Melanins seem to be everywhere in the body, but for what?

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Mammals and birds produce two major types of melanin, brown to black eumelanin and yellow to reddish pheomelanin. Both types of melanin pigments are synthesized in melanocytes and in retinal pigment epithelium cells. In those pigment cells, a specific enzyme tyrosinase oxidizes L-tyrosine to a highly reactive intermediate dopaquinone (Ito and Wakamatsu, 2008). Dopaquinone undergoes a series of spontaneous reactions, leading to the production of eumelanin via 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid (DHICA). When L-cysteine intervenes with this process, 5-*S*- and 2-*S*-cysteinyldopa are produced, whose oxidation gives rise to pheomelanin. However, most natural melanin pigments are actually produced by the integration of eumelanin and pheomelanin (mixed melanin). A closely related brown pigment, neuromelanin, is produced in the substantia nigra and in other regions in the catecholaminergic neurons of the brain (Zecca et al., 2008). Dopamine (and/or dopa) acts as a precursor of neuromelanin with a partial incorporation of cysteine.

A major biological function of melanin appears to protect the skin from UV-induced DNA damage because melanocytes are mostly found in the skin and in the eyes where melanocytes are exposed to harmful UV radiation. However, melanocytes are also found in the inner ear and in the leptomeninges of the brain where UV radiation cannot elicit any harmful roles to the host. How melanin (or melanocytes) functions in those tissues is not so clear at present. But any roles that melanin plays there may be related to the chemical properties of melanins which effectively scavenge reactive oxygen species (ROS), toxic free radicals and metal ions. Other functions have also been suggested for melanin, including thermoregulation, camouflage, sexual attraction, and others (see below).

Visceral organs usually do not contain melanin under healthy conditions. Exceptions are found in pigmented macrophages of the spleen and the liver of amphibians and in the abdomen of chickens (Crespo and Pizarro, 2006). However, melanin pigments had not been previously found in visceral organs of mammals. A recent study by Randhawa et al. (2008) has clearly demonstrated that the biosynthesis of melanin does take place in visceral adipose tissue of morbidly obese patients. As this was the first time that a melanin pigment was detected in visceral tissues, the identification of melanin (and melanogenesis) should be rigorous and unambiguous. This goal was realized through carefully designed methodology. An accumulation of black pigment in the periphery of adipocytes in morbidly obese patients was revealed by Fontana–Masson staining. The black pigment was chemically identified as eumelanin by permanganate degradation of melanin coupled with the liquid chromatography/ultraviolet/mass spectrophotometry determination of pyrrole-2,3,5-tricarboxylic acid (PTCA). PTCA serves as a specific marker of DHICA-derived units in eumelanin (Wakamatsu and Ito, 2002). PTCA contents in visceral adipose sediments of three morbidly obese subjects ranged from 0.19 to 0.12 ng/μl, while those in non-obese subjects were 0.05 ng/μl.

Randhawa et al. (2008) then moved on to comparing levels of melanogenesis in adipose tissue samples between obese and non-obese subjects. Melanin biosynthetic activity in adipose tissue samples was assessed by the L-[U-^{14}C] tyrosine assay. Activities in seven obese subjects were three times greater than in a non-obese subject (0.17 pmol product/μg/h versus 0.05 pmol product/μg/h), which seems to parallel the melanin content. Expression levels of melanogenesis-related genes in adipose tissue samples were then compared between seven obese and two non-obese subjects by real-time PCR quantification. *TYR, TYRP1, TYRP2,* and *MC1R* genes were expressed at relatively higher levels in obese subjects. *TYR* mRNA signals were also detected by in situ hybridization in visceral adipocytes. Finally, immunohistochemical staining of adipose tissue samples was performed. TYR, TYRP1, and TYRP2 were found to be restricted to the periphery of the adipocytes. Expression of those three melanogenesis-related proteins was higher in the adipose tissue from morbidly obese subjects compared to non-obese subjects.

The presence of melanin (and melanogenesis) in human adipose tissue, especially of morbidly obese subjects, was thus unambiguously confirmed by Randhawa et al. (2008). Some technical difficulties may have arisen from the low melanogenic activities in the adipose tissues, as evidenced by about 20 times lower levels of melanin biosynthetic activity compared to melanoma cells. Another problem is that this study analyzed only one of the two types of melanin pigments, eumelanin. The other type of melanin, pheomelanin, can easily be analyzed in the form of a specific product, 4-amino-3-hydroxyphenylalanine (4-AHP), after hydrolysis with hydroiodic acid (Wakamatsu and Ito, 2002). Especially when we consider that melanogenesis in adipocytes takes place in the cytosol (not in melanosomes), it is highly possible that the adipocytic melanogenesis involves the participation of cysteine (or glutathione), because the addition reaction of thiol

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compounds to dopaquinone proceeds extremely fast (Ito and Wakamatsu, 2008).

What is the function of melanin in the adipose tissue? The authors hypothesized that the ectopic synthesis of melanin in obese adipose tissues may serve as a compensatory mechanism advantageous for its anti-inflammatory and oxidative damage-absorbing properties. It seems that with the progression of obesity and the increase of cellular fat deposition, adipocytes become more exposed to endogenous apoptotic signals, especially ROS, and to counteract those proapoptotic ROS effects, the adipocytes may ectopically activate melanogenesis, thus neutralizing excess ROS (Randhawa et al., 2008). Adipocytic melanin might also suppress the secretion of proinflammatory molecules, thereby decreasing the proinflammatory background in obese subjects and alleviating the metabolic syndrome. The authors also reported a preliminary but intriguing observation that fasting glucose levels correlated well to total outputs of the melanogenic pathway in adipose tissues of obese patients. A larger study is certainly necessary to confirm a link of the melanin biosynthesis to the morbid obesity and the possible protective role of melanin (Randhawa et al., 2008).

How is the melanogenic pathway in adipose tissue activated? This may be related to the three-fold elevated levels of an endogenous melanogenic peptide α-MSH in the serum of obese subjects compared with lean subjects (Hoggard et al., 2004). Human adipocytes are known to express the melanocortin receptor MC1-R, which may trigger the α-MSH-induced melanogenesis in melanocytes.

The authors admit that their study raises more questions than it answers. Their future research aimed at developing an appropriate cellular system to allow the study of adipocytic melanogenesis in vitro is highly warranted. This will allow us to explore the connection between melanogenesis and the metabolic syndrome.

Finally, with respect to the finding of melanin in visceral organs, it should be mentioned that melanocytes have recently been found to be present in the valves and septa of the heart (Brito and Kos, 2008; Yajima and Larue, 2008). Cardiac melanocytes depend on the same signaling pathways that are crucial for cutaneous melanocytes and their numbers appear to reflect that of the skin. The function of cardiac melanocytes, if any, is not known at present. Rather, cardiac melanocytes are not essential in a healthy and nonstressful environment. Chemical and biochemical confirmation of melanin and melanogenesis such as performed for the adipose tissue, if feasible, should be warranted for the cardiac melanocytes.

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