Published in final edited form as:

Expert Rev Dermatol. 2008 December ; 3(6): 649–656. doi:10.1586/17469872.3.6.649.

Ribosomal stress, p53 activation and the tanning response

Graeme Walker¹ and Neil Box²

¹Oncogenomics Laboratory, Queensland Institute of Medical Research, 300 Herston Rd, Herston, 4029, Qld, Australia

²Department of Dermatology, University of Colorado at Denver, 12800 E. 19th Ave, Aurora, CO, 80045, USA

Abstract

Melanocytes (MC) sit along the epidermal basal layer, largely quiescent except for constitutive melanin production. They are usually only activated after sun exposure. The recent paper by McGowan et al. (1) describes a novel mechanism by which melanocytes are induced to proliferate upon p53 activation in adjacent keratinocytes (KC). In this study, small subunit ribosomal protein mutations cause a dramatic activation of p53 that we propose mimics important aspects of the skin sunburn response after ultraviolet radiation (UVR) exposure. McGowan et al. show that the phenotype of their hyperpigmented mouse mutants results from p53-dependent upregulation of KITLG, a cytokine that binds to the KIT receptor on melanocytes and influences melanin synthesis, melanocyte proliferation, and dictates MC localization at the dermo-epidermal junction. These findings extend our knowledge about skin stress responses, in particular, how p53 activity in keratinocytes is central to the regulation of melanocyte behaviour.

Keywords

melanocyte; keratinocyte; melanoma; tanning; p53; MC1R; KIT

BACKGROUND

The study of coat color in animals has been critical in the discovery of genes that control pigmentation in humans. In both humans and animals fully differentiated MCs can be divided into two major groups, those in the hair bulb, whose function is mainly to produce pigment for hair color, and epidermal MCs, which supply the epidermis with melanin and are involved in the tanning response. One of the central themes of research into the genetics of malignant melanoma (MM) is the question of how genes influencing pigment switching (e.g. the melanocortin receptor gene, MC1R), and/or melanocyte (MC) development (e.g. KIT), play a role in both pigmentation and MM development).

UVR and the tanning response

After an erythematous UVR exposure, basal keratinocytes proliferate (resulting in epidermal hyperplasia), to replace apoptotic “sunburn cells” that express p53 (2). Skin melanocytes are also highly responsive to UVR, causing increased pigmentation in two ways. The first, termed immediate pigment darkening, is due to oxidation of existing melanin, and fades rapidly. The second, delayed tanning, appears primarily due to increased melanogenesis and

Correspondence to, Tel: 1-303-724-0160, Neil.Box@ucdenver.edu.

Financial & competing interests disclosure

The authors report no conflict of interest. No writing assistance was utilized in the production of this manuscript.

other changes that are evident a few days post-UVR and can last for weeks (3–5). Melanin synthesis occurs in MCs, within melanosomes that are translocated via dendritic processes into the surrounding KCs. Thus melanin performs its primary role in KCs. Tanning may also involve a second response: recruitment of new MCs to the burned skin area. UVR appears to induce an increase in epidermal MC numbers in human (6–9), and mouse (e.g. 10–12) skin. This increase can result from activation of pre-existing *in situ* precursors not expressing MC pigmentation or differentiation antigens until exposed to UVR (i.e. not a true change in MC density). However MCs positive for proliferation markers can be observed after UVR exposure (12–14), suggesting that at least some of the increase results from cell division. Recent work staining for multiple MC markers further supports this notion (e.g. 9, 12). Epidermal grafting experiments, while not confirming proliferation, indicate that MCs can migrate through the epidermis (15–18). In practice it is difficult to disprove that precursor activation may play some role, yet the evidence suggests that proliferation and migration may be largely responsible for the increase in MC numbers.

Melanocytes are under the control of mitogens and melanogens produced by keratinocytes

The role of KCs in determining MC localization and function is becoming increasingly recognized. KCs largely regulate the melanocytic component of the tanning response (19, 20) via the paracrine release of melanogenic and mitogenic factors (Figure 1), including KIT ligand (KITLG), fibroblast growth factor 2 (FGF2), endothelins (EDNs), and MC stimulating hormone (α -MSH). These cytokines signal through their respective receptors on MCs (KIT, FGFR, EDNRB and MC1R respectively) to regulate intracellular signaling cascades, including the mitogen activated protein kinase, protein kinase C and protein kinase A pathways. Part of the tanning response is dependent upon p53, which transcriptionally upregulates the *pro-opiomelanocortin* (POMC) gene after UV exposure (21). POMC is a multicomponent precursor for α -MSH (melanotropic), ACTH (adrenocorticotrophic), and β -endorphin. Since p53 has a wide array of target genes, it is likely that p53 regulates additional KC-derived growth factors that influence MCs. The study by McGowan et al. (1) has revealed a novel mechanism by which p53 is upregulated in KCs to regulate MC numbers in the basal epidermis.

SUMMARY OF METHODS AND RESULTS

In a large scale *N-ethyl-N-Nitrosourea* (ENU) mouse mutagenesis screen for hyperpigmentation phenotypes, mutants fell into three categories: adult onset dark skin with epidermal thickening, accumulation of dermal MCs during development, and accumulation of epidermal MCs during development. While the first two categories of mouse mutants are characterised (22, 23), the third is described in McGowan et al. (1), including two lines with the same phenotype, dark skin 3 and 4 (*Dsk3* and *4*). These phenotypes became obvious around 3 weeks of age and included hyperpigmentation of the footpads, ears and tail. To allow assessment of MC numbers during development, *Dsk3* and *4* mice were bred with a transgenic mouse expressing β -galactosidase under the control of a MC-specific gene promoter. *Dsk3* and *4* mice had decreased MC numbers during embryogenesis, but increased basal layer MCs postnatally, only marginally at P3, but about 5-fold by P30.

Using a meiotic mapping approach, *Dsk3* and *4* were localized to small intervals on chromosomes 7 and 4 respectively. Candidate gene sequencing identified a T316A point mutation (predicting a Y54N amino acid substitution) in the small ribosomal subunit gene, *Rps19*, in the *Dsk3* mutant, and a T209C (L32P) mutation in *Rps20* in *Dsk4* mice. The authors then utilised mice carrying a conditional knockout allele for another small subunit ribosomal protein, *Rps6*. When the *Rps6^{lox}* allele was crossed with a transgenic that expresses Cre recombinase specifically in KCs, the resultant progeny (*Rps6^{lox/+} Tg.K5Cre/*

+) presented with a phenocopy of the *Dsk3* and *Dsk4* mice (hyperpigmented footpads, ears and tails), indicating that KC-specific haploinsufficiency of ribosomal proteins was driving the increase in epidermal MC numbers. The *Rps6^{lox}* allele was also crossed with MC-specific Cre (*Tg.MitfCre*) mice to direct in deletion of *Rps6* specifically to MCs. The *Rps6^{lox/+} Tg.MitfCre/+* mice presented with slightly diminished epidermal pigmentation, the opposite to the effect observed with KC-specific haploinsufficiency for *Rps6*.

The fact that this was essentially a KC-directed pigmentation phenotype prompted an investigation of KC-derived cytokines that signal to MCs. RNA was isolated from footpads of *Rps6^{lox/+} Tg.K5Cre/+* (mutant) and *Rps6^{lox/+}* (wild type) mice and expression levels of *Kitlg*, *Pomc*, and *Edn1* measured. *Kitlg* expression in the mutant mouse footpads was increased 8.3 fold at P3 and persisted at 3.8 fold at P30, compared to wild type footpads. Elevated *Kitlg* signaling was also observed in *Rps19^{Dsk3}* and *Rps20^{Dsk4}* footpads. For a number of reasons, the *Rps6^{lox/+} Tg.K5Cre/+* mice were introduced to the conventional *p53^{+/-}* and *p53^{-/-}* backgrounds and it was apparent that the dark skin phenotype was ameliorated on the haploinsufficient *p53* background and completely reversed on the *p53* null background. Hence the dark skin phenotype is entirely *p53* dependent, and activation of KC *p53* through haploinsufficiency of ribosomal proteins causes increased release of the *Kitlg* signaling factor, which in turn drives epidermal melanocytosis.

DISCUSSION AND SIGNIFICANCE

Ribosomal proteins, ribosomal stress and *p53*

The ribosome is a multiprotein-RNA complex that is essential for cellular protein synthesis. Almost 80% of the energy of a proliferating cell is spent on synthesis of nearly 80 ribosomal proteins (and approximately 150 rRNAs) that make up the ribosome (24). Ribosomal proteins are synthesized in the cytoplasm, imported into the nucleolus for partial assembly of ribosomal subunits, then exported back to the cytoplasm as mature ribosomes. During cellular transformation, the system undergoes characteristic changes, including structural changes in the nucleolus (25). Increased cell cycling associated with the tumorigenic tissue necessitates increased ribosomal protein synthesis. These activities are subjected to *p53* dependent surveillance that aims to prevent the rapid S phase transition synonymous with cellular transformation. Defects that alter ribosomal protein levels (dubbed “ribosomal stress”) invoke a powerful *p53* response. In 1994 Arnold Levine suggested that *p53* protein levels are coupled to ribosomal protein levels. He showed that RPL5 forms a ternary protein complex with the mouse double minute 2 (Mdm2) ubiquitin ligase and with *p53* itself (26). Other studies later confirmed that reduced RPL5 expression decreases *p53* protein level, while over-expression of RPL5 causes *p53* to increase (27). Eighteen other cytoplasmic/nucleolar ribosomal proteins (26–38) have been shown to impact on *p53* via diverse mechanisms (Table 1). These ribosomal proteins fall into 2 groups. The first group includes RPL4, L5, L6, L11, L12, L13, L19, L21, L22, L26 and RPS7, S9, S16, and S25, where *p53* levels are reduced concomitant with ribosomal protein gene knockdown or haploinsufficiency. Here the ribosomal protein is acting as a positive regulator of *p53* – consistent with a tumor suppressive role. In the second group (RPL23, Rps6, Rps19 and Rps20), reduced expression results in increased *p53* levels. In the case of RPL23, *p53* increased with both knockdown and over-expression of the same gene.

No clear picture has emerged to explain how alterations in ribosomal protein levels can effect *p53*. It is possible that *p53* levels decrease with ribosomal protein insufficiency simply due to reduced synthesis of the *p53* protein (28, 34). However a reduction in levels of a particular ribosomal protein may also result in a completely opposite effect - increased *p53* protein levels. It has been shown using siRNA knockdown of *RPL23* in cancer cell lines that nucleophosmin and other nucleolar components shuttle from the nucleolus to the

nucleoplasm, where they are capable of activating p53 by interacting with MDM2 (33). The observations reported for Rps6 (35–37) and for Rps19 and Rps20 (1) add these three small subunit ribosomal proteins to the list of those capable of activating p53 through this potential mechanism. Whether these proposed mechanisms can be validated *in vivo* or not remains to be seen.

As both increased and reduced ribosomal protein gene expression can activate p53, it is not possible to predict the functional status of ribosomal proteins simply based on their effect on p53 levels; one must determine whether the *Rps* mutation is a loss of function, dominant negative, or gain of function allele. McGowan et al report amino acid substitutions in Rps19 and Rps20. They infer that since *Rps6* haploinsufficiency induces hyper-pigmented footpads that all of these mutations are loss of function alleles similar to *Rps6*. While this may be the case, these mutations could equally be gain of function or dominant negative alleles that induce a phenotypically identical p53 pathway response. Notably, they do not mention the effects of homozygosity of the *Rps6* allele (*Rps6^{Lox/Lox}*) when inherited with the *Tg.K5Cre* or *Tg.MitfCre* mice. A true determination of the genetic nature of the mutation may well yield important insights into the mechanism of action by which these mutations cause p53 activation.

KITLG drives MC migration to the epidermal basal layer

The next novel aspect of this study is the upregulation of *Kitlg* expression by p53. It is unsurprising that upregulation of Kitlg signaling can effect MC development and localisation. Overexpression of the membrane bound form of Kitlg resulted in a human-like skin structure in mice, i.e., with MCs in the epidermal basal layer (39). Injection of KITLG into human skin also results in increased MC numbers, as well as changes in their size and dendricity (40). Although it is not clear precisely how p53 upregulates KITLG, it is becoming increasingly evident that, based on p53 binding site analysis, most paracrine factors that are released from KCs after UVR exposure are potentially regulated by p53 (reviewed in 41). Indeed, the *KITLG* gene has a p53 binding site in the first intron (42), thus it may be directly transcriptionally upregulated by p53.

Ribosome deregulation mimics UVR damage?

It is well known that both p53 and KITLG expression are increased in KCs after UVR exposure (43). Our own studies with neonatal UVR in both wild type mice, and MC-specific *Ras* overexpressors with dermal melanocytosis, suggest the MC response to UVR is invariably characterized by their attraction to the epidermal basal layer (12). Thus we believe that the upregulation of p53 (and Kitlg) resulting from decreased ribosome function is effectively mimicking UVR-induced damage to KCs (Figure 2). As is the case with the *Rps*-defective mice, if Kitlg signaling is blocked after UVR using a Kit-blocking antibody, the MC proliferative response is substantially diminished (44). We hypothesize that KITLG plays a major role in increasing basal MC numbers, as well as increasing pigment synthesis (probably in collaboration with other cytokines) following UVR. The study of McGowan et al. may be only the beginning of an expanded role for p53 in the regulation of MC response to UVR as part of the delayed tanning response.

p53 regulation of both KITLG and KIT

Whereas KC-specific *Rps* mutation results in hyperpigmentation, MC-specific mutation results in slight hypopigmentation. This suggests that post-natally the mutant MCs are slightly less responsive to normal KITLG levels. Given the published data suggesting disparate cell type-specific p53 responses to *Rps* downregulation, we have to ask is whether *Rps6* downregulation also results in p53 upregulation in MCs? This seems likely, but is not known. Again inferring from skin response to UVR, there is some limited evidence that

UVR damage – hence increased p53 expression - in MCs can result in down regulation of the MC KIT receptor (44, 45). Some of the other paracrine signaling factors and cognate receptors also appear to be subjected to a co-ordinated regulation by p53. For example, *HGF* has a confirmed p53 regulatory sequence in its immediate promoter (46) while the HGF receptor *c-MET* is similarly regulated (47). This paradigm would predict that c-MET, KIT and MC1R are subjected to similar downregulation in MCs after p53 activation. Although the study of McGowan et al. point to a downregulation of Kt in MCs due to p53 activation, it is known that increased KC KITLG expression can upregulate KIT in MCs (48). Very little is known about how any of these MC cell surface receptors are regulated *in vivo* at various timepoints after UVR exposure, and this warrants further investigation.

Attraction of MCs specifically to the epidermal basal layer

The location of the murine MCs in the basal layer is intriguing, reflecting their position in human skin. This location normally makes perfect sense - MCs sit along the basement membrane and supply melanosomes/melanin to adjacent KCs to protect against future UVR damage. KITLG is constitutively expressed in the human (thus probably keeps MCs constitutively in the basal layer), but not the adult mouse epidermis. It is likely that the reason epidermal MC number returns to normal some weeks after UVR exposure is that the signals from proliferating KCs (e.g., Kitlg) have subsided. Notably, Kitlg does not return to normal in the *Dsk3/4* mutants, hence MCs remain in the basal layer. Interestingly, two other dark skinned phenotypes with increased basal MCs isolated from the ENU mutagenesis screen, *Dsk2* and *Dsk5* (22), were shown to result from gain-of-function mutations in the *keratin 2E* and *Egfr* genes respectively. In these cases the attraction of MCs to the basal layer seems to be secondary to epidermal KC hyperproliferation (which also occurs after UVR exposure). Whether this also involves increased KC p53 and Kitlg expression in the proliferating KCs causing MCs to be attracted to the region will be interesting to ascertain.

Pomc/αMsh and Edn1 are not upregulated in *Dsk* mice

If p53 activation mimics UVR-induced DNA damage, why is *Pomc* (and hence α-Msh and Acth) not upregulated in the study of McGowan et al. (1), as in the study of Cui et al. (21). There are two major differences between the studies. The latter used primarily murine ear skin, whereas McGowan et al. used footpad skin. There may be anatomical site-specific differences in *Pomc* regulation that prevents it being activated in footpad skin. Alternatively, it may be that a significantly higher level of p53 (for instance after UVR) is needed to upregulate *Pomc* expression, whereas lower levels (as in the *Dsk* mice) are sufficient for *Kitlg* upregulation. The situation is unclear. In addition, although endothelins also have a recognised role as MC mitogens and melanogens, *Edn1* is not upregulated in the *Dsk3* or *Dsk4* epidermis. Interestingly, mice with KC-specific overexpression of another endothelin family member, *Edn3*, exhibit dermal, not basal layer melanocytosis (or hyperpigmented footpads) (49). Thus *Kitlg* and *Edn1* may have a different role in the response of MCs to epidermal stress. Certainly, *Edn1* is not needed to keep MCs at the basal layer. Furthermore, following UVR *EDN1* is induced much later than *KITLG*, and its upregulation in KCs may be via an autocrine mechanism dependent upon prior expression of IL-1α (43). As expected, *KITLG* is the critical cytokine for the localisation of MCs to the epidermal basal layer.

Do keratinocytes regulate melanocyte number in different anatomical locations?

Another critical question is why Rps-p53-Kitlg signaling only causes hyperpigmentation in the non-hairy (glabrous) skin? This is probably explained by the fact that murine dorsal interfollicular epidermis is devoid of MCs, except during the first week of life. Similarly, UVR exposure of adult mice UVR also does not result in migration of MCs to the basal layer (12), even though epidermal p53 is upregulated. The follicular microenvironment only produces *Kitlg* during anagen, to activate bulge MC stem cells to resupply the hair bulb.

Thus the bulb MCs and bulge precursors are normally unresponsive to Kitlg, including its promiscuous production due to the *Rps* defects. However the wild type murine footpad, ear and tail already have low numbers of epidermal MCs, thus in this case the epidermal melanocytosis probably requires pre-existing epidermal MCs. As previously mentioned, footpad skin is analogous to human palmoplantar skin, which also normally contains very low levels of MCs. In humans this may be due to the expression of Dickopf (DKK), a secreted antagonist of the Wnt/ β -catenin pathway, in mesenchymal fibroblasts, that is critical in suppressing MC number (50). Recently it has been shown that palmoplantar skin has very low levels of KITLG and EDN1 in epidermal KCs (51). Therefore there may be complex means of regulation of MC number – possibly involving KIT signaling – that dictates MC density and/or differentiation status in different anatomical locations.

KITLG signaling and melanoma

In line with a protective response of increased p53 signaling, mice that are null for *p53* are highly susceptible to the development of MM if they also carry a *Ras* mutation in their MCs (52). We suspect that this phenotype is more dependent on p53 ablation in the MC than in the KC, although no studies have been performed to dissect the role of *p53* nullizygosity of the respective cell types in MM development. Paradoxically, upregulation of p53 may also be involved in development of some pigmented lesions. Overexpression of KITLG in KCs is observed in senile keratoses that are characterised by increased epidermal basal layer MCs (43). Furthermore, somatic gain-of-function *KIT* mutations are found in some forms of human MM (53), specifically, in terms of cutaneous lesions, MMs arising as a result of chronic sun exposure. These hypermorphic *KIT* mutations should theoretically be equivalent to constitutive overexpression of KITLG. This raises the question of whether other mutations that upregulate KITLG-KIT signaling may play a role in the development of some MMs. We speculate that increased levels of p53 in KCs may well sensitize MCs to transformation, providing a signaling environment more tolerant for tumor promotion. If this hypothesis does indeed turn out to be true, it would be the first example to our knowledge of p53 acting like a tumor promoter instead of a tumor suppressor.

Five year view

Genes involved in MC development (e.g. *KIT*) and pigment type switching (e.g. *MC1R*) are also involved in melanoma tumorigenesis. The KC-derived cytokines that stimulate these receptors (KITLG and α -MSH respectively) are directly regulated by p53. We envisage that future studies will reveal other cytokines that are induced by p53 in keratinocytes, and that p53 is a central player in regulating constitutive and facultative pigmentation. Paradoxically, while lack of p53 can dramatically increase MM penetrance in mice, constitutive overexpression of p53, such as by upstream deregulation (e.g. ribosomal stress), or chronic UVR exposure, may also be involved in the induction of some melanocytic lesions. Further elucidation of pathways involved in KC-MC signaling should increase our understanding of how variation in tanning response to sun exposure influences our susceptibility to MM development.

Acknowledgments

The authors work was supported by the Cancer Council of Queensland (GW), the Dermatology Foundation (NB) and the American Skin Association (NB).

References

1. McGowan KA, Li JZ, Park CY, et al. Ribosomal mutations cause p53-mediated dark skin and pleiotropic effects. *Nat. Genet.* 2008; 40(8):963–970. [PubMed: 18641651]

2. Ziegler A, Jonason AS, Leffell DJ, et al. Sunburn and p53 in the onset of skin cancer. *Nature*. 1994; 372(6508):773–776. [PubMed: 7997263]
3. Caswell M. The kinetics of the tanning response to tanning bed exposures. *Photodermatol. Photoimmunol. Photomed*. 2000; 16(1):10–14. [PubMed: 10721858]
4. Sheehan JM, Potten CS and Young AR. Tanning in human skin types II and III offers modest photoprotection against erythema. *Photochem. Photobiol*. 1998; 68(4):588–592. [PubMed: 9796443]
5. Sheehan JM, Young AR. The sunburn cell revisited: an update on mechanistic aspects. *Photochem. Photobiol. Sci*. 2002; 1(6):365–377. [PubMed: 12856704]
6. Quevedo WC, Jr, Szabo G, Virks J, Sinesi S. Melanocyte populations in UV-irradiated human skin. *J. Invest. Dermatol*. 1965; 45(4):295–298. [PubMed: 5320054]
7. Scott GA, Haake AR. Keratinocytes regulate melanocyte number in human fetal and neonatal skin equivalents. *J. Invest. Dermatol*. 1991; 97(5):776–781. [PubMed: 1919043]
8. Stierner U, Rosdahl I, Augustsson A, Kagedal B. UVB irradiation induces melanocyte increase in both exposed and shielded human skin. *J. Invest. Dermatol*. 1989; 92(4):561–564. [PubMed: 2703724]
9. Yamaguchi Y, Coelho SG, Zmudzka BZ, Takahashi K, Beer JZ, Hearing VJ, Miller SA. Cyclobutane pyrimidine dimer formation and p53 production in human skin after repeated UV irradiation. *Exp. Dermatol*. 2008 [Epub ahead of print].
10. Rosdahl I, Szabo G. Mitotic activity of epidermal melanocytes in UV-irradiated mouse skin. *J. Invest. Dermatol*. 1978; 70(3):143–148. [PubMed: 632619]
11. Quevedo W, Fleischmann R. Developmental biology of mammalian melanocytes. *J. Invest. Dermatol*. 1980; 75(1):116–120. [PubMed: 6771335]
12. Walker G, Kimlin M, Hacker E, et al. Murine neonatal melanocytes exhibit a heightened proliferative response to ultraviolet radiation and migrate to the epidermal basal layer. *J. Invest. Dermatol*. 2008 [Epub ahead of print].
13. Jimbow K, Uesugi T. New melanogenesis and photobiological processes in activation and proliferation of precursor melanocytes after UV-exposure: ultrastructural differentiation of precursor melanocytes from Langerhans cells. *J. Invest. Dermatol*. 1982; 78(2):108–115. [PubMed: 7057046]
14. van Schanke A, Jongsma MJ, Bisschop R, van Venrooij GM, Rebel H, de Gruij FR. Single UVB overexposure stimulates melanocyte proliferation in murine skin, in contrast to fractionated or UVA-1 exposure. *J. Invest. Dermatol*. 2005; 124:241–247. [PubMed: 15654980]
15. Billingham R, Silvers W. Studies on the migratory behaviour of melanocytes in guinea pig skin. *J. Exp. Med*. 1970; 131(1):101–117.
16. Horikawa T, Mishima Y, Nishino K, Ichihashi M. Horizontal and vertical pigment spread into surrounding piebald epidermis and hair follicles after suction blister epidermal grafting. *Pigment Cell Res*. 1999; 12(3):175–180. [PubMed: 10385913]
17. Nishimura EK, Jordan SA, Oshima H, et al. Dominant role of the niche in melanocyte stem-cell fate determination. *Nature*. 2002; 416(6883):854–860. [PubMed: 11976685]
18. Yonetani S, Moriyama M, Nishigori C, Osawa M, Nishikawa S. In vitro expansion of immature melanoblasts and their ability to repopulate melanocyte stem cells in the hair follicle. *J. Invest. Dermatol*. 2008; 128(2):408–420. [PubMed: 17657242]
19. Hirobe T. Role of keratinocyte-derived factors involved in regulating the proliferation and differentiation of mammalian epidermal melanocytes. *Pigment Cell Res*. 2005; 18(1):2–12. [PubMed: 15649147]
20. Lin J, Fisher DE. Melanocyte biology and skin pigmentation. *Nature*. 2007; 445(7130):843–850. [PubMed: 17314970]
21. Cui R, Widlund HR, Feige E, et al. Central role of p53 in the suntan response and pathologic hyperpigmentation. *Cell*. 2007; 128(5):853–886. [PubMed: 17350573]
22. Fitch KR, McGowan KA, van Raamsdonk CD, et al. Genetics of dark skin in mice. *Genes Dev*. 2003; 17(2):214–212. [PubMed: 12533510]
23. Van Raamsdonk CD, Fitch KR, Fuchs H, de Angelis MH, Barsh GS. Effects of G-protein mutations on skin color. *Nat. Genet*. 2004; 36(9):961–968. [PubMed: 15322542]

24. Thomas G. An encore for ribosome biogenesis in the control of cell proliferation. *Nat. Cell Biol.* 2000; 2(5):E71–E72. [PubMed: 10806485]
25. Gani R. The nucleoli of cultured human lymphocytes. I. Nucleolar morphology in relation to transformation and the DNA cycle. *Exp. Cell Res.* 1976; 97(2):249–258. [PubMed: 1248517]
26. Marechal V, Elenbaas B, Piette J, Nicolas JC, Levine AJ. The ribosomal L5 protein is associated with mdm-2 and mdm-2-p53 complexes. *Mol. Cell Biol.* 1994; 14(11):7414–7420. [PubMed: 7935455]
27. Dai MS, Lu H. Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. *J. Biol. Chem.* 2004; 279(43):44475–44482. 2004. [PubMed: 15308643]
28. Castro ME, Leal JF, Leonart ME, Ramon Y Cajal S, Carnero A. Loss-of-function genetic screening identifies a cluster of ribosomal proteins regulating p53 function. *Carcinogenesis.* 2008; 29(7):1343–1350. [PubMed: 18515283]
29. Bhat KP, Itahana K, Jin A, Zhang Y. Essential role of ribosomal protein L11 in mediating growth inhibition-induced p53 activation. *EMBO J.* 2004; 23(12):2402–2412. [PubMed: 15152193]
30. Zhang Y, Wolf GW, Bhat K, Jin A, Allio T, Burkhardt WA, Xiong Y. Ribosomal protein L11 negatively regulates oncoprotein MDM2 and mediates a p53-dependent ribosomal-stress checkpoint pathway. *Mol. Cell Biol.* 2003; 23(23):8902–8912. [PubMed: 14612427]
31. Lohrum MA, Ludwig RL, Kubbutat MH, Hanlon M, Vousden KH. Regulation of HDM2 activity by the ribosomal protein L11. *Cancer Cell.* 2003; 3(6):577–587. [PubMed: 12842086]
32. Anderson SJ, Lauritsen JP, Hartman MG, et al. Ablation of ribosomal protein L22 selectively impairs alphabeta T cell development by activation of a p53-dependent checkpoint. *Immunity.* 2007; 26(6):759–772. [PubMed: 17555992]
33. Jin A, Itahana K, O'Keefe K, Zhang Y. Inhibition of HDM2 and activation of p53 by ribosomal protein L23. *Mol. Cell Biol.* 2004; 24(17):7669–7680. [PubMed: 15314174]
34. Takagi M, Absalon MJ, McLure KG, Kastan MB. Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. *Cell.* 2005; 123(1):49–63. [PubMed: 16213212]
35. Sulic S, Panic L, Barkic M, Mercep M, Uzelac M, Volarevic S. Inactivation of S6 ribosomal protein gene in T lymphocytes activates a p53-dependent checkpoint response. *Genes Dev.* 2005; 19(24):3070–3082. [PubMed: 16357222]
36. Panic L, Tamarut S, Sticker-Jantscheff M, et al. Ribosomal protein S6 gene haploinsufficiency is associated with activation of a p53-dependent checkpoint during gastrulation. *Mol. Cell Biol.* 2006; 26(23):8880–8891. [PubMed: 17000767]
37. Panic L, Montagne J, Cokaric M, Volarevic S. S6-haploinsufficiency activates the p53 tumor suppressor. *Cell Cycle.* 2007; 6(1):20–24. [PubMed: 17245121]
38. Chen D, Zhang Z, Li M, Wang W, et al. Ribosomal protein S7 as a novel modulator of p53-MDM2 interaction: binding to MDM2, stabilization of p53 protein, and activation of p53 function. *Oncogene.* 2007; 26(35):5029–5037. [PubMed: 17310983]
39. Kunisada T, Lu SZ, Yoshida H, et al. Murine cutaneous mastocytosis and epidermal melanocytosis induced by keratinocyte expression of transgenic stem cell factor. *J. Exp. Med.* 1998; 187(10):1565–1573. [PubMed: 9584135]
40. Grichnik JM, Burch JA, Burchette J, Shea CR. The SCF/Kit pathway plays a critical role in control of normal human melanocyte homeostasis. *J. Invest. Dermatol.* 1998; 111(2):233–238. [PubMed: 9699723]
41. Box NF, Terzian T. The role of p53 in pigmentation, tanning and melanoma. *Pigment Cell Melanoma Res.* 2008; 21(5):525–533. [PubMed: 18761658]
42. Wei CL, Wu Q, Vega VB, Chiu KP, et al. A global map of p53 transcription-factor binding sites in the human genome. *Cell.* 2006; 124(1):207–219. [PubMed: 16413492]
43. Imokawa G. Autocrine and paracrine regulation of melanocytes in human skin and in pigmentary disorders. *Pigment Cell Res.* 2004; 17(2):96–110. [PubMed: 15016298]
44. Kawaguchi Y, Mori N, Nakayama A. Kit⁺ melanocytes seem to contribute to melanocyte proliferation after UV exposure as precursor cells. *J. Invest. Dermatol.* 2001; 116(6):920–925. [PubMed: 11407982]

45. Hosaka E, Soma Y, Kawa Y, et al. Effects of ultraviolet light on melanocyte differentiation: studies with mouse neural crest cells and neural crest-derived cell lines. *Pigment Cell Res.* 2004; 17(2):150–157. [PubMed: 15016304]
46. Metcalfe AM, Dixon RM, Radda GK. Wild-type but not mutant p53 activates the hepatocyte growth factor/scatter factor promoter. *Nucl. Acids Res.* 1997; 25(5):983–986. [PubMed: 9023107]
47. Seol DW, Chen Q, Smith ML, Zarnegar R. Regulation of the c-met proto-oncogene promoter by p53. *J. Biol. Chem.* 1999; 274(6):3565–3572. [PubMed: 9920903]
48. Hasegawa J, Goto Y, Murata H, Takata M, Saida T, Imokawa G. Downregulated melanogenic paracrine cytokine linkages in hypopigmented palmpplantar skin. *Pigment Cell Mel. Res.* 2008 [Epub ahead of print].
49. Garcia RJ, Ittah A, Mirabal S, Figueroa J, Lopez L, Glick AB, Kos L. Endothelin 3 induces skin pigmentation in a keratin-driven inducible mouse model. *J Invest Dermatol.* 2008; 128(1):131–142. [PubMed: 17611578]
50. Yamaguchi Y, Passeron T, Hoashi, et al. Dickkopf 1 (DKK1) regulates skin pigmentation and thickness by affecting Wnt/beta-catenin signaling in keratinocytes. *FASEB J.* 2008; 22(4):1009–1020. [PubMed: 17984176]
51. Bardeesy N, Bastian B, Hezel A, Pinkel D, DePinho R, et al. Dual inactivation of RB and p53 pathways in RAS-induced melanomas. *Mol. Cell. Biol.* 2001; 21(6):2144–2153. 2001. [PubMed: 11238948]
52. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. *J. Clin. Oncol.* 2006; 24(26):4340–4346. [PubMed: 16908931]

Key Issues

- Ribosomal stress in keratinocytes results in an increase in p53 protein levels possibly through interaction with MDM2.
- The increased keratinocyte p53 directly induces KITLG expression, which in turn drives melanocyte proliferation/migration at the epidermal basal layer.
- Paradoxically, ribosomal stress in melanocytes slightly decreases basal melanocyte number probably through down regulation of the KIT receptor.
- Ribosome deregulation may mimic UVR damage to keratinocytes by inducing p53 as a stress response.
- Increased melanocyte numbers in the basal layer is largely non-cell autonomous – determined at least in part by p53-KITLG upregulation in keratinocytes.
- Variation in levels of KITLG signaling may regulate melanocyte number in different anatomical locations.
- Although p53 classically acts as a tumor suppressor, in some circumstances, for instance with constitutive activation or chronic UVR exposure, we speculate that it may act as a promoter of melanocytic lesions.

Figure 1. Schematic depiction of UVR-treated skin

Damaged keratinocytes release various cytokines that bind to their cognate receptors on the melanocytes to induce pigmentation and proliferation.

Figure 2. Primacy of KITLG in controlling melanocyte proliferation and migration

As a result of ultraviolet UVR-induced damage to keratinocytes (A), or a ribosomal protein defect in keratinocytes (B), subsequent p53 induction in these cells can drive melanocyte proliferation via KITLG signalling. Whether αMSH signalling also plays a role in the melanocyte proliferative response after UVR exposure is unknown.

Table 1

A literature survey of p53 protein level changes after ribosomal protein knockdown or over-expression

	Reduced RP Expression	RP Over-Expression	Reference
L4 ^a	↓*	?	Castro et al. (28)
L5 ^{a,b}	↓	↑	Dai et al. 2004 (27) Castro et al. (28)
L6 ^a	↓	?	Castro et al. (28)
L11 ^{a,b}	↓	↑	Bhat et al. (29) Zhang et al. (30) Lohrum et al. (32)
L12 ^a	↓	?	Castro et al. (28)
L13 ^a	↓	?	Castro et al. (28)
L19 ^a	↓	?	Castro et al. (28)
L21 ^a	↓	?	Castro et al. (28)
L22 ^c	↓	?	Anderson et al. (32)
L23 ^b	↑	↑	Jin et al. (33).
L26	↓	↑	Takagi et al. (3), 2005
S6 ^{a,c}	↑↓	↑	Panic et al. (36). Sulic et al. (35) Castro et al. (28)
S7 ^b	↓	↑	Chen et al. (38).
S9 ^a	↓	?	Castro et al. (28)
S16 ^a	↓	?	Castro et al. (28)
S19 ^d	↑	?	McGowan et al. (1)
S20 ^{a,d}	↑↓	?	McGowan et al. (1) Castro et al. (28)
S25 ^a	↓	?	Castro et al. (28)

* Arrows indicate direction of change of p53 protein levels.

^a: Castro et al. (28) performed antisense knockdown in a mouse embryonic fibroblast (MEF) gene knockdown screen that identified genes that allowed MEFs to overcome a p53 dependent cell cycle arrest. Discrepancies with mouse phenotypic data (S6 and S20) suggest that some ribosomal proteins may act differently on p53 in different tissues.

^b: siRNA knockdown and over-expression studies were performed in U2OS and other cancer cell lines.

^c: Anderson et al. (32) generated a conditional knockout mouse that was defective in T-/B- cell development.

^d: McGowan et al. (1) identified mutations in *Rps19* and *Rps20*. They assume these mutations are loss-of-function/haploinsufficiency alleles.

RP: Ribosomal Protein