melanosomes [29]; *MC1R*, which codes for the melanocortin 1 receptor in the membrane of melanocytes to which melanocortins bind and change intracellular cAMP levels, and *ASIP*, which codes for the antagonist agouti signaling protein [30]; and *SLC45A2* (solute carrier family 45 member 2), whose function is not clear but has also been suggested to code for a transporter of cysteine into melanosomes [31]. Our results suggest that a high cysteine transport activity carried out by *SLC7A11* in normal skin explains the incidence of melanoma.

2. Results

Our study comprised 45 patients of cutaneous melanoma and 50 healthy individuals that served as controls. We could measure mRNA levels in normal skin in 53 individuals for *SLC7A11* (23 melanoma and 30 controls), 45 individuals for *CTNS* (19 melanoma and 26 controls), 87 individuals for *MC1R* (38 melanoma and 49 controls), 77 individuals for *ASIP* (36 melanoma and 41 controls), 80 individuals for *NFE2L2* (35 melanoma and 45 controls), and 15 individuals for *SLC45A2* (6 melanoma and 9 controls). Expression of the β -actin (*ACTB*) gene, which was detected in the skin of all individuals, was used for normalization, although we corroborated results by normalizing with a different gene (the glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*, gene). The fact that mRNA levels

for some genes were not found in some cases indicates that some patients were not expressing some genes in normal skin or were expressing those genes in undetectable low levels. mRNA levels were highly correlated between most genes ($0.25 < r < 0.91$, $11 < n < 73$, $0.0001 < p < 0.091$), thus we tested the predictive capacity of melanoma incidence for each gene separately using generalized linear models.

Expression levels of the studied genes in the normal skin of controls and melanoma patients are shown in Fig. 1. The full model testing for the predictive capacity of *SLC7A11* mRNA levels, which included all phenotypic and behavioral factors potentially affecting melanoma risk and *NFE2L2* expression as predictors (Table 1), resulted in a significant effect of *SLC7A11* expression ($\chi^2_1 = 5.15$, $p = 0.023$). After removal of non-significant predictors, the final model explained a significant proportion of variability in melanoma incidence among individuals ($\chi^2_4 = 10.52$, $p = 0.032$), and again resulted in a significant effect of *SLC7A11* expression ($\chi^2_1 = 6.02$, $p = 0.014$). This effect indicated that expression levels were higher in the normal skin of melanoma patients than in controls (parameter estimate = -10.41; Fig. 1A). In addition to *SLC7A11* expression, a significant effect of Fitzpatrick skin type ($\chi^2_2 = 6.95$, $p = 0.031$) and a marginally non-significant effect of *NFE2L2* expression ($\chi^2_1 = 2.76$, $p = 0.096$), indicating a tendency of melanoma patients to show low expression levels (parameter estimate = 7.86), were other predictors remaining in the final model. A similar significant model ($\chi^2_4 = 11.03$, $p = 0.026$) was obtained when gene expression was normalized with *GAPDH* instead of *ACTB*, as it resulted in a significant effect of *SLC7A11* expression (parameter estimate = -13.19, $\chi^2_1 = 7.19$, $p = 0.007$), a marginally non-significant effect of Fitzpatrick skin type ($\chi^2_2 = 5.28$, $p = 0.071$) and a marginally significant effect of *NFE2L2* expression ($\chi^2_1 = 3.86$, $p = 0.049$). This indicates that results were independent of the housekeeping gene used for normalization.

The same final model, i.e. controlling for the effect of Fitzpatrick skin type, did not result in a significant effect of expression levels when tested on *CTNS* (*ACTB*-

179 normalization: $\chi_1^2 = 1.70$, $p = 0.192$; *GAPDH*-normalization: $\chi_1^2 = 1.68$, $p = 0.195$), *MC1R*
 180 (*ACTB*-normalization: $\chi_1^2 = 0.17$, $p = 0.680$; *GAPDH*-normalization: $\chi_1^2 = 0.08$, $p = 0.770$) or
 181 *ASIP* (*ACTB*-normalization: $\chi_1^2 = 0.97$, $p = 0.324$; *GAPDH*-normalization: $\chi_1^2 = 0.24$, $p =$
 182 0.625). In *SLC45A2*, in contrast, the model resulted in a significant effect of expression
 183 level when normalized with *ACTB* ($\chi_1^2 = 4.71$, $p = 0.030$) similar to the effect found in
 184 *SLC7A11*, as *SLC45A2* expression was higher in the normal skin of melanoma patients
 185 than in controls (parameter estimate = -12.03; Fig. 1B). This result, however, should be
 186 taken with caution given the low sample size, as we only detected expression of *SLC45A2*
 187 in the skin of 15 individuals. Indeed, the effect of *SLC45A2* expression was not significant
 188 when normalized with *GAPDH* ($\chi_1^2 = 2.44$, $p = 0.118$).

189 In melanoma patients, *SLC7A11* expression was not related to the stage of
 190 melanoma (*ACTB*-normalization: $r = 0.002$, $n = 23$, $p = 0.993$; *GAPDH*-normalization: $r =$
 191 0.09 , $n = 24$, $p = 0.671$). *SLC7A11* expression was neither related to the thickness of
 192 melanoma lesions (*ACTB*-normalization: $r = 0.02$, $n = 23$, $p = 0.908$; *GAPDH*-
 193 normalization: $r = 0.19$, $n = 24$, $p = 0.369$).

194

195 3. Discussion

196 Our findings indicate that patients of cutaneous melanoma exhibit high *SLC7A11*
 197 expression in skin not affected by the tumor. According to our sample (Fig. 1A), ΔC_t values
 198 for *SLC7A11* below 6.5 cycles after normalization with *ACTB* or below 8.2 cycles after
 199 normalization with *GAPDH* should be considered as indicative of high risk of melanoma.
 200 No mutations in *SLC7A11* coding sequence are known to affect pigmentation in humans
 201 [32], suggesting that physiological effects promoted by alterations on the protein are more
 202 likely to be exerted by changes in its gene expression than by changes in its structure.
 203 Indeed, no single-nucleotide polymorphisms (SNP) in *SLC7A11* are related to melanoma
 204 risk [33]. Although a high *SLC7A11* expression has been reported as a causative factor

205 leading to the incidence of different types of cancer [18-20], this is, to our knowledge, the
206 first time that it is found associated to melanoma. Notably, this is the first time that
207 *SLC7A11* expression in non-tumorous tissues is related to the incidence of a cancer,
208 opening a new alternative for the interpretation of the role of this gene in
209 melanomagenesis. It must be considered, however, that only future prospective studies in
210 which patients with different levels of *SLC7A11* expression are followed up will be able to
211 establish the expression of this gene in epidermal melanocytes as a causative factor of
212 skin melanoma.

213 High *SLC7A11* expression in normal skin may be the result of a high expression at
214 the level of organism that is also present in melanoma cells, which may thus exhibit a high
215 antioxidant capacity and resistance to ferroptosis derived from an impediment of p53 anti-
216 tumor activity [17]. This may thus explain the association between high *SLC7A11*
217 expression and melanoma incidence. However, the effect of *SLC7A11* expression was
218 independent of the intrinsic antioxidant capacity of patients as given by the expression of
219 the gene *NFE2L2*, meaning that, for a given level of antioxidant capacity, the incidence of
220 melanoma increases with *SLC7A11* expression. Therefore, *SLC7A11* expression might
221 have physiological consequences other than effects on the antioxidant capacity of cells,
222 which makes likely an alternative explanation for the association between high *SLC7A11*
223 expression and melanoma incidence. This explanation may be given by the fact that
224 melanocytes use cysteine provided during *SLC7A11* activity not only for GSH synthesis
225 but also for the synthesis of pheomelanin, which can potentially produce chronic oxidative
226 stress [12, 13]. This makes that, in melanocytes, a high *SLC7A11* expression can lead to
227 increasing oxidative stress instead of decreasing it as in other types of cells [18-20]. This
228 may explain why pigmentation phenotypes produced by large amounts of pheomelanin
229 favor oxidative DNA damage and melanoma progression [10] and, similarly, the
230 association that we found between high *SLC7A11* expression and melanoma incidence in

231 normal skin. The lack of association that we found between *SLC7A11* expression and
232 stage and thickness of melanoma lesions supports the view that the expression of this
233 gene in normal skin increases the susceptibility to begin a tumorous process, but does not
234 affect the progression of melanoma once the process is initiated.

235 The effect of *SLC7A11* expression was also independent of phenotypic and
236 behavioral factors known to affect melanoma risk, especially the Fitzpatrick skin type,
237 which increase with decreasing melanoma risk [27], and the frequency of sun exposure
238 and sunburns [23]. Our results suggest that *SLC7A11* expression is a better predictor of
239 melanoma incidence than these factors, which supports a direct biological relevance of
240 this gene in melanomagenesis. This is further reinforced by the fact that the effect of
241 *SLC7A11* explaining variability in melanoma incidence was significant while that of other
242 genes involved in pheomelanin synthesis (*CTNS*, *MC1R* and *ASIP*) was not, despite a
243 high correlation between the expression levels of all genes. Although other genes can
244 influence the switch from the synthesis of pheomelanin to eumelanin in melanocytes, no
245 other genes are known to be specifically involved in the synthesis of pheomelanin [34],
246 thus our result points to a direct effect exerted by the activity of the antiporter xCT in
247 melanocytes. The expression of *SLC45A2*, whose function is not clear but has been
248 suggested to be involved in pheomelanin synthesis by transporting cysteine into
249 melanosomes [31], also tended to be higher in melanoma patients than in controls, but this
250 should be taken with caution as the low sample size prevented us from testing the relative
251 importance of this gene's expression in relation to the other factors. Furthermore, no
252 differences in *SLC45A2* expression between melanoma patients and controls were found
253 when expression values were normalized with the gene *GAPDH* instead of *ACTB*. It is
254 thus possible that the contribution of *SLC45A2* to melanoma risk is better explained by
255 one SNP in this gene [33] than by its expression level.

256 These results may not only be used to determine melanoma risk. Most importantly,
257 inducing downregulation of *SLC7A11* levels in individuals with a high expression of this
258 gene should be considered as a new avenue to prevent melanoma progression. Recent
259 findings in birds, which share the molecular pathway of pheomelanin synthesis with
260 mammals, show that some species are able to downregulate *SLC7A11* in melanocytes
261 when exposed to exogenous factors that generate oxidative stress and thus avoid
262 oxidative damage [35]. Other species, in contrast, do not show lability in *SLC7A11* in
263 response to environmentally induced oxidative stress and consequently exhibit a
264 constrained antioxidant capacity [36]. This means that the downregulation of *SLC7A11* in
265 melanocytes may actually be physiologically advantageous against oxidative stress
266 induced by pheomelanin synthesis. Future studies should therefore propose alternatives to
267 downregulate *SLC7A11* expresion in normal epidermal melanocytes.

268

269 **4. Material and methods**

270 **4.1 Study design and skin sampling**

271 This study was approved by the Research Ethics Committee of Virgen Macarena-Virgen
272 del Rocío University Hospitals, Junta de Andalucía, on January 2017. Informed consent
273 was obtained from all individuals included in the study after the nature and possible
274 consequences of the study were explained, and the research was carried out according to
275 the World Medical Association Declaration of Helsinki. All patients were attended at the
276 Dermatology Department of the University Hospital Virgen del Rocio of Seville from
277 January to December 2017. Epidermal samples were taken from normal remaining skin
278 after surgical procedures during daily clinical practice. We included patients with
279 cutaneous melanoma (cases) and patients with benign skin tumors (controls). In the case
280 of melanoma patients, epidermal samples were obtained from skin regions near the
281 melanoma lesion but not affected by the tumor (i.e., healthy skin). Those patients affected

with non-melanoma skin cancer were excluded to avoid confusing factors due to the similarities between basal cell carcinoma and melanoma etiology.

Fitzpatrick skin type (from I to IV) [27], age, sex, gender, frequency of sun exposure, history of sunburns and number of melanocytic nevi were registered for all patients (Table 1). The frequency of sun exposure was quantified as the total number of occasions in which patients are exposed to solar radiation during a year (considering exposure during leisure time, work, practicing sports, indoor tanning and phototherapy). The history of sunburns was considered as the total number of sunburns experienced by patients since childhood. Lastly, the number of melanocytic nevi was the total number of nevi considering all types observed in the patients, including congenital, compound, intradermal, dysplastic and junctional nevi. The number of melanocytic nevi was divided into three categories: 0 (< 50 nevi), 1 (≥ 50 nevi) and 2 (≥ 100 nevi) (Table 1).

We conducted histopathological analyses of the melanoma lesions of patients to determine the pathologic T stage of melanoma and the thickness of the lesions, the latter expressed as a measure of Breslow thickness (in mm). These analyses revealed that, out of 45 melanoma patients, 16 were in stage pT0 (melanoma *in situ*), 18 were in stage pT1a, two were in stage pT1b, three were in stage pT2a, one was in stage pT2b, two were in stage pT3a, one was in stage pT3b, and two were in stage pT4b. These analyses also revealed that most cases (30) corresponded to superficial spreading melanomas, while six corresponded to *in situ* melanomas, four to nodular melanomas, three to Spitzoid melanomas, one was a lentigo maligna melanoma, and one was an acral lentiginous melanoma.

A mean ($\pm$ sem) amount of 36.4 ± 4.4 mg of epidermis was collected from each individual and immersed in RNAlater solution (Ambion, Thermo Fisher Scientific, Waltham, MA, USA). The samples were stored at -80°C until analyses of gene expression.

308 4.2 Gene expression in skin

309 Although keratinocytes constitute the most abundant cellular component of the epidermis,
 310 its basal layer represents the main reservoir of melanocytes [14], thus the genetic material
 311 obtained from these samples corresponds to these cells to a large extent. Total RNA was
 312 extracted using TRI Reagent (Ambion), and residual genomic DNA carry over was
 313 removed using the TURBO DNA-free kit (Ambion). The amount of extracted RNA was
 314 quantified with a Qubit 4 Fluorometer (Invitrogen, Thermo Fisher Scientific).

315 Complementary DNA (cDNA) was prepared from total RNA using RevertAid
 316 Reverse Transcriptase provided in the RevertAid First Strand cDNA Synthesis kit (Thermo
 317 Scientific, Thermo Fisher Scientific). Quantitative real-time PCR (qPCR) was performed on
 318 cDNA for *SLC7A11*, *SLC45A2*, *CTNS*, *MC1R*, *ASIP* and *NFE2L2*. Reactions were
 319 performed using SYBR Green I Master in a LightCycler 480 System (Roche, Basel,
 320 Switzerland). Cycle threshold (C_t) levels were used as a measure of gene expression, with
 321 lower C_t values indicating higher mRNA levels and higher gene expression levels. C_t
 322 values for the housekeeping β -actin (*ACTB*) gene were used for normalization by
 323 subtraction to the C_t values for the other genes in the same samples (ΔC_t). Additionally,
 324 we normalized C_t values with other housekeeping gene (the glyceraldehyde-3-phosphate
 325 dehydrogenase gene, *GAPDH*) to corroborate the results obtained with *ACTB*-normalized
 326 C_t values.

327 Two replicates were obtained for each sample, and the mean value was used in the
 328 statistical analyses. Gene expression levels were unrelated to RNA concentration used in
 329 the analysis of the different genes (*SLC7A11*: $r = 0.12$, $n = 33$, $P = 0.487$; *SLC45A2*: $r =$
 330 0.18 , $n = 7$, $P = 0.691$; *CTNS*: $r = 0.02$, $n = 32$, $P = 0.894$; *MC1R*: $r = -0.21$, $n = 62$, $P =$
 331 0.100 ; *ASIP*: $r = -0.09$, $n = 51$, $P = 0.514$; *NFE2L2*: $r = -0.15$, $n = 45$, $P = 0.311$; *ACTB*: $r =$
 332 -0.22 , $n = 61$, $P = 0.090$; *GAPDH*: $r = 0.07$, $n = 62$, $P = 0.597$; RNA concentration could not

333 be quantified in all samples). Sample sizes for gene expression levels differ between
 334 genes because we did not find detectable expression levels in some patients.

335 Gene primers were designed based on human refseq sequences (GenBank) using
 336 the Primer-BLAST tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). The
 337 oligonucleotide primer sets used were as follows: 5'-CATCGTCCTTTCAAGGTGC-3' and
 338 5'-ATAGAGGGAAAGGGCAACC-3' for *SLC7A11*; 5'-TTTCATGGGCCAGATTGTG-3' and
 339 5'- GACTCCTCTTTCGTAGATGAG -3' for *SLC45A2*; 5'- CGAAATGTGAGTCAAGCGTC-
 340 3' and 5'- GGTGTCATTTAATGGTGGC-3' for *CTNS*; 5'-CATCTCACACTCATCGTCC-3'
 341 and 5'-AGTTCTTGAAGATGCAGCC-3' for *MC1R*; 5'-GAGATGACAGGAGCCTGAG-3' and
 342 5'-ATTTCTTGTTTCAGCGCCAC-3' for *ASIP*; 5'-ATGACAATGAGGTTTCTTCGG-3' and 5'-
 343 CAATGAAGACTGGGCTCTC-3' for *NFE2L2*; 5'-GAAGATCAAGATCATTGCTCCTC-3'
 344 and 5'- ATCCACATCTGCTGGAAGG-3' for *ACTB*; and 5'-TCAAGATCATCAGCAATGCC-
 345 3' and 5'- CGATACCAAAGTTGTCATGGA-3' for *GAPDH*.

346

347 **4.3 Statistical analysis**

348 Generalized linear models assuming a binomial response distribution and a logit link
 349 function were used to test the capacity of the expression level of each gene (ΔC_t ;
 350 covariate) to explain variability in melanoma incidence (binomial response variable; 0-
 351 control, 1-melanoma) among individuals. Fitzpatrick skin type and gender were added as
 352 fixed factors to the models, and age, body mass, frequency of sun exposure, frequency of
 353 sunburns and number of melanocytic nevi were added as covariates. In the model for
 354 *SLC7A11*, ΔC_t for *NFE2L2* was also added as a covariate to control for the intrinsic
 355 antioxidant capacity of the individuals. Starting with the full model, non-significant
 356 predictors were removed by a backward stepwise approach, establishing a *P*-value of 0.1
 357 to abandon the model. Gene expression values were $\log_{10}$ -transformed prior to analyses.
 358 As only one individual was assigned a Fitzpatrick skin type 1, it was considered a

359 Fitzpatrick skin type 2 in the analyses. Pearson's correlation tests were used to investigate
360 possible correlations between gene expression and the stage (considering increasing
361 values from pT0 to pT4b) and thickness (in $\log_{10}$) of melanoma lesions.

362 Generalized linear models were checked for deviations from canonical assumptions
363 using the over-dispersion coefficients. An over-dispersion value different from unity
364 appears when the deviance of an observed response variable exceeds the nominal
365 deviance, given the respective assumed distribution of errors and the proper link function.
366 In our case, over-dispersion coefficients were close to unity (1.08 and 1.06 in the models
367 for *SLC7A11* expression after normalization by *ACTB* and *GAPDH*, respectively), meaning
368 that parameter estimates and related statistics are reliably computed. Analyses were made
369 with STATISTICA 12.0 (StatSoft, Inc., Tulsa, OK, USA).

370

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Table 1. Phenotypic and behavioral characteristics of healthy individuals (control) and cutaneous melanoma patients included in the study. Mean $\pm$ sem values and ranges (in parentheses) are shown. For Fitzpatrick skin type and gender, the median (mode in parentheses) and the proportion of men, respectively, are shown instead.

	Control	Melanoma
Fitzpatrick skin type	III (III)	III (III)
Age (years)	62.16 $\pm$ 2.58 (18-92)	56.47 $\pm$ 2.54 (31-91)
Gender (% men)	56 % (28/50)	55 % (25/45)
Body mass (kg)	78.81 $\pm$ 1.92 (50-110)	76.05 $\pm$ 2.56 (50-115)
Frequency of sun exposure	1.48 $\pm$ 0.18 (0-6)	1.23 $\pm$ 0.14 (0-6)
History of sunburns	6.43 $\pm$ 2.54 (0-100)	7.00 $\pm$ 1.33 (0-30)
No. melanocytic nevi (0: < 50, 1: $\geq$ 50, 2: $\geq$ 100)	0.19 $\pm$ 0.08 (0-2)	0.93 $\pm$ 0.14 (0-2)

503 **Legend to figure:**

504

505 **Figure 1.** Expression levels of the genes *SLC7A11* (A), *SLC45A2* (B), *CTNS* (C), *MC1R*
506 (D), *ASIP* (E) and *NFE2L2* (F) in normal skin. Mean $\pm$ sem values corresponding to
507 healthy individuals (control) and patients of cutaneous melanoma are shown. Expression
508 was measured by means of mRNA amounts quantified by cycle threshold (C_t) levels and
509 normalized by subtraction of C_t values corresponding to the gene *ACTB* in the same
510 samples (ΔC_t). Lower C_t values indicate higher mRNA levels and higher gene expression
511 levels. Differences between controls and melanoma patients were statistically significant
512 only in A and B.