Supplementary material

Py-GC/MS/MS analysis of melanin from the cultured melanocytes

The total amount of each of the isolated pigments was placed into a microfurnace-type pyrolyser (Pyrojector II, SGE Analytical Science) and thermally degraded at 500°C. Pyrolysis products were transferred with a stream of helium directly to the split/splitless injector of a Agilent Technologies 7890A gas chromatograph. The GC separations were performed on Agilent HP–5ms capillary column (5% diphenyl, 95% dimethylpolysiloxane, 60 m × 0.32 mm i.d. × 0.5 μm film thickness) with helium (96.5 kPa) as the carrier gas. The GC oven temperature was programmed from 35°C (held for 5 min) to 100°C at a rate of 5°C/min and then to 260°C at a rate of 10°C/min. The final temperature was constant for 16 min. The GC column outlet was connected directly to the ion source of an Agilent Technologies 7000 GC/MS Triple Quad mass spectrometer. The temperatures of the GC/MS interface, the ion source, and the quadrupoles were fixed at 240°C, 230°C, and 150°C, respectively. A standard electron ionization (70 eV) was applied. The mass spectrometer was operated in a multiple reaction monitoring (MRM) mode with nitrogen (constant flow rate 1.5 ml/min) and helium (2.25 ml/min) as the collision and the quench gas, respectively. MassHunter GC/MS Acquisition B.07.01 and MassHunter Workstation Qualitative Analysis B.07.00 (Agilent Technologies) software were used for data acquisition and processing.

The list of the thermal degradation products that were monitored in melanin pyrolysates by tandem MS, along with the optimal MRM settings, are shown in the Table S1 (see below). The 2 distinct groups of products were monitored in each pyrolysate: heterocyclic compounds with 1,3-thiazole or 1,4 thiazine rings, which are characteristic of thermally degraded pheomelanin (the pheomelanin markers, P) and the products devoid of sulphur (NS). The latter group of compounds are always formed in high yields during the pyrolysis of melanin, irrespective of its structural type and origin, and therefore we use them for normalization of pyrolysis data.

For pheomelanin quantitation, a standard curve was prepared, based on a series of synthetic melanins with known percentages of incorporated pheomelanin. The standard pigments were pyrolysed as described above. The ratio of the total peak area of all the monitored pheomelanin markers and the total peak area of all non-sulphur-containing pyrolysis products, taken from the recorded MRM pyrograms, was plotted against the percentage of the pheomelanin component in the melanin standard (0.5-20%), and a high degree of linearity was achieved ($R^2 >$ 0.995). The linear regression equation was then used to calculate the pheomelanin content (%) of pigments isolated from the cultured melanocytes.

Table S1. Multiple reaction monitoring (MRM) settings for the analysis of melanin pyrolysate by GC/MS/MS

Time segment	Retention time [min]	Compound (type[b])	MS transition		Collision energy [V]
			Precursor ion [*m/z*]	Product ion [*m/z*]	
1	9.7	Thiazole (P)	85	58	15
			58	57	12
	11.1	Toluene (NS)	92	91	15
			91	65	16
2	16.2	Styrene (NS)	104	77	27
			104	78	15
			103	77	14
3	19.2	Phenol (NS)	94	65	26
			94	66	16
	21.4–22.0	Methylphenol[a] (NS)	108	107	16
			107	77	16
4	24.9	1,2-Benzenediol (NS)	110	63	30
			110	64	20
			110	92	15
	25.8	Benzothiazole (P)	135	108	18
			108	82	12
5	26.9	Indole (NS)	117	89	30
			117	90	16
			90	63	27
6	28.1	4-Hydroxybenzothiazole (P)	151	96	23
			151	123	12
			123	96	10
	28.5	Methylindole (NS)	130	77	27
			130	103	14
			103	77	10
7	29.3–29.9	2,3-Dihydro-5H-1,4-benzothiazin-5-one[a] (P)	165	110	18
			165	136	22
			136	109	16
8	30.7–30.9	7-Methyl-2,3-dihydro-5H-1,4-benzothiazin-5-one[a] (P)	179	110	15
			179	150	20
			178	109	20
	31.0–31.3	4-Hydroxy−6-ethylbenzothiazole[a] (P)	179	164	15
			164	109	20
			164	136	15
9	31.8	7-Methyl-5H-1,4-benzothiazin-5-one (P)	177	121	30
			177	148	20
			148	77	27
	31.9–32.3	7-Ethyl-2,3-dihydro-5H-1,4-benzothiazin-5-one[a] (P)	193	178	15
			192	164	10
			178	150	13
10	32.8	7-propyl-2H-1,4-benzothiazine (P)	191	78	27
			191	121	26
			191	163	12
11	35.0, 36.1	Thiazoloisoquinoline[a] (P)	186	142	18
			186	159	19
			159	132	13
	35.9, 37.0	2-Methyl-thiazoloisoquinoline[a] (P)	200	154	18
			200	159	22

[a]Isomers. [b]P – pheomelanin marker, NS – non-sulphur-containing pyrolysis product.