MELANOCYTES/MELANOGENESIS

Molecular Aspects of Tanning

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Tanning is commonly understood as increased melanization of the epidermis observed in skin following UV exposure. It is further understood to represent a host response that protects against future UV-induced damage. Although best studied in humans, tanning can also be observed in other mammals and even in more primitive animals such as sharks.

The molecular basis of tanning was poorly understood well into the 1990s, although UV irradiation of murine melanoma cells had been shown to increase the number of cell surface receptors to α-melanocyte–stimulating hormone (α-MSH) and to increase the melanogenic response to α-MSH via increased protein levels and activity of tyrosinase, the rate-limiting enzyme in melanin synthesis (Bolognia *et al.*, 1989; Chakraborty *et al.*, 1991). However, the initiating molecular events were unknown. Identification of the responsible molecules ultimately evolved from studies of the UV action spectrum for critical events in human skin, as well as from a philosophical appreciation of tanning as a genome protective response.

In the 1980s, investigators at Harvard's Wellman Laboratories exposed normal skin of volunteers to a wide range of light doses using narrow band sources across the UV spectrum and observed them for several days to determine the lowest dose at each wavelength capable of producing a delayed tan (Parrish *et al.*, 1982). In other experiments, they used the same approach to define the action spectrum for induction of cyclobutane pyrimidine dimers (CPDs), the most common form of DNA damage following UV exposure (Freeman *et al.*, 1989). The spectra were virtually superimposable, peaking at ~300 nm, suggesting a cause-and-effect relationship between the immediate DNA damage and subsequent increased production and dispersion of epidermal melanin.

Eller *et al.* (1996) first documented that agents acting exclusively on DNA, such as DNA restriction enzymes, stimulate melanin production in cultured pigment cells, at least in part by increasing α-MSH binding and the melanogenic response to α-MSH. Although this did not exclude a role for UV-mediated membrane effects, it did directly implicate UV-mediated DNA damage in the tanning response. Similarly, accelerating DNA damage repair by providing UV-irradiated pigment cells with T4 endonuclease V, the bacterial enzyme that catalyzes the first step in CPD resolution, also enhanced the melanization response (Gilchrest *et al.*, 1993).

By the 1990s, DNA damage responses were recognized to be largely mediated by the transcription factor and tumor suppressor protein p53, also termed the ''guardian of the genome'' (Lane, 1992), and two groups independently asked whether p53 was involved in mediating the tanning response. Using p53 null osteosarcoma cells transfected with wild-type p53, Nylander *et al.* (2000) showed that p53 activation increased read-out from a transfected tyrosinase promoter linked to a reporter gene, implying that p53 could directly or indirectly stimulate tyrosinase transcription. This link between p53 and tanning was expanded and refined using both human melanoma cells and a mouse model. Compared with a p53-null parental melanoma line and to the same line transfected with an empty vector, melanoma cells transfected with wild-type p53 increased their tyrosinase mRNA levels progressively over 72 hours following p53 activation (Khlgatian *et al.*, 1999; Khlgatian *et al.*, 2002). Confirming an earlier report (Kichina *et al.*, 1996), these studies also showed an inverse relationship between total p53 protein (inactive) and tyrosinase levels. The requirement for p53 activation to increase tyrosinase protein level and epidermal melanin content *in vivo* was confirmed by documenting the tanning ability of wild type versus p53 knockout mice (Khlgatian *et al.*, 2002). The question remained whether p53 directly increased tyrosinase transcription, as no p53 consensus sequence had been identified in its promoter region.

The key observation that the UV action spectrum for tanning is virtually identical to that for CPD production, had suggested that thymidine dinucleotide (abbreviated ''TT''), the obligate substrate for the majority of CPDs, might itself serve as a molecular signal for increased melanogenesis (tanning). Using various models, the responses to UV irradiation versus this DNA fragment were therefore compared. Indeed, TT increased mRNA and protein expression of tyrosinase as well as melanin content of cultured human and murine pigment cells and of intact guinea pig skin-containing melanocytes in the interfollicular epidermis, in a time frame similar to that observed after UV irradiation (Eller *et al.*, 1994). As well, in guinea pig skin, the histological features were virtually identical following either treatment: increased total melanin with prominent

supranuclear melanin ''caps'' in the keratinocytes, the familiar distribution pattern for UV-tanned human skin, and understood to maximally protect nuclear DNA from further UV damage. Similar to UV irradiation, TT supplementation also increased cell surface binding of α-MSH in cultured pigment cells, presumptively through increased α-MSH production by the cultured pigment cells and/or increased binding affinity for α-MSH to its cognate cell surface receptor, the melanocortin 1 receptor (MC1R). In other experiments, TT was observed to upregulate and activate p53 in a variety of cell types (Eller *et al*., 1997), bringing together the two paths of investigation.

THE ROLE OF TELOMERE DISRUPTION

During the 1990s, initially outside of the dermatological and pigment cell biology communities, there was great interest in the biological role of telomeres. Telomeres, the terminal portion of all mammalian chromosomes, consist of short-tandem repeats of a noncoding DNA sequence, TTAGGG, in all mammalian species. The de Lange group observed that telomeres are not linear but rather form a loop structure and that disruption of this telomere loop structure, exposing the single-stranded TTAGGG overhang, that is, normally concealed within the proximal double-stranded telomere, leads to DNA damage signaling (Karlseder *et al*., 1999). These investigators determined that, depending on the experimental manipulation, loop disruption led to activation of ataxia telangiectasia-mutated (Karlseder *et al*., 1999) or ataxia telangiectasia-related (Denchi and de Lange, 2007) kinases known to mediate responses to DNA damage due to gamma irradiation or UV, respectively, followed by activation of p53 and ultimately by apoptosis or senescence of the treated cells, depending on cell type. Thus, experimental disruption of the telomere loop with exposure of the TTAGGG repeat sequence rapidly led to the same cellular responses as observed after acute DNA damage due to UV (among other injurious agents). This suggested that introduction of DNA damage such as CPDs, or influx of DNA repair proteins to sites of such damage, might also expose single-stranded TTAGGG repeats and, hence, that TTAGGG-containing DNA sequences might alone suffice as a cellular DNA damage signal (Gilchrest *et al*., 2009).

Interestingly, TT, one-third of the repeat sequence, is the favored substrate for CPDs; guanine (GGG, one-half the sequence) is the substrate for almost all oxidative damage; and G residues or adjacent adenine–guanine (AG) residues are the favored site for formation of chemical adducts to DNA, making telomeres an ideal target for DNA damage (Gilchrest *et al*., 2009). For example, it has been shown that following UV irradiation, CPDs are seven times more frequent in telomeric DNA than in other portions of the genome (Rochette and Brash, 2010). It was therefore tempting to ask whether TT was effective in stimulating melanogenesis precisely because it was a part of the telomere repeat sequence and whether full telomere repeat sequences would be even more effective. Experiments demonstrated that an 11 base oligonucleotide GTTAGGGTTAG was indeed far more effective on a molar basis than TT in stimulating tanning of cultured pigment cells or of human skin supplemented *ex vivo* (Gilchrest *et al*., 2009). Furthermore, disrupting the telomere loop by the same method as in the de Lange group experiments also upregulated tyrosinase and tanned cultured human melanocytes (Gilchrest *et al*., 2009). Furthermore, telomere homolog oligonucleotides, termed T-oligos, increase expression not only of tyrosinase, but also of other melanogenic gene products such as TRP1, Mart1, and gp100 (Puri *et al*., 2004).

TYROSINASE ACTIVATION

It had long been recognized that the rate of melanin production at baseline and following UV exposure in either cultured pigment cells or intact skin depends on tyrosinase activity, rather than simply on total tyrosinase protein. Experiments by Park *et al*. (1999) established that tyrosinase is a phosphoprotein, active only when phosphorylated on serine residues in its cytoplasmic domain that extends beyond the melanosome membrane. Only after this phosphorylation event, mediated by protein kinase C-β (PKC-β), does the intra-melanosomal portion of tyrosinase catalyze the production of eumelanin. Interestingly, PKC-β, which is activated by diacylglycerol generated from UV-irradiated cell membranes, is among the gene products transcriptionally upregulated following UV irradiation (Park *et al*., 2006).

MELANOCYTE–KERATINOCYTE INTERACTIONS

Tanning occurs to a far greater degree in intact skin or in cocultures of melanocytes or keratinocytes than in isolated melanocyte cultures, suggesting that keratinocyte-derived products contribute to the UV-induced tanning response (Archambault *et al*., 1995). Accordingly, many laboratories have identified keratinocyte-derived gene products that are upregulated by UV irradiation and then act as paracrine factors in the skin to stimulate survival, melanogenesis, and/or melanin transfer by melanocytes, including many induced by p53 and/or T-oligos (reviewed in Park *et al*., 2009).

Using an elegant mouse model in which UV irradiation causes tanning when the melanocytes express wild-type MC1R, but not when they express loss-of-function MC1R variants associated in man with blond or red hair and poor tanning ability, the Fisher group demonstrated that the MC1R ligand α-MSH, a cleavage product of pro-opiomelanocortin (POMC), is regulated in keratinocytes by p53 via a p53 consensus sequence in the POMC gene promoter (Cui *et al*., 2007). This observation expanded the concept that tanning is an integrated response of the epidermis to DNA damage and identified the first direct target of p53 transcriptional activation involved in melanogenesis. POMC production, cleavage to α-MSH and other peptides, upregulation by UV exposure, and extracellular release had been documented in human keratinocytes by several groups (Wintzen *et al*., 1996), but the precise mechanism of UV induction had been unknown. Whether

the increased melanogenesis observed in UV-irradiated cultured pigment cells, in the absence of keratinocytes, reflects POMC and subsequent α-MSH upregulation by p53 in melanocytes (an autocrine rather than paracrine event) or the existence of additional as yet unrecognized p53-regulated genes involved in melanogenesis remains to be determined (Park *et al.*, 2009).

Binding of keratinocyte-derived α-MSH by wild-type MC1R on the melanocyte surface results in increased cyclic AMP production that in turn leads to protein kinase A-mediated transcriptional upregulation of the microphthalmia transcription factor (MITF; reviewed in Park *et al.*, 2009). MITF in turn transcriptionally upregulates the tyrosinase, *TRP1*, *TRP2*, and *PKC-β* genes, increasing production of melanin and specifically of the photoprotective brown–black eumelanin polymer, highly capable of absorbing UV photons that might otherwise damage DNA (Park *et al.*, 2009).

"TANNING" INCLUDES ENHANCED DNA REPAIR CAPACITY

As noted above, it has long been appreciated that tanning is the skin's major protective response against acute and chronic UV damage. However, the increased epidermal melanin content associated with a ''good tan'' offers at best a modest degree of photoprotection. For example, compared with lightly pigmented epidermis, an excised darkly pigmented epidermis placed over intact viable skin reduces UV damage by at best a factor of 4 (Halder and Bridgeman-Shah, 1995). This suggests that the tanning response might consist of numerous changes within the viable epidermis in addition to enhanced melanogenesis. This speculation is made more appealing by the recognition that p53 activation is a critical initial tanning step, as described above. Indeed, it was possible to document that within 24 hours UV irradiation of human or murine skin or of cultured skin-derived cells resulted in a 2- to 3-fold increase in the level of several nucleotide excision repair proteins (Goukassian *et al.*, 1999), many but not all of which are known to be p53 regulated, as well as upregulation of superoxide dismutases (SOD 1 and 2; Leccia *et al.*, 2001), the enzymes responsible for reduction of oxidative damage due to UV or other insults. This results in an accelerated rate of repair for DNA damage following a subsequent UV exposure, compared with that after an initial UV exposure (Arad *et al.*, 2007), as well as a reduction in mutations and photocarcinogenesis (Goukassian *et al.*, 2004; Arad *et al.*, 2008). At least some of these effects appear to be mediated through α-MSH binding to MC1R (Kadekaro *et al.*, 2005), followed by induction of cAMP (D'Orazio *et al.*, 2006; Passeron *et al.*, 2009) rather than through p53-stimulated direct upregulation of DNA repair proteins. Thus, it seems that the UV-induced increase in epidermal melanin content (conventionally termed ''tanning'') is, but one aspect of a p53-mediated DNA damage response that includes upregulation of DNA repair capacity and antioxidant defenses in addition to an increased rate of melanogenesis, a protective adaptation strikingly reminiscent of the SOS response first identified in bacteria (Eller *et al.*, 2008).

To date, the major molecular variations identified as impacting the tanning response are MC1R polymorphisms and splice variants (Rouzaud *et al.*, 2006; Cui *et al.*, 2007). However, it seems likely that variations in p53 and other DNA damage response genes will also be found to influence the tanning response.

CONFLICT OF INTEREST

The author states no conflict of interest.

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