Long noncoding RNA lincRNA-p21 is the major mediator of UVB-induced and p53-dependent apoptosis in keratinocytes

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LincRNA-p21 is a long noncoding RNA and a transcriptional target of p53 and HIF-1α. LincRNA-p21 regulates gene expression in cis and trans, mRNA translation, protein stability, the Warburg effect, and p53-dependent apoptosis and cell cycle arrest in doxorubicin-treated mouse embryo fibroblasts. p53 plays a key role in the response of skin keratinocytes to UVB-induced DNA damage by inducing cell cycle arrest and apoptosis. In skin cancer development, UVB-induced mutation of p53 allows keratinocytes upon successive UVB exposures to evade apoptosis and cell cycle arrest. We hypothesized that lincRNA-p21 has a key functional role in UVB-induced apoptosis and/or cell cycle arrest in keratinocytes and loss of lincRNA-p21 function results in the evasion of apoptosis and/or cell cycle arrest. We observed that lincRNA-p21 transcripts are highly inducible by UVB in mouse and human keratinocytes in culture and in mouse skin in vivo. LincRNA-p21 is regulated at the transcriptional level in response to UVB, and the UVB induction of lincRNA-p21 in keratinocytes and in vivo in mouse epidermis is primarily through a p53-dependent pathway. Knockdown of lincRNA-p21 blocked UVB-induced apoptosis in mouse and human keratinocytes, and lincRNA-p21 was responsible for the majority of UVB-induced and p53-mediated apoptosis in keratinocytes. Knockdown of lincRNA-p21 had no effect on cell proliferation in untreated or UVB-treated keratinocytes. An early event in skin cancer is the mutation of a single p53 allele. We observed that a mutant p53R172H allele expressed in mouse epidermis (K5Cre+/tg;LSLp53+/R172H) showed a significant dominant-negative inhibitory effect on UVB-induced lincRNA-p21 transcription and apoptosis in epidermis. We conclude lincRNA-p21 is highly inducible by UVB and has a key role in triggering UVB-induced apoptotic death. We propose that the mutation of a single p53 allele provides a pro-oncogenic function early in skin cancer development through a dominant inhibitory effect on UVB-induced lincRNA-p21 expression and the subsequent evasion of UVB-induced apoptosis.

Cell Death and Disease (2015) 6, e1700; doi:10.1038/cddis.2015.67; published online 19 March 2015

The Human Genome Project revealed that only ~3% of human genome encodes protein.1 The remaining 97% of the human genome is referred to as noncoding DNA. Initially, much of the intergenic noncoding sequence was referred to as ‘junk DNA’ as it was considered to have no function. Although some intergenic sequences contain DNA elements important in gene regulation, it is now known that many intergenic sequences can be transcribed into RNA.2–5 In fact, ~85% of the human genome is transcribed into RNA.6,7 RNA transcripts that lack protein-coding function are referred to as noncoding RNAs (ncRNAs) and of these the long ncRNAs (lncRNAs; ≥200 nt) represent the majority. Most lncRNAs are transcribed from intergenic or intronic regions of the genome or overlap with or are transcribed antisense to protein-coding genes.8 LncRNAs are one of the largest and more diverse classes of cellular transcripts with over 10,000 lncRNA transcripts reported in the human genome.6,7

Only a handful of lncRNAs have been studied to date and mostly in cell culture. These lncRNAs are involved in regulating gene expression through a variety of mechanisms including epigenetic silencing, transcriptional regulation, RNA processing, RNA modification and translation.4,9 Emerging evidence indicates that IncRNAs are associated with human diseases such as cancer,10,11 Alzheimer’s12 and heart disease.13 In lung, liver, prostate and breast cancer, the expression of certain IncRNAs correlates with tumor development, progression or survival.10,11 Half of all the trait-associated SNPs identified in GWAS are located in non-coding DNA intergenic sequences, and many of the intergenic regions may function by encoding IncRNAs.14 These results point to important roles of IncRNAs in human disease. It is critical to determine whether associations of IncRNAs with specific diseases are functionally significant and to develop mouse genetic models to define and characterize the function of IncRNAs in disease in vivo. LncRNAs could represent diagnostic markers and/or possible therapeutic targets. Moreover, because the etiology of most chronic human diseases involves interactions with the environment,15 it is important to...
determine whether environmental factors can impact the expression, activity and function of lncRNAs to contribute to disease pathogenesis.

Nonmelanoma skin cancer (NMSC) is the most common cancer in the United States. The majority of NMSCs is environmentally induced and caused by solar UVB radiation which produces DNA damage and mutations. Each year, there are more cases of NMSC than all cases of breast, prostate, lung and colon cancers combined. For skin squamous cell carcinomas (SCCs), the incidence of p53 mutations ranges from 50 to 90% in both humans and mice. UVB-induced mutation of p53 allows keratinocytes upon successive UVB exposures to evade apoptosis and cell cycle arrest. These defects have a critical role in skin cancer development. Mice lacking one or both copies of p53 as well as mutant p53 mice all display increased susceptibility to UVB-induced skin cancer and greatly decreased apoptosis in response to UVB.

LincRNA-p21 is a long intergenic non-coding RNA (3100 nt). Because of lincRNA-p21’s location on chromosome 17, approximately 15 kb upstream from the Cdkn1a (p21) gene, it was named lincRNA-p21. LincRNA-p21 was first reported to be a direct transcriptional target of p53 and to mediate p53-dependent apoptosis but not cell cycle arrest in doxorubicin-treated mouse embryo fibroblasts (MEFs). Another more recent report indicates that lincRNA-p21 has no role in apoptosis and has an important role in regulating p53-dependent cell cycle arrest in doxorubicin-treated MEFs. The former report states that lincRNA-p21 regulates global gene expression in trans whereas the latter report indicates that lincRNA-p21 only regulates nearby Cdkn1a (p21) in a cis manner. Thus, there are conflicting reports on the role of lincRNA-p21 in MEFs. LincRNA-p21 can also regulate mRNA translation and protein stability. Recently, LincRNA-p21 transcripts were shown to be upregulated in livers of mice treated with the carcinogen furan and lincRNA-p21 was shown to be hypoxia-responsive and promote glycolysis and regulate the Warburg effect independent of p53.

Because of the key role of p53 in UVB-induced apoptosis, cell cycle arrest and skin cancer, we reasoned that lincRNA-p21 could have a critical functional role in UVB-induced apoptosis and/or cell cycle arrest in keratinocytes and its loss in the evasion of apoptosis and/or defective cell cycle control and the pathogenesis of skin cancer. Our results reveal that lincRNA-p21 is highly inducible by UVB through a p53-dependent mechanism and that lincRNA-p21 has a key role in triggering UVB-induced apoptosis in human and mouse keratinocytes.

**Results**

LincRNA-p21 transcripts are highly inducible by UVB in mouse and human keratinocytes in culture and in mouse skin in vivo. Treatment of Balb/MK2 mouse keratinocytes with UVB radiation resulted in increased levels of p53 protein and its target gene, p21 (Figure 1a) demonstrating that Balb/MK2 keratinocytes are responsive to UVB radiation. To determine whether UVB radiation can increase lincRNA-p21 transcript levels, Balb/MK2 keratinocytes were treated with various doses of UVB radiation. UVB treatment was an potent inducer of lincRNA-p21 transcripts producing up to a ~60-fold increase in lincRNA-p21 (Figure 1b). UVB treatment...
increased lincRNA-p21 in a dose-dependent manner (Figure 1b). Time-course studies revealed that UVB increased lincRNA-p21 levels as early as 4 h post UVB treatment with peak transcript levels occurring at ~16 h (Figure 1c). Normal human epidermal keratinocytes (NHEK) also displayed a UVB dose-dependent induction of lincRNA-p21 (Figure 1d) and a similar time course and magnitude of induction of lincRNA-p21 (~50-fold) (Figure 1e) as mouse keratinocytes. To determine whether UVB radiation was capable of inducing lincRNA-p21 in vivo in skin (epidermis), we utilized SKH-1 hairless mice and environmentally relevant doses of UVB. SKH-1 mice are an experimental model used to study the effects of UVB in skin and are relevant to UVB-induced human SCC skin cancer as the UV-induced tumors in these mice resemble, both at the morphologic and molecular levels, the UVB-induced SCC skin cancers in humans. The minimal erythema dose (MED) of UVB treatment in skin is defined as the minimal dose that produces a just-perceptible erythema (redness) at 24 h. SKH-1 mice were treated with UVB doses that correspond to 0.5 (100 mJ/cm²) and 1.0 MED (200 mJ/cm²). LincRNA-p21 was highly inducible by UVB radiation in SKH-1 mouse epidermis in vivo (Figures 1f and g). Collectively, these data demonstrate that lincRNA-p21 transcripts are highly inducible by UVB in mouse and human keratinocytes in culture and in mouse skin in vivo.

LincRNA-p21 is transcriptionally upregulated in response to UVB and is p53-dependent in keratinocytes in culture and in mouse skin in vivo. To determine whether lincRNA-p21 is regulated at the transcriptional level in response to UVB treatment, we treated Balb/MK2 keratinocytes with actinomycin D, an inhibitor of transcription, and then exposed the cells to UVB and collected the cells 12 h later. As shown in Figure 2a, actinomycin D blocked the increase in UVB-induced lincRNA-p21 transcripts indicating that lincRNA-p21 is regulated at the transcriptional level in response to UVB treatment. Next, we examined the role of p53 on the regulation of UVB-induced lincRNA-p21 expression in Balb/MK2 keratinocytes in culture. UVB-treated p53 knockdown keratinocytes (Figure 2b) were significantly impaired in their ability to induce lincRNA-p21 transcripts (Figure 2c). Next, we examined the role of p53 on the regulation of UVB-induced lincRNA-p21 expression in vivo in mouse epidermis using K5Cre⁺/⁻;p53foxfloxfoxflox mice. In this mouse model, the keratin 5 (K5) promoter directs Cre recombinase expression to the epidermis. UVB-treated K5Cre⁺/⁻;p53foxfloxfoxflox mice SKH-1 mice lacking p53 in their epidermis (Figure 2d) displayed significantly decreased levels of epidermal lincRNA-p21 transcripts compared with UVB-treated K5Cre⁺/⁻ mice (Figure 2e). Although the majority (~85%) of UVB-induced lincRNA-p21 in mouse skin in vivo occurs through a p53-dependent pathway, there also appears to be a minor role of lincRNA-p21 in keratinocyte apoptosis
p53-independent pathway involved in the UVB regulation of lincRNA-p21 transcript levels (Figures 2e). We also observed that mouse skin SCC lines that are defective in p53 signaling (MT2.5 and MT2.6) were unable to induce lincRNA-p21 in response to UVB radiation compared with mouse keratinocytes (Figure 2f). Likewise, HaCaT cells which are a spontaneously immortalized human keratinocyte cell line that contain two alleles of mutant p53 also displayed significantly impaired UVB induction of lincRNA-p21 when compared with similarly treated NHEKs (Figure 2g). Collectively, these data demonstrate that lincRNA-p21 is regulated at the transcriptional level in response to UVB treatment and that the UVB induction of lincRNA-p21 in keratinocytes and in vivo in mouse epidermis is dependent upon p53.

**LincRNA-p21 is a critical regulator of apoptosis in UVB-treated mouse and human keratinocytes.** To begin to determine the functional role of lincRNA-p21 in UVB-treated keratinocytes, we used an siRNA approach to knockdown lincRNA-p21. As shown in Figure 3a, we were able to knockdown lincRNA-p21 transcript levels in UVB-treated mouse keratinocytes by >80% using two different siRNA sequences. Knockdown of lincRNA-p21 had no effect on cell viability in untreated keratinocytes (Figure 3b); however, UVB-treated lincRNA-p21-depleted keratinocytes displayed an increased viability (Figure 3c). These results suggest that in UVB-treated keratinocytes, lincRNA-p21 functions to inhibit keratinocyte cell cycle progression and/or to induce keratinocyte apoptosis in response to UVB. Earlier studies showed that lincRNA-p21 has an important role in regulating p53-dependent cell cycle arrest involving p21 in doxorubicin-treated MEFs. Therefore, we first examined the levels of p53 and p21 in control and lincRNA-p21-depleted keratinocytes and observed that the levels of p53 and p21 were not affected by the knockdown of lincRNA-p21 (Figure 3d). These results indicate that the knockdown of lincRNA-p21 does not interfere with p53 protein levels or the regulation of p21 in response to UVB. In accord with these results, the knockdown of lincRNA-p21 had no effect on the cell cycle distribution and the DNA damage checkpoint as determined by FACS analysis (Figures 3e and f). In contrast to the lack of effect on cell proliferation and DNA damage checkpoint function, lincRNA-p21-depleted keratinocytes displayed striking decreases in UVB-induced apoptosis as determined by decreased cleaved caspase-3 levels (Figure 4a) and decreased annexin-V staining (Figure 4b). On the basis of annexin V single positive staining, there was a ~10-fold decrease in early apoptotic cells in lincRNA-p21-depleted keratinocytes at 18 h post UVB (Figure 4b). Similar results were obtained with a second lincRNA-p21 siRNA sequence (Figure 4c). These data demonstrate that lincRNA-p21 is a critical regulator of UVB-induced apoptosis and regulates at least 75% of the apoptosis induced by UVB in mouse keratinocytes (Figures 4b and c). Knockdown of lincRNA-p21 resulted in altered expression of several genes associated with apoptosis in response to UVB radiation indicating that lincRNA-p21 can both repress the expression of anti-apoptotic and activate the expression of pro-apoptotic genes in response to UVB (Figure 4d). The levels of Cdkn1a mRNA
were not decreased by knockdown of lincRNA-p21 in UVB-treated keratinocytes; in fact, there was an unexpected ~ two fold increase. Additionally, the protein levels of the pro-apoptotic factors Noxa and Bax were significantly reduced in lincRNA-p21 knockdown cells following UVB exposure and despite changes in Stat3 mRNA, no changes in Stat3 protein were observed (Figure 4e). Next, we examined the role of lincRNA-p21 in UVB-induced apoptosis in human keratinocytes. The knockdown of human lincRNA-p21 in NHEKs (Figure 4f) resulted in decreased apoptosis as determined by annexin V staining (Figure 4g). Thus, lincRNA-p21 is a key mediator of UVB-induced apoptosis in human keratinocytes and is responsible for ~70% of UVB-induced apoptosis (Figure 4g). Knockdown of lincRNA-p21 in NHEKs resulted in the altered expression of apoptosis-related genes in response to UVB; lincRNA-p21 represses the expression of anti-apoptotic genes and activates the expression of pro-apoptotic genes in response to UVB and had no effect on...
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Role of lincRNA-p21 in keratinocyte apoptosis

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**CDKN1A** mRNA levels (Figure 4h). These data reveal that lincRNA-p21 has a major and critical role in UVB-induced apoptotic death in human and mouse keratinocytes.

Mutant p53<sup>+/R172H</sup> allele displays a significant dominant-negative inhibitory effect on UVB-induced lincRNA-p21 transcription and apoptosis in epidermis. Because the development of UVB-induced skin cancer often entails early oncogenic events involving the mutation of a single p53 allele and the escape from UVB-induced apoptosis, we examined whether a dominant inhibitory mutation in a single p53 allele (p53<sup>+/R172H</sup>) could greatly impair UVB-induced lincRNA-p21 transcript levels and apoptosis when compared with loss-of-function mutation involving deletion of a single p53 allele (p53<sup>−/−</sup>). Previous studies have shown that missense mutants of p53 in the heterozygous state can function as dominant-negative inhibitors of certain tumor-suppressive functions and retain normal wild-type p53 of others. For example, in p53<sup>+/R172H</sup> cells, the mutant p53 does not have a dominant-negative effect on DNA damage-induced G1 arrest or p21 gene expression but does have a dominant-negative effect on gamma radiation-induced apoptosis. Therefore, we generated two genetically engineered mouse models (K5Cre<sup>+/tg</sup>;p53<sup>+/flox</sup> and K5Cre<sup>+/tg</sup>;LSLp53<sup>+/R172H</sup>) to test whether the mutation of a single p53 allele (p53<sup>+/R172H</sup>) as opposed to the loss of a single p53 allele (p53<sup>−/−</sup>), both expressed under normal physiological control in vivo in epidermis of SKH-1 mice, will have a dominant-negative effect on UVB-induced lincRNA-p21 expression and keratinocyte apoptosis. In this mouse model, the keratin 5 (K5) promoter directs Cre recombinase expression to the keratinocytes during the development of skin cancer. We have identified lincRNA-p21 as the key mediator of UVB-induced apoptosis in human and mouse keratinocytes.

Several lncRNAs have been shown to be regulated by p53 or to modulate apoptosis in response to DNA damage, for example, Pint<sup>41</sup> and Panda<sup>42</sup> have anti-apoptotic activity, whereas INXS<sup>43</sup>, MEG3<sup>44</sup>, lincRNA-p21<sup>28</sup> and Gas5<sup>45</sup> all have pro-apoptotic activity. It is generally considered that p53-regulated lncRNAs serve to fine tune p53-regulated apoptosis and/or cell cycle arrest and function as low copy number lncRNAs. Our results indicate that lincRNA-p21 does much more than fine tune the p53 response in UVB-treated keratinocytes as lincRNA-p21 is responsible for the majority of UVB-induced apoptosis in both human and mouse keratinocytes.

In sun-exposed areas of the human skin, p53 plays an important role in skin cancer prevention through its regulation of keratinocyte cell cycle arrest and apoptosis in response to UVB radiation-induced DNA damage. These p53-regulated responses serve to prevent UVB-induced mutagenesis by producing cell cycle arrest to allow time for DNA repair and by triggering apoptotic death of DNA-damaged keratinocytes. We have identified lincRNA-p21 as the key mediator of UVB-induced apoptosis in human and mouse keratinocytes. Our results demonstrate that p53 plays a key role in regulating lincRNA-p21 transcript levels in response to UVB in keratinocytes and that lincRNA-p21 has a critical function in triggering apoptosis in UVB-treated keratinocytes. In fact, lincRNA-p21 is responsible for the majority of UVB-induced apoptosis in both human and mouse keratinocytes.

**Discussion**

In sun-exposed areas of the human skin, p53 plays an important role in skin cancer prevention through its regulation of keratinocyte cell cycle arrest and apoptosis in response to UVB radiation-induced DNA damage. These p53-regulated responses serve to prevent UVB-induced mutagenesis by producing cell cycle arrest to allow time for DNA repair and by triggering apoptotic death of DNA-damaged keratinocytes. We have identified lincRNA-p21 as the key mediator of UVB-induced apoptosis in human and mouse keratinocytes. Our results demonstrate that p53 plays a key role in regulating lincRNA-p21 transcript levels in response to UVB in keratinocytes and that lincRNA-p21 has a critical function in triggering apoptosis in UVB-treated keratinocytes. In fact, lincRNA-p21 is responsible for the majority of UVB-induced apoptosis in both human and mouse keratinocytes.

**Figure 5**

Mutation of single p53 allele has a dominant-negative effect in vivo in mouse epidermis. (a) K5Cre<sup>+/tg</sup>, K5Cre<sup>+/tg</sup>;LSLp53<sup>+/R172H</sup>, K5Cre<sup>+/tg</sup>;p53<sup>−/−</sup> and K5Cre<sup>−/−</sup>;p53<sup>−/−</sup> SKH-1 mice were treated with 200 mJ/cm<sup>2</sup> UVB and epidermal lincRNA-p21 transcripts measured 9 h post UVB treatment. (b) K5Cre<sup>+/tg</sup>, K5Cre<sup>+/tg</sup>;LSLp53<sup>+/R172H</sup>, K5Cre<sup>−/−</sup>;p53<sup>−/−</sup> and K5Cre<sup>−/−</sup>;p53<sup>−/−</sup> SKH-1 mice were treated with 100 mJ/cm<sup>2</sup> UVB and the number of apoptotic interfollicular basal epidermal keratinocytes/cm skin were scored at 9 h post UVB treatment. Data are expressed as the mean ± S.D. *P < 0.05 significantly different compared with UVB-treated K5Cre mice as determined by the student
in mouse epidermis is primarily through a p53-dependent pathway. We observed that UVB is an extremely potent inducer of lincRNA-p21, producing ~50–60-fold increase in mouse and human keratinocytes. Thus, lincRNA-p21 transcript levels rise from low copy number of transcripts to very significant transcript levels in response to UVB to trigger apoptotic keratinocyte death. Evaluation of candidate gene expression in UVB-treated keratinocytes revealed that lincRNA-p21 represses the expression of anti-apoptotic genes Mcl1, Stat3 and Atf2 and activates the expression of pro-apoptotic genes Noxa and Bax. Future studies are required to understand how lincRNA-p21 is repressing and activating gene expression in keratinocytes in response to UVB treatment.

As mentioned in the 'Introduction', there are conflicting reports on the functional role for lincRNA-p21 in MEFS. Huarte et al.28 reported that lincRNA-21 functions as the global trans regulator of gene expression, has no effect on p21 expression and mediates p53-dependent apoptosis but not cell cycle arrest in doxorubicin-treated MEFS. On the other hand, Dimitrova et al.29 report lincRNA-p21 does not regulate apoptosis but regulates p53-mediated cell cycle arrest through the regulation of p21 in cis in doxorubicin-treated MEFS and that lincRNA-p21 indirectly regulates genes associated the Polycomb Repressive Complex 2 through a p21-dependent mechanism. A major technical difference between these studies is that Huarte et al.28 used an RNAi knockdown approach and Dimitrova et al.29 employed knockout MEFS. Dimitrova et al. suggested that this difference in depleting lincRNA-p21 could be responsible for the different cellular outcomes. Our results agree with the findings of Huarte et al.28 and like their study, our studies utilized an RNAi knockdown approach. We found lincRNA-p21 has a key role in regulating UVB- and p53-mediated apoptosis in both human and mouse keratinocytes using three different RNAs. These results argue against off-target effects and further studies in lincRNA-p21 knockout keratinocytes will be required to address method of depletions and whether lincRNA-p21 functions in cis or trans to regulate gene expression in UVB-treated keratinocytes.

Depletion of lincRNA-p21 allows keratinocytes to evade UVB-induced apoptosis suggesting a possible tumor suppressor function for lincRNA-p21 in NMSC. In skin cancer, the incidence of p53 mutations ranges from 50 to 90% in humans and mice.17,18 Mutant p53 provides keratinocytes an advantage over normal keratinocytes in response to successive UVB exposure by evading cell cycle checkpoints and by allowing mutant keratinocytes to evade UVB-induced apoptosis.17,19–23,40 These events promote genomic instability, clonal expansion and the development of skin cancer. In early skin cancer development, a single p53 allele is mutated or deleted as an early initiating oncogenic event.17 Patch(es) of clones of mutant p53 keratinocytes (considered to be the precursor lesion to skin cancer) can be detected in sun-exposed areas of human skin and in UVB-treated mouse skin long before tumors form.17,46,47 Most of these clones have a single mutant allele of p53 (97% missense and 3% nonsense).17 Collectively, these studies indicate that mutation of a single p53 allele provides a pro-oncogenic function/advantage early in skin cancer development. Our results demonstrate a potent dominant-negative inhibitory effect of a single mutant p53 allele (p53<sup>±R172H</sup>) on UVB-induced lincRNA-p21 transcript levels and UVB-induced apoptosis. We propose that the mutation of a single p53 allele provides a pro-oncogenic function early in skin cancer development through a dominant inhibitory effect on UVB-induced lincRNA-p21 expression and the subsequent evasion of UVB-induced apoptosis.

The etiology of most chronic human diseases involves complex interactions among environmental factors and an individual’s genetic and epigenetic makeup. However, these gene × environment interactions are poorly understood, leading to a deficit in our understanding of how these interactions contribute to adverse health outcomes. Our study demonstrates for the first time that exposure to an environmental factor, in this case solar UVB radiation, can impact the expression, activity and function of a lncRNA. Moreover, lincRNA-p21 expression is induced in vivo in mouse skin by environmentally relevant doses of UVB. We speculate that lincRNA-p21 may function as a tumor suppressor gene in UVB-induced non-melanoma skin cancer where the loss of lincRNA-p21 expression results in the evasion of apoptosis.

Materials and Methods

**Cells and mice.** Bab/mk2 keratinocytes were a gift from B. E. Weissman (UNC)46 are maintained in Ca<sup>2+</sup>-free EMEM (Lonza, Walkersville, MD, USA), 8% Chex-lax-treated FBS (F2442, Sigma Aldrich, St. Louis, MO, USA), 4 ng/ml HEGF (Life Technologies, Carlsbad, CA, USA) and 0.05 mM CuCl<sub>2</sub> NHEKs were purchased from Lonza and maintained in KGM-Gold keratinocyte medium (Lonza). 1295-p53LSL.R172H (LSLp53<sup>+/R172H</sup>) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA),<sup>38</sup> FVB129-Tp53<sup>129M18B</sup>/p53<sup>fox/fox</sup>) were obtained through the NCi Mouse Repository.<sup>39</sup> To generate SKH-1 mice, LSLp53<sup>+/R172H</sup> and p53<sup>fox/fox</sup> male mice were mated to SKH-1 (Charles River Labs, Wilmington, MA, USA) females. Male LSLp53<sup>+/R172H</sup> and p53<sup>fox/fox</sup> males were backcrossed to SKH-1 females five times to obtain SKH-1 LSLp53<sup>+/R172H</sup> and p53<sup>fox/fox</sup> SKH-1 mice. KSCre, KSCre<sup>LSLp53<sup>+/R172H</sup></sup>, KSCre<sup>p53<sup>+/R172H</sup></sup> and KSCre<sup>p53<sup>fox/novo</sup></sup> littermates that were used in this study were obtained by crossing LSLp53<sup>+/R172H</sup> and p53<sup>fox/fox</sup> SKH-1 female mice with KSCre<sup>LSLp53<sup>+/R172H</sup></sup> and KSCre<sup>p53<sup>+/R172H</sup></sup> male mice. KSCre mice were a gift from Angel Ramirez and Jose Jorcano<sup>34</sup> and detailed information on control (K5Cre<sup>+/tg</sup>) SKH-1 mice have been described.45 All aspects of animal care and experimentation described in this study were conducted according to the NIH guidelines and approved by the Institutional Animal Care and Use Committee of NCSU.

**UVB treatment and chemicals.** Bab/mk2 keratinocytes were exposed at less than 50% confluence to 10 mJ/cm<sup>2</sup> UVB with a calibrated UVB lamp as previously described.<sup>49</sup> SKH-1 mice were treated with a single dose of 100 or 200 mJ/cm<sup>2</sup> UVB as described.<sup>50</sup>
Antibodies. Antibodies against p21 (sc-471) and Bax (sc-493) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Noxa antibody (ab13654) was purchased from Abcam (Cambridge, UK). p35 antibody (1C12) was purchased from Cell Signaling (Danvers, MA, USA). β-Actin antibody (A5441) was purchased from Sigma Aldrich. Caspase-3 antibody (CS-9665S) and cleaved caspase-3 (Cleaved Caspase-3 (CS-9661S)) were purchased from Cell Signaling and generously provided by Dr. Jun Tsujii (NCSU).

RNA and quantitative PCR. Total RNA was isolated using QiZOL lysis (Qiagen, Valencia, CA, USA), and further purified using Qiagen RNeasy columns. cDNA was prepared from RNA by ImProm-II Reverse Transcription System (Promega, Madison, WI, USA). Quantitative PCR for mouse LincRNA-p21 (FAM probe TGGCGCAACACTGGTG, forward primer GAGGCTCTTCTGGTAGACTAAAA, reverse primer CCACCAGGTAGAAACTACGAAA) and human LincRNA-p21 (FAM probe ATGGGCTCTTCAGG, forward primer CCCGGGCTGGTCTTIGTTGTT, reverse primer GATGTGCTGCTACTCTTCG) was performed using Custom TaqMAN Gene Expression Assays (Life Technologies). Additional mouse TaqMAN RT-PCR assays include p53 Mm441964_g1, Gadd45a Mm00083380_g1, Survivin Mm05997947_m1, Mcl1 Mm00725832_s1, Bax Mm00432051_s1, Puma Mm00833804_m1, Mmp7 Mm00725398_m1, Atf2 Mm00833804_m1, Nt5e Mm00725398_m1, Noxa Mm00451763_m1, Atf2 Mm0019268_m1, Cdk1a Mm00432448_m1 and Stat3 Mm01219775_m1. Additional human TaqMAN RT-PCR assays include GAPDH Hs03929097_g1, β-Actin Hs00607939_s1, Noxa Mm00432051_m1, β-Actin Mm00607939_s1, Noxa Mm00432051_m1, Atf2 Mm00833804_m1, Survivin Mm05997947_m1, Mcl1 Mm00725832_s1, Bax Mm00432051_m1, Puma Mm00519268_m1, Cdk1a Mm00432448_m1 and Stat3 Mm01219775_m1. Additional human TaqMAN RT-PCR assays include GAPDH Hs03929097_g1, β-Actin Hs00607939_s1, Noxa Mm00432051_m1, β-Actin Mm00607939_s1, Noxa Mm00432051_m1, Atf2 Hs01095345_m1, Cdk1a Mm00355782_m1 and Stat3 Hs01047580_m1 (Life Technologies). TaqMan Gene Expression Assays were used in combination with FastStart Universal Probe Master Mix (Roche). Data were analyzed using the comparative ΔΔCt method.

Detection of apoptotic keratinocytes. H&E-stained mouse skin sections were used to quantify the presence of apoptotic keratinocytes. As previously described, apoptotic keratinocytes in the interfollicular basal epidermis were scored positive if all three of the following criteria were present: dark pyknotic nuclei, cytoplasmic eosinophilia and absence of cellular contacts.23 Data are presented as the average number of apoptotic basal interfollicular epidermal keratinocytes/cm skin.

BrdU staining. Cells were pulsed-labeled with 10 μM BrdU for 1 h before harvest. Collected cells were washed twice with ice-cold PBS and then fixed by the addition of ice-cold 75% ethanol. Staining with anti-BrdU FITC was performed as previously described.17 Cells were analyzed by flow cytometry at the NCSU Flow Cytometry and Cell Sorting Laboratory.

Annexin-V staining. Harvested cells were washed twice with ice-cold PBS and then resuspended in Annexin V Binding Buffer (BioLegend, San Diego, CA, USA) at a concentration of 1 × 106 cells/ml. Five microliters of Annexin V Pacific Blue was added to 100 μl of the cell suspension, followed by the addition of 10 μl propidium iodide (PI) solution (BioLegend). Cells were incubated for 15 min at room temperature in the dark, followed by the addition of 400 μl Annexin V Binding Buffer. Cells were analyzed by flow cytometry at the NCSU Flow Cytometry and Cell Sorting Laboratory. Data were collected and presented on a scatter plot with Annexin V Pacific Blue intensity on the x-axis and PI intensity on the y-axis.

Statistical analysis. Differences between groups were evaluated by two-sided t-tests for paired data with the significance level set to P < 0.05.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements. This research was supported by grants from the National Institute of Environmental Health Sciences (ES017734 to JRH, ES12473 awarded to RCS, and ZUM was supported by training grant ES007046). Additional support for this research was provided by a NC State University Center for Human Health and Environment pilot project grant awarded to JRH and RCS.

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