# IS MELANIN A RADIOPROTECTOR OR RADIOSENSITIZER? IT'S IMPLICATION FOR RADIOTHERAPY

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It continues to be debated whether melanins are photoprotecting or photosensitizing in human skin cells. Epidemiologic studies have demonstrated that dark-skinned people are much less susceptible than Caucasians to ultraviolet (UV)-induced skin damage including both non-melanoma and melanoma skin cancer. On the other hand, melanin, its intermediates and precursors can generate reactive-oxygen species when irradiated with UV irradiation and have been shown to be effective photosensitizers.

The last decade has witnessed a renewed and unprecedented impetus in melanin research that has significantly expanded our knowledge of this field. Melanin is a highly irregular heteropolymer consisting of monomeric units derived from the enzymatic oxidation of the amino acid tyrosine. There are two distinct types of melanin, eumelanin and pheomelanin. Now, it is proved that eumelanins are the main photoprotective pigment in the epidermis and pheomelanins are phototoxic upon irradiation.

The same situation is also true for ionizing radiation that eumelanin not only possesses radiation protective effect but also contains photoprotective ability. Since malignant melanoma is resistant to radiation therapy, therapeutic strategies targeted to the melanogenesis of eumelanin may have potential benefit for the radiation therapy of malignant melanoma.

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Key word: Eumelanin, Pheomelanin, Radioprotector, Radiosensitizer, Radiotherapy, Malignant melanoma

## INTRODUCTION

Many lines of evidence have indicated that melanin is probably a photoprotector and a radioprotector. Dark-skinned people are much less susceptible than Caucasians to ultraviolet (UV)-induced skin damage including both non-melanoma and melanoma skin cancer. The difference can to a large degree be explained by the superior optical filtering provided by the large amount of melanin in the epidermis of black skin [20]. These findings seem to indicate that melanin plays an important role to protect people from UV irradiation.

Another example supporting that melanin is a potential radiation protector originates from clinical experience in the radiotherapy of malignant melanoma. The survival curves of

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melanoma cells displays a broad shoulder with conventional radiation [7, 12], which means that these cells have higher ability to repair sublethal damage after irradiation than other cells. All of the evidences above show that melanin may be a photoprotector and radioprotector.

However, melanin, its intermediates and precursors can generate reactive-oxygen species when irradiated with UV irradiation and these have been shown to be effective photosensitizers [22, 34, 36]. In addition, experimental studies conducted on different pigmented and non-pigmented melanoma cell lines did not show any differences in radiosensitivity [4, 11, 33, 38]. Taken together, the long-lasting question of whether melanin is photoprotective or phototoxic and even radioprotective is still a controversy.

In this review, we will focus on the structures, properties, and synthesis of melanin; its relationship with UV irradiation and ionizing radiation, and finally its implication for radiotherapy.

## STRUCTURES OF MELANIN

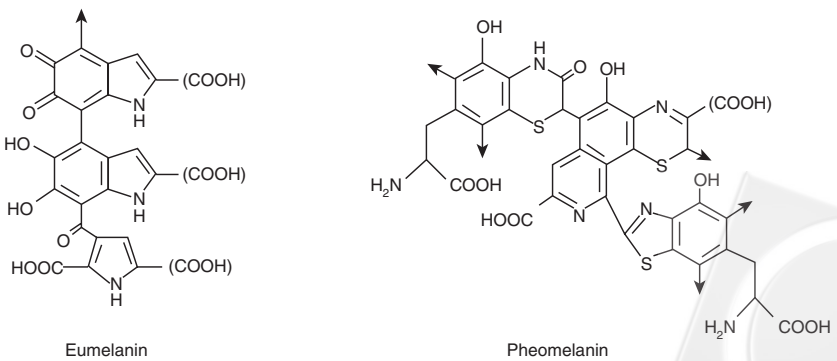
Melanin, a predominantly indolic polymer, is the major pigment in surface structure of vertebrates. The term "melanin" has been used fairly indiscriminately to mean any dark pigment but the nomenclature has been refined in the case of mammalian melanin to include eumelanin and pheomelanin [17].

The basic structural unit of melanins is usually represented by covalently linked indoles. Most melanins appear to be mixed polymers based on indoles but containing variable amounts of other pre-indolic products of the synthetic pathway. The indolic domains may be stacked by van der Wall's interactions giving approximately 3.4Å interlayer spacing in X-ray diffraction [39]. The basic structures of eumelanin and pheomelanin are shown in Fig. 1.

## PROPERTIES OF MELANIN

The melanin polymer has many interesting properties, among which the most conspicuous is the wide spectral absorbance of light due to the high degree of conjugation in the molecule. The darkness of the pigment is a result of the fact that much of the visible spectrum is absorbed, including radiation with low quantal energy. Melanins also absorb in the ultra-violet region of the spectrum, which occur in unsaturated carbon bonds. Melanins with high level of indole quinones (the eumelanins) appear darker because of the strong absorbance in the red part of the spectrum. Melanins with fewer carbonyl groups are paler and appear more yellow or red as is the case in the pheomelanin.

Melanins, especially eumelanins, exhibit marked redox properties, and electron delocalization between orthoquinone and catecholic moieties of the polymer give rise to semiquinone free radicals, which can be detected by electron spin resonance spectroscopy [37].




Eumelanin Pheomelanin

Fig 1. The basic structures of eumelanin and pheomelanin

Melanins also have powerful cation chelating properties through the anionic functions such as the carboxyl and the deprotonated hydroxyl groups [35].

## SYNTHESIS OF MELANIN

The melanin pigments are of no fixed molecular weight but are all derived by enzymatic oxidation of the amino acid tyrosine. Major advances in the understanding of the chemistry and enzymology of the biosynthetic pathway involved in the synthesis of the eumelanin (black and brown) and pheomelanin (red and yellow) have now been made [28, 31].

Tyrosinase catalyses the first two steps, the hydroxylation of tyrosine to 3,4-dihydroxphenylalanine (DOPA) and oxidation of DOPA to DOPAquinone (Fig.2). Eumelanins are derived from the metabolites of DOPAchrome, whereas pheomelanins derived from metabolites of 5-S-cysteinylDOPA. The isomerization of DOPAchrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA) is catalysed by DOPAchrome tautomerase (tyrosinase-related protein-2; TRP-2) and the oxidation of DHICA is performed by a DHICA-oxidase enzyme (tyrosinase-related protein-1; TRP-1). Eumelanin consists of 5,6-dihydroxyindole (DHI) and DHICA units in a reduced or oxidized state, and some indole rings are split to

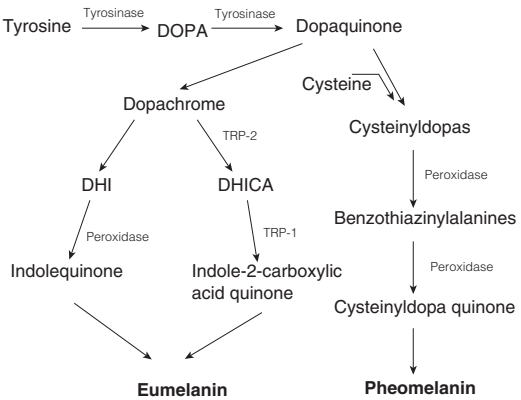



Fig 2. Overview of biosynthesis of eumelanin and pheomelanin

give pyrrole rings [27]. On the other hand, pheomelanin consists mostly of benzothiazine units, but those units are degraded to benzothiazole units to some extent. Mice with mutant forms of these enzymes have been found in the *albino, brown* and *slaty* coat color loci proposed to produce tyrosinase, and tyrosinase related proteins TRP-1 and TRP-2, respectively [6].

In vertebrates these reactions take place in specialized membrane-bound organelles, melanosome, and the current view is that the group of melanogenic enzymes is present as a complex associated with a protein matrix [42]. The process of melanin synthesis is under the control of the tyrosinase promoter. Tyrosinase promoter is only activated in specialized melanogenic cells [8] including retinal pigment epithelium and melanocytes.

## MELANIN COMPOSITIONS IN SKIN, HAIR AND MELANOMA

The human pigmentary system is dependent on the production of the light absorbing biopolymer, melanin, within epidermal, ocular and follicular melanocytes. Melanocytes within the skin are situated in the basal layer between the dermis and epidermis and have a number of dendritic processes that interdigitate with the surrounding keratinocytes. While pigment synthesis occurs within the melanocyte, the majority of pigment within the skin is found in melanin laden vesicles known as melanosomes located within the keratinocytes. Mammalian melanocytes produce two types of melanin, the brownish black eumelanin and the reddish yellow pheomelanin. To elucidate the melanin composition in normal skin, we used the skin typing according to sun-reaction.

The concept of sun-reactive "skin-typing" was created in 1975 [9] for a specific need to classify persons with white skin in order to correct initial doses of ultraviolet A in the newly developed treatment for psoriasis—oral

methoxsalen photochemotherapy (PVUA) [30]. A simple working classification was proposed based, not on the hair and eye color, but on what patient says their responses are to an initial sun exposure. Individuals with skin type I have no inherent melanin pigmentation and develop a marked tender sunburn or erythema following short exposures to ultraviolet radiation and are absolutely incapable of tanning. Persons with skin type IV, on the other hand, although exhibiting white skin with no clinical evidence of inherent melanin pigmentation, seldom get sunburn and tan very well. People with skin type II most often have sunburn and will only develop a light tan after several exposures. The person with skin type III will develop some nontender sunburn after 45 minutes of initial sun exposure but can develop a quite dark tan. Later, in additional to white-skinned persons, brown- and black-skinned persons were classified as type V and VI skin by Pathak et al [29] and Fitzpatrick [10](Table 1).

To clarify whether both melanin pigments are simultaneously present in human epidermis, Thody et al. [40] collected skin samples of 13 Caucasian subjects with skin types I, II, and III and analyzed for the contents of both eumelanin and pheomelanin. It demonstrated that eumelanin and pheomelanin were found in all epidermal samples and the lowest concentrations of eumelanin were found in subjects of skin type I, with higher levels in skin types II and III. The concentrations of pheomelanin were more variable and showed no relationship to skin type. Alaluf et al. [1] found that total epidermal melanin content is significantly elevated in photoexposed type V and VI skin. Further analysis suggests that pheomelanin content increases only slightly, whereas eumelanin content is markedly elevated. It also suggests that eumelanin formation is the dominant pathway for melanin synthesis in heavily pigmented (Fitzpatrick V and VI) skin type, and is the favored pathway when melanin production is increased in chronically photoexposed skin.

Table 1. Sun-Reactive Skin Type*

| Skin Color (Unexposed Skin) | Skin Type | Sunburn | Tan |
|---|---|---|---|
| White | I | Yes | No |
| | II | Yes | Minimal |
| | III | Yes | Yes |
| | IV | No | Yes |
| Brown | V | No | Yes |
| Black | VI | No | Yes |

* Based on verbal response regarding first, moderate (three minimal erythema dose) unprotected sun exposure for a period of 45 to 60 minutes.

Ito and Jimbow [18] found a good correlation between the eumelanin and pheomelanin contents with the type of melanogenesis in normal mouse hair. Eumelanin was found in black hair at a high level whereas pheomelanin was found in yellow hair at a higher level. However, the two types of melanogenesis can be switched from one to another under certain physiologic and pathologic conditions. Ito [16] and Ando et al. [2] demonstrated that the switching between the two types of melanogenesis is mainly controlled by the level of tyrosinase activity: higher activity leads to eumelanogenesis and lower activity leads to pheomelanogenesis. Thus, different melanoma cells will possess quite different combination of melanin contents due to their intrinsic tyrosinase activity.

## MELANIN AND UV IRRADIATION

In humans, one obvious effect of UV irradiation is increased cutaneous pigmentation. A direct relationship exists between the constitutive skin pigmentation and the ability of individuals to synthesize melanin in response to UV irradiation [19]. Barker et al. [3] found that, after UVB irradiation (280-320 nm), heavily pigmented melanocytes had the same percent survival as, but a greater capacity to resume proliferation than, their lightly pigmented counterpart. The lightly pigmented melanocytes also suffers prolonged induction of p53 and G1

arrest than heavily pigmented melanocytes, suggesting the need for lightly pigmented melanocytes to repair extensive DNA damage. The differences might partially explain the increased susceptibility of individuals with lightly pigmented skin compared to individuals with dark skin to the photodamaging and photocarcinogenic effects of sun exposure.

Skin cells irradiated by UVA (320-380 nm) could induce 8-hydroxy-deoxyguanosine (8-OHdG) which is a premutagenic oxidative DNA base damage [24]. Three melanoma cells, GLL19, IGR1 and B16, were used to study the role of natural melanin the induction of oxidative DNA base damage. Treatment of human GLL19 melanoma cells with phosphotyrosine increased the melanin content 6.9 times. Confluent IGR1 cells developed a 7.5-fold increased melanin content compared with non-confluent cells. The mouse melanoma cells B16 increased their melanin content 5.4 times after treatment of α-melanocyte stimulating hormone [25]. Increased melanin synthesis clearly did not protect the cells against UVA-induced oxidative DNA base damage in GLL9 and IGR1 cells because two times more 8-OHdG was produced after UVA irradiation when comparing with its non-stimulated parental ones. However, UVA induction of 8-OHdG in the mouse melanoma B16 cells was not changed by the induction of melanin synthesis. Thus total melanin content in the melanoma cells is not representatives of photoprotector or photosensitizer.

To discriminate the role of different types of melanin in the photosensitivity of UV irradiation, Wenczl et al. [41] elucidated, for the first time, the correlation between UVA-induced genotoxicity and pheo-/total melanin ratio. They found that within both skin types I and VI cell cultures, after increasing the melanin content, the melanin composition (pheo-/total melanin ratio) appears to be a more important factor with regard to the change in UVA sensitivity than the absolute quantity of melanin. They concluded that pheomelanin acts as a photosensitizer upon UVA irradiation, resulting in increased DNA single strand break in skin type I melanocytes following increased melanization; however, the UVA sensitivity of skin type VI melanocytes was not influenced by the increase in their melanin contents.

Kowalczuk et al. [23] examined the effect of increasing the intracellular melanin content on cell survival in a human melanoma cell line, G361, following exposure to UVA, UVB and UVC irradiation. A human melanoma cell line, G361, was manipulated to increase melanin production by growth in a tyrosine phosphate and glutamine-rich medium. Melanin content was increased about 2-fold compared with controls, with eumelanin levels increased proportionately more than pheomelanin. They demonstrated no evidence of any photosensitizing effect on the cell survival following exposure to UVC, UVB or broadband UVA radiation. A slight protective effect seen following exposure to long wavelength (311 nm) UVB radiation may have been due to increased scavenging of reactive-oxygen species, particularly by eumelanin.

In sum, the origin of UV sensitivity differences have focused on variation in the cutaneous levels of dark eumelanins, generally regarded as the main photoprotective pigment in the epidermis. Yet, the skin of red-haired fair-complexioned individuals contains, in addition to eumelanin, significant amounts of a lighter variant of melanin pigments, the sulfur-containing pheomelanin [40], which appear less photoprotective than eumelanin and even phototoxic upon irradiation as evidenced by in vitro experiments. These pigments may therefore play a crucial role in determining the overall photobiologic response.

## MELANIN AND IONIZING RADIATION

The clinical observation of the relative

radiation resistance of pigmented melanomas has led to the suggestion that melanin may protect against radiation damage. Zhdanova et al. [43] first found that two fungal allomelanins isolated from Cladosporium were differently protective against γ-ray-induced damage, suggesting that different melanin pigment can vary in their protective ability. Similar results were also presented by Hill and Hill [13], who demonstrated that endogenous melanin was protective against radiation-induced damage in murine S91 melanoma cells.

The relationship between cell pigmentation and radiosensitivity was investigated in mouse melanoma cells, in which melanogenesis was suppressed by glucosamine to inhibit the glucosylation-dependent melanization [26]. It was found that X-irradiation of melanotic B-16 melanoma cells and their amelanotic counterparts, obtained by glucosamine treatment, showed an inverse correlation between radiosensitivity and melanin contents. Since melanogenesis interruption by glucosamine does not affect the DNA repair capacity of non-pigmented cells, it is likely that intracellular melanins play a role in the relative resistance of pigmented cells to X-irradiation. Although some previous studies failed to show any dose-response correlation between pigmentation and radioresistance [14, 32, 33], they were probably due to the design of the experimental model used. For examples: Raaphorst and Azzam [32] used a melanoma-like cell line obtained by transformation of C3H 10T1/2 mouse embryo cells in which melanin content increased as a function of incubation time in culture. Hopwood et al. [14] used Chinese hamster ovary (CHO) cells incubated with exogeneous melanin. Rofstad et al. [33] also observed differences in radiosensitivity between two of three human melanoma xenograft lines that varied in visible pigmentation and were established from different lesions in the same patient. All these three studies determined intracellular melanin content by optical microscopy and thus could not detect pheomelanin, and these differences are attributed to clonal phenotypic heterogeneity and not to differences in melanin synthesis [21].

In view of human melanoma cell model, Barranco et al. [4] failed to show a relationship between pigmentation and radiosensitivity for three human melanoma cell lines that varied in pigmentation. A possible explanation for these results would be the low plating efficiency of the clonogenic assay. Recently, Kinnaert et al. [21] selected two human melanoma cell lines with different melanin content (mixed type: eumelanin and pheomelanin, and pheomelanotic phenotype) to check the possibility that the type of melanin may influence the response of melanoma cells to X-irradiation. The results showed that radiation survival response of the two melanoma cell lines appeared to be correlated with the type of melanin; i.e., the phenomelanotic cell line was more sensitive to X-irradiation than the mixed type. Therefore, the presence of eumelanin might confer some protection against radiation. The protective role of melanin against X-ray-induced damage is generally attributed to the free radical scavenging ability of melanin.

We [15] have also investigated the protective effect of melanin against radiation in prokaryotic cells. The strategy of our study is the construction of a recombinant E coli with melanin production. Plasmids pIJ702 and pGEM-3Z were treated with sph I restriction enzyme and then relegated to construct a plasmid pTSH-1 with mel gene. It was then transformed into JM109 to construct a recombinant JM109-pTSH-1 expressing melanin. The other recombinant JM109-pTSH-R1with reversely inserted mel gene that can not express melanin was constructed, too. The survival rate of JM109-pTSH-1 was significantly higher than the control group when they were treated with γ- ray irradiation. A survival rate of 40% for JM109-pTSH-1 and 5% for JM109-pTSH-R1

and JM109 were observed when treated with 4000 cGy of γ- ray irradiation (Fig. 3). The results indicate that melanin is effective for the protection of the cell from damage of γ- ray irradiation.

Taken together, the radioprotective ability of melanin to ionizing radiation has been demonstrated in fungus, murine and human melanoma cell line. The intracellular content of melanin in melanocyte or melanoma cell line could determine the radiosensitivity of these different melanin-containing cells. Moreover, the type of melanin could also contribute to this radioresistent effect. Based on previous researches, eumelanin, but not pheomelanin, is both photoprotector and radioprotector.

## IMPLICATION TO RADIOTHERAPY

The role of radiotherapy in the treatment of melanoma patients is still a matter of debate. The individual response of malignant melanoma to radiotherapy is apparently radioresistant, reflecting the different radiobiological characteristics of the tumor, especially the ability of tumor cells to repair radiation damage. To overcome the radiation-resistance of malignant melanoma, interventions to decrease intracellular eumelanin content of melanoma cells seems to be a novel strategy to optimize the therapeutic gain in melanoma radiotherapy. With the rapid advances in recombinant DNA technology and gene delivery methods, transduction of antisense oligonucleotide (ASO) or small interfering RNA (siRNA) to target the melanogenesis genes, such as tyrosinase, TRP-1 and TRP-2, might decrease the intracellular content of melanin and thus sensitizes the melanoma cells to radiotherapy.

In the opposite direction, eumelanin might be a radioprotector for normal tissue under radiotherapy. Bouchard et al. [5] had demonstrated the induction of pigmentation in mouse

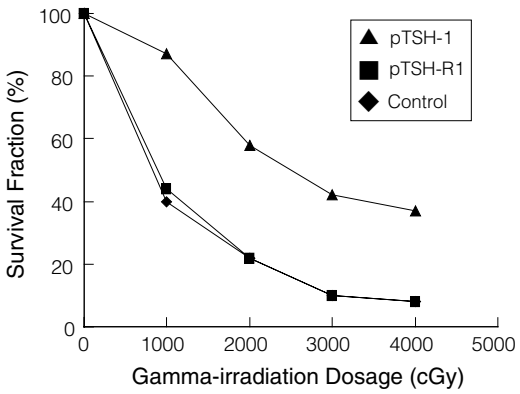



Fig 3. Survival curve of E. coli JM109 containing various plasmid after γ-ray irradiation

fibroblasts by expression of human tyrosinase cDNA. It also demonstrated that melanin synthesis can take place in cells that do not have melanosome. Further understanding of the melanogenesis can help to develop a new strategy to protect critical organs from radiation damage using transgene technique to induce intracellular melanins production in normal cells.

## ACKNOWLEDGEMENTS

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# 黑色素是輻射保護劑或輻射致敏劑？及其在放射治療上的運用

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黑色素對於人類皮膚細胞是光照保護劑（Photoprotector）或光照致敏劑（Photosensitizer），長久以來仍存有許多爭議。流行病學的研究顯示：深膚色人種與白種人相較，因紫外線引發皮膚損傷，包括黑色素瘤或其他皮膚腫瘤的機會較少。然而，過去的研究也發現：黑色素及其中間產物與前驅物在接受紫外線照射後會產生反應性氧化物質（reactive-oxygen species），因此顯示黑色素也是有效的光照致敏劑。

經過十年來不斷創新與前所未有的研究，使我們更加擴展對黑色素的認知與瞭解。黑色素是由酥胺酸（tyrosine）氧化所形成之單元體所建構之高度不規則異化之聚合物，其包含兩種構型： eumelanin 及 pheomelanin 。目前證實： eumelanin 是具有光照保護效果的色素，而 pheomelanin 在紫外線照射後會產生細胞毒性。

黑色素與游離輻射也存在有類似的效應： eumelanin 不僅有光照保護效果，同時也有輻射保護效果，這與臨床上所觀察到惡性黑色素瘤細胞之輻射抵抗性的現象相符合。因此若能阻斷 eumelanin 色素的合成過程，對於增進惡性黑色素瘤的放射治療效果當有潛在的意義。
[ 放射治療與腫瘤學 2003; 10(4): 237-246 ]

關鍵詞：黑色素、輻射保護劑、輻射致敏劑、放射治療、惡性黑色素瘤