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Slug Expression in Mouse Skin and Skin Tumors Is Not Regulated by p53

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TO THE EDITOR

Slug (Snai2), a highly conserved transcriptional repressor important in epithelial-mesenchymal transformation (EMT) in development, has also been implicated in EMT-like processes during tumor progression (Hemavathy *et al.*, 2000). *Slug* null mice chronically exposed to ultraviolet radiation (UVR) develop a lower skin tumor burden than wild type mice, with fewer aggressive spindle cell tumors (Newkirk *et al.*, 2007). Slug expression is transiently induced in keratinocytes by UVR and Slug expression is persistently elevated in UVR-induced skin tumors (Kusewitt *et al.*, 2009; Newkirk *et al.*, 2007). Slug expression is regulated both at the transcriptional and post-translational levels, and there are conflicting reports about the role of the p53 tumor suppressor in this regulation, as discussed below. In the present studies, we investigated the relationship between Slug and p53 expression in UVR-exposed skin and UVR-induced skin tumors by immunohistochemistry using a specific anti-Slug antibody (Cell Signaling, Danvers, MA), the CM5 antibody (Leica Microsystems, Buffalo Grove, IL) that recognizes all forms of p53, and the PAb240 antibody (Novus, Littleton, CO) that recognizes p53 in the unfolded conformation assumed by many mutant forms. Animal studies were performed in accordance with all applicable state and national animal welfare guidelines.

Several studies have suggested that wild type p53 suppresses Slug expression and enhances Slug degradation (Rinon *et al.*, 2011; Wang *et al.*, 2009; Zhang *et al.*, 2011); however, we demonstrated that induction of wild type p53 by UVR in keratinocytes did not prevent concurrent Slug induction. In the epidermis of wild type 129 mice exposed 1 time to 4800 J/m^2 UVR and harvested 24 hours after exposure, both Slug and p53 were expressed

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predominantly in basal keratinocytes, with some nuclei clearly expressing high levels of both p53 and Slug (Figure 1).

Slug has been reported to be positively regulated by p53 in hematopoietic cells in response to ionizing radiation (Wu *et al.*, 2005). To determine if Slug is a p53 target gene, we quantified Slug expression in *p53* null mice (Donehower, 1996) following a single exposure to 2400 J/m^2 UVR. We observed no significant difference in the number of Slug-positive nuclei in the unexposed skin of *p53* null FVB mice compared to wild type FVB skin (Figure 2a). Moreover, in both wild type and *p53* null mice, UVR exposure significantly ($p \leq 0.05$) enhanced Slug expression, and there was no significant difference between Slug induction in wild type versus *p53* null epidermis (Figure 2a). Thus wild type p53 was not required for Slug induction by UVR.

A number of reports indicate that wild type p53 suppresses while mutant p53 enhances Slug expression (Rinon *et al.*, 2011; Roger *et al.*, 2010; Wang *et al.*, 2009; Zhang *et al.*, 2011). We therefore examined the relationship between Slug and p53 expression in skin lesions expressing mutant p53. After repeated UVR exposures, hairless mice develop focal aggregates of p53-positive keratinocytes; many of these foci harbor p53 mutations and some represent precursors of squamous cell carcinomas (de Gruijl and Rebel, 2008; Rebel *et al.*, 2005; Rebel *et al.*, 2001). Virtually all UVR-induced tumors in the skin of SKH-1 hairless mice express 1 or more mutant p53 alleles (Melnikova *et al.*, 2005). We examined the skin of 5 SKH-1 hairless mice exposed 3 times weekly to UVR for a total of 10 weeks to induce the formation of preneoplastic p53-positive foci. At the time of sacrifice, 20 weeks after the last UVR exposure, UVR-exposed skin without gross abnormalities was examined. Of the 30 foci identified using the CM5 antibody, only 14 of these were also immunoreactive with the PAb240 antibody. Examination of skin from similarly treated mice at earlier time points revealed that CM5-positive foci appeared before Pab240-positive foci were detectable, suggesting that some CM5-positive foci expressed stabilized wild type rather than mutant p53. We also examined p53 expression in a total of 32 squamous cell carcinomas and spindle cell tumors induced by UVR in SKH-1 mice. Four of the squamous cell carcinomas did not express immunohistochemically detectable p53; the p53 gene was likely deleted in these tumors. Of the remaining 23 squamous cell carcinomas, 14 expressed p53 detectable using both Pab240 and CM5 antibodies. Another 9 squamous cell carcinomas expressed p53 detectable only with the CM5 antibody. All 5 spindle cell tumors examined had adjacent or intermingled neoplastic epithelial components and all spindle cell components were immunoreactive with both p53 antibodies.

Slug expression in these UVR-induced preneoplastic foci and skin tumors was compared to p53 expression (Figure 2b). Both p53-negative epidermis and p53-positive preneoplastic foci in UVR-exposed skin had significantly fewer Slug-positive nuclei than p53-positive epithelial tumors ($p \leq 0.05$). There was no significant difference between non-tumor skin, whatever the p53 status, and p53-negative epithelial tumors or p53-positive spindle cell tumors. Spindle cell tumors had significantly lower Slug expression than CM5-postive epithelial tumors ($p \leq 0.05$), but did not differ significantly from PAb240-positive epithelial tumors. P53-positive epithelial tumors expressed higher levels of Slug than p53-positive preneoplastic foci and there was reduced expression of Slug in in p53-positive spindle cell

tumors. Thus it appeared that p53 mutation was not the critical factor driving Slug expression during skin tumor progression.

Our results failed to demonstrate a relationship between Slug and p53 expression in acutely UVR-exposed skin, UVR-induced preneoplastic foci, or UVR-induced skin tumors. Activation of wild type p53 did not prevent Slug induction, p53 was not required for UVR induction of Slug, and mutant p53 expression did not enhance Slug expression. *Indeed, in tumors, expression of mutant p53 detectable with pAB240 or complete absence of p53 expression was actually associated with somewhat decreased Slug expression.* Our findings are consistent with a recent report showing no correlation between p53 and Slug immunoreactivity in human squamous cell carcinomas (Chen *et al.*, in press) and suggest that the relationship between Slug expression and p53 status *in vivo* requires additional investigation.

Slug interactions with the p53 protein are believed to be mediated via Mdm2, with formation of a wild type p53-Mdm2-Slug complex leading to degradation of Slug (Wang et al., 2009). However the relationship between Slug and Mdm2 expression is also unresolved. Some studies have shown that decreased Mdm2 expression is correlated with increased Slug expression (Wu et al., 2013; Kim et al., 2010). On the other hand, another study indicates that Mdm2 actually enhances Slug mRNA expression, although Mdm2 increases Slug protein levels only in the absence to wild type p53 (Jung et al., 2013). Studies in Mdm2 knockout mice like those performed in the present study in p53 knockout mice will be required to determine the interrelationship of Mdm2 and Slug in vivo.

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Abbreviations used

EMT	epithelial-mesenchymal transformation
UVR	ultraviolet radiation

References

Chen H, Takahara M, Xie L, Takeuchi S, Tu Y, Nakahara T, Uchi H, Moroi Y, Furue M. Levels of the EMT-related protein Snail/Slug are not correlated with p53/p63 in cutaneous squamous cell carcinoma. J Cutan Pathol. 2013 Mar 6. Epub ahead of print.

de Gruijl FR, Rebel H. Early events in UV carcinogenesis--DNA damage, target cells and mutant p53 foci. Photochem Photobiol. 2008; 84:382–7. [PubMed: 18221455]

Donehower LA. The p53-deficient mouse: a model for basic and applied cancer studies. Semin Cancer Biol. 1996; 7:269–78. [PubMed: 9110404]

Hemavathy K, Ashraf SI, Ip YT. Snail/slug family of repressors: slowly going into the fast lane of development and cancer. Gene. 2000; 257:1–12. [PubMed: 11054563]

Jung CH, Kim J, Park JK, Hwang SG, Moon SK, Kim WJ. Um HD. Mdm2 increases cellular invasiveness by binding to and stabilizing the Slug mRNA. Cancer Lett. 2013; 335:270–7. [PubMed: 23438693]

Kim D, Song J, Jin EJ. MicroRNA-221 regulates chondrogenic differentiation through promoting proteosomal degradation of slug by targeting Mdm2. J Biol Chem. 2010; 285:26900–7. [PubMed: 20576614]

Kusewitt DF, Choi C, Newkirk KM, Leroy P, Li Y, Chavez MG, et al. Slug/Snai2 is a downstream mediator of epidermal growth factor receptor-stimulated reepithelialization. J Invest Dermatol. 2009; 129:491–5. [PubMed: 18685621]

Melnikova VO, Pacifico A, Chimenti S, Peris K, Ananthaswamy HN. Fate of UVB-induced p53 mutations in SKH-hr1 mouse skin after discontinuation of irradiation: relationship to skin cancer development. Oncogene. 2005; 24:7055–63. [PubMed: 16007135]

Newkirk KM, Parent AE, Fossey SL, Choi C, Chandler HL, Rajala-Schultz PJ, et al. Snai2 expression enhances ultraviolet radiation-induced skin carcinogenesis. Am J Pathol. 2007; 171:1629–39. [PubMed: 17916597]

Rebel H, Kram N, Westerman A, Banus S, van Kranen HJ, de Gruijl FR. Relationship between UV-induced mutant p53 patches and skin tumours, analysed by mutation spectra and by induction kinetics in various DNA-repair-deficient mice. Carcinogenesis. 2005; 26:2123–30. [PubMed: 16051635]

Rebel H, Mosnier LO, Berg RJ, Westerman-de Vries A, van Steeg H, van Kranen HJ, et al. Early p53-positive foci as indicators of tumor risk in ultraviolet-exposed hairless mice: kinetics of induction, effects of DNA repair deficiency, and p53 heterozygosity. Cancer Res. 2001; 61:977–83. [PubMed: 11221893]

Rinon A, Molchadsky A, Nathan E, Yovel G, Rotter V, Sarig R, et al. p53 coordinates cranial neural crest cell growth and epithelial-mesenchymal transition/delamination processes. Development. 2011; 138:1827–38. [PubMed: 21447558]

Roger L, Jullien L, Gire V, Roux P. Gain of oncogenic function of p53 mutants regulates E-cadherin expression uncoupled from cell invasion in colon cancer cells. J Cell Sci. 2010; 123:1295–305. [PubMed: 20332115]

Wang SP, Wang WL, Chang YL, Wu CT, Chao YC, Kao SH, et al. p53 controls cancer cell invasion by inducing the MDM2-mediated degradation of Slug. Nat Cell Biol. 2009; 11:694–704. [PubMed: 19448627]

Wu WS, Heinrichs S, Xu D, Garrison SP, Zambetti GP, Adams JM, et al. Slug antagonizes p53-mediated apoptosis of hematopoietic progenitors by repressing puma. Cell. 2005; 123:641–53. [PubMed: 16286009]

Wu DW, Lee MC, Wang J, Chen CY, Cheng YW, Lee H. DDX3 loss by p53 inactivation promotes tumor malignancy via the MDM2/Slug/E-cadherin pathway and poor patient outcome in non-small-cell lung cancer. Oncogene. 2013 Apr 15. Epub ahead of print. 10.1038/onc.2013.107

Zhang Y, Yan W, Chen X. Mutant p53 disrupts MCF-10A cell polarity in three-dimensional culture via epithelial-to-mesenchymal transitions. J Biol Chem. 2011; 286:16218. [PubMed: 21454711]

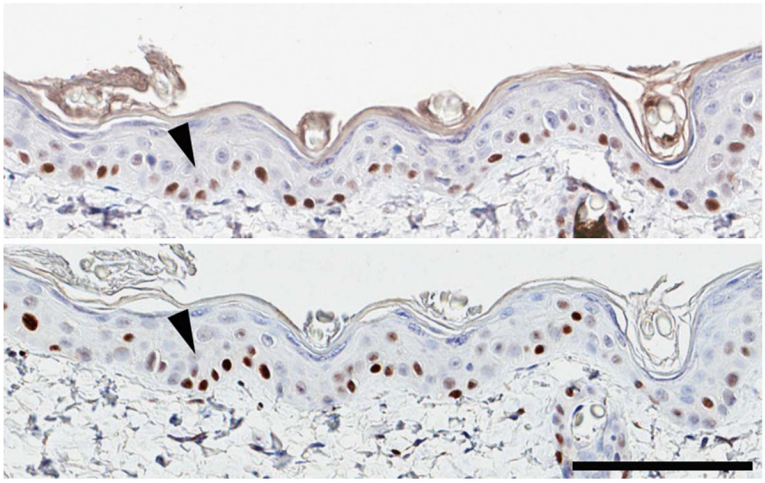

Figure 1. Slug and p53 are co-expressed in UVR-exposed skin

Wild type 129 mice were exposed to 4800 J/m^2 UVR obtained from UVB sunlamps that emitted wavelengths in the 290–340 nm range, with peak emission at 310 nm. Skin was collected 24 hours after exposure. Adjacent sections were stained for Slug (upper panel) and for p53 using the CM5 antibody (lower panel). Arrowheads indicate an area in which a number of cells are immunopositive for both Slug and p53. These findings are representative of those in several 129 mice. Scale bar = 100 μm.

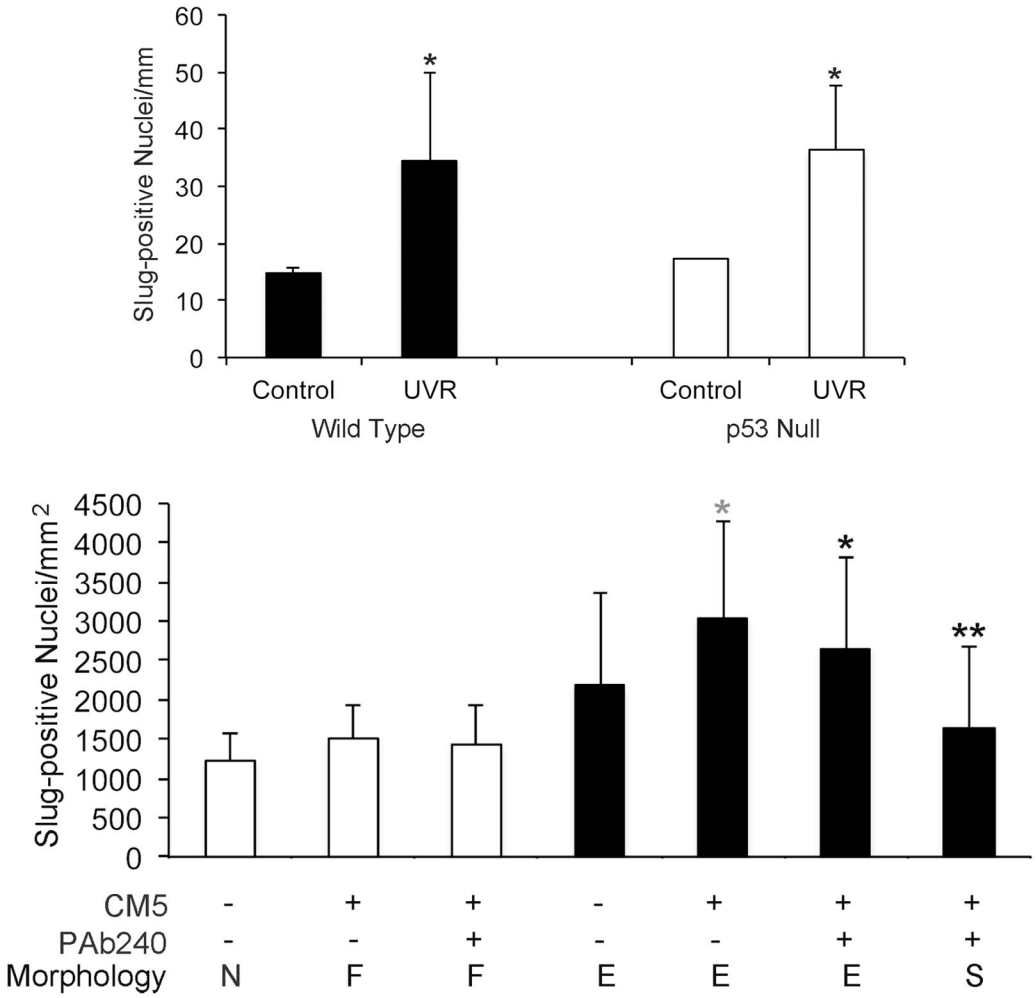

Figure 2. Slug induction by UVR is independent of p53 status

a, Wild type and p53 null mice were exposed to 2400 J/m^2 UVR; skin was collected 24 hours later. Asterisks indicate significantly elevated Slug-positive nuclei/mm in UVR-exposed versus unexposed epidermis (Mann-Whitney). **b,** For p53-positive foci, SKH-1 hairless mice were exposed to 2240 J/m^2 UVR 3 times weekly for 10 weeks, with skin harvested 20 weeks later. For skin tumors, SKH-1 mice were exposed to 2240 J/m^2 UVR 3 times weekly for 25 weeks. Morphology is indicated as N (p53-negative skin), F (preneoplastic foci), E (epithelial tumor), or S (spindle cell tumor). Asterisks indicate a significant difference in Slug-positive nuclei/mm^2 compared to p53-negative skin and preneoplastic foci (*) or to CM5-positive tumors (**) (ANOVA).