Human red hair pheomelanin is a potent pro-oxidant mediating Uv-independent mechanisms of oxidative stress in red hair phenotypes

Traditionally, the positive correlation between red hair and melanoma has been attributed to both the poor antioxidant and photoprotective properties of pheomelanins compared to the dark eumelanins, and the capacity of pheomelanin to act as photosensitizer inducing generation of reactive oxygen species upon UV irradiation. Recently, the occurrence of UV-independent pathways of carcinogenesis was provided by a series of experiments showing that induction of an activating mutation of BRAF into red mice resulted in a high incidence of invasive melanomas in the absence of UV stimulation . In addition, the skin of pheomelanic mice contained higher levels of oxidative DNA and lipid damage with respect to albino-Mc1re/e mice (Mitra et al, Nature 2012, 491, 449-453). These data clearly showed that the pheomelanin pathway could mediate oxidative stress and melanomagenesis ; however the mechanisms have not so far addressed at molecular level. To get an insight into these processes we investigated the reactivity of pheomelanin pigment isolated from human red hair (RHP) and synthetic pheomelanins from cysteinyldopa (CD-Mel) toward critical cellular antioxidants such as GSH and NAD(P)H. Data showed a remarkable increase in the oxidation rate of both compounds in the presence of RHP compared to the controls, whereas much less marked variations were noticed with the eumelanin extracted from black hair. HPLC analysis consistently indicated a rise in GSH disulfide (GSSG) levels with GSH decrease, confirming a redox reaction. Formation of NAD(P)+ was similarly observed in the reaction mixture of NAD(P)H with RHP. In the absence of oxygen, GSH and NAD(P)H depletion was not observed while the presence of enzymes as superoxide dismutase and catalase did not modify the effect of pheomelanin suggesting a ROS independent mechanism. Similar effects were obtained with CD-Mel confirming that the prooxidant effects are due to the pheomelanic component. The mechanism of GSH oxidation by RHP was investigated by EPR spectroscopy. Besides inducing antioxidant depletion, RHP, like CD-Mel, proved also capable of promoting autoxidation of melanin precursors, such as 5-S-cysteinyldopa and dopa, under conditions of exclusion of light, with formation of pheomelanin or eumelanin pigments. This effect is oxidative in character, since it depends on oxygen, revealing a remarkable prooxidant capacity of the pigment serving as a biocatalyst for non-enzymatic melanogenesis (Panzella et al., PCMR 2014, 27,244-252; Greco et al. Chem Comm. 2011,47, 10308-10310). A mechanism has been proposed to account

for the observed effects in which GSH, NAD(P)H and melanin precursors oxidation is not mediated by ROS, but is the result of a direct redox exchange with oxidized 1,4-benzothiazine units within the pigment scaffold. Re-oxidation of reduced pheomelanin by oxygen with formation of ROS would ensure shuttling of pheomelanin between the redox states. Overall these results provide a unifying chemical framework into which to explain the mechanisms underlying the relationships between pheomelanin pigmentation and oxidative stress.