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Inverse relationship between p53 and phospho-Chk1 (Ser317) protein expression in UVB-induced skin tumors in SKH-1 mice

Jamie J. Bernard¹, You-Rong Lou¹, Qing-Yun Peng, Tao Li, Allan H. Conney, and Yao-Ping Lu*

Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, United States

Abstract

Immunohistochemical evaluation of serial stored paraffin sections from 42 keratoacanthomas and 11 squamous cell carcinomas demonstrated that skin tumors from UVB-exposed mice showed an inverse relationship (>95%) between p53 protein expression and phospho-Chk1 (Ser317), but not phospho-Chk1 (Ser345) protein expression. Tumors expressing high levels and large areas of p53 protein had no detectable phospho-Chk1 (Ser317), whereas tumors expressing high levels and large areas of phospho-Chk1 (Ser317) protein had no detectable p53. Squamous cell carcinomas that demonstrated heterogeneous p53 and phospho-Chk1 (Ser317) protein expression within the same tumor showed that areas expressing p53 were negative for phospho-Chk1 (Ser317) immunostaining while areas expressing phospho-Chk1 (Ser317) were negative for p53. Similar patterns were observed for keratoacanthomas. These findings were also observed in epidermal areas distant from tumors that demonstrated no detectable phospho-Chk1 (Ser317), but appreciable p53 protein in the basal layer. Tumors from congenic hairless p53 knockout mice had elevated levels of phospho-Chk1 (Ser317) compared to tumors from p53 wild-type SKH-1 controls. After a single exposure to UVB, normal epidermal cells from a p53 knockout mouse expressed a relatively high level of phospho-Chk1 (Ser317) where as epidermal cells from a p53 wild-type littermate induced p53 protein and expressed a relatively low level of phospho-Chk1 (Ser317). These data illustrate the dynamic regulation of checkpoint function, suggesting that phosphorylation of Chk1 on Serine 317 is regulated by p53 status and that p53 may act as a molecular on/off switch for phosphorylation at this site.

Keywords

Skin; Tumor; p53; Phospho-chk1; UVB; Cancer

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*Corresponding author at: Susan Lehman Cullman Laboratory for Cancer, Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020, United States. Fax: 732 445 0687. yaoping.lu@pharmacy.rutgers.edu (Y.-P. Lu).

¹Co-first authors.

Conflict of interest statement

The authors declare no conflict of interest.

Introduction

Sunlight-induced nonmelanoma cancer is the most common form of human cancer with upwards of 2 million new cases diagnosed per year in the United States (Kripke, 1986; Rogers et al., 2010). UVB-induced DNA damage activates the ATR signaling pathway leading to elevated p53 and phospho-Chk1 (Ser317) and cell cycle arrest, thereby allowing time for DNA repair. However, continued UVB exposure increases the frequency of p53 mutant clones in skin, which can lead to the selective loss of the G1 checkpoint pathway, thereby sensitizing the cells to UV-damage and enhancing carcinogenesis.

Recent mechanistic studies from our laboratory showed that caffeine inhibited the ATR/Chk1 pathway, increased the number of apoptotic cells and reduced tumor formation in UVB-exposed epidermis (Huang et al., 1997; Lu et al., 2000). Continuous treatment of mice with topical caffeine during an UVB-induced carcinogenesis study significantly inhibited tumor formation, diminished phospho-Chk1 (Ser317) immunostaining and increased the number of mitotic cells expressing both cyclin B1 and caspase 3 in tumors (Lu et al., 2011). These results suggested that caffeine induced apoptosis in tumors by inhibiting the ATR/Chk1 pathway and by promoting lethal mitosis. In other studies, we found that a single irradiation with UVB in p53 knockout mice markedly decreased the number of mitotic cells with cyclin B1 and sensitized these mice to caffeine-induced lethal mitosis by several-fold (Lou et al., 2010), leading to the hypothesis that p53 plays a role in the ATR/Chk1 pathway (Lou et al., 2010; Lu et al., 2011). In the present study, we used the stored paraffin sections from UVB-induced skin tumors as described in Lu, Y.P. et al. *Cancer Prev Res* 4:1118–1125, 2011 (Lu et al., 2011) to evaluate the relationship between p53 and phospho-Chk1 (Ser317). The phospho-Chk1 (Ser317) staining was previously published (Lu et al., 2011).

Materials and methods

Chemicals and animals

Female SKH-1 hairless mice (6–7 weeks old) were purchased from the Charles River Breeding Laboratories (Kingston, NY) and the animals were maintained in our animal facility for at least 1 week before use. Congenic hairless p53 knockout mice were bred in our animal facility as previously described (Lu et al., 2004). Mice were given water and Purina Laboratory Chow 5001 diet from Ralston-Purina ad libitum, and maintained on a 12-h light/12-h dark cycle.

UVB irradiation

The UV lamps used (FS72T12-UVB-HO; National Biological) emitted UVB (280–320 nm; 75%–80% of total energy) and UVA (320–375 nm; 20%–25% of total energy). There was little or no radiation below 280 nm or above 375 nm. The dose of UVB was quantified with a UVB Spectra 305 dosimeter (Daavlin). The radiation was further calibrated with a model IL-1700 research radiometer/photometer (International Light).

Treatment of mice with UVB and preparation of skin sections

All histopathology examinations and immunohistochemical determinations were made using the stored paraffin blocks from a previous study (Lu et al., 2011). Briefly, mice were irradiated with UVB (30 mJ/cm²) twice a week for 20 weeks, and UVB treatment was stopped. After 20 weeks of UVB irradiation, these mice showed no tumor formation, but will develop skin tumors over the next several months. Mice were sacrificed 21 weeks after the last UVB treatment and dorsal skins both with and without tumors were removed and stapled flat to a plastic sheet and placed in 10% phosphate-buffered formalin at 4 °C for 24 h. Skin samples were then dehydrated in ascending concentrations of ethanol (80%, 95%, and 100%), cleared in xylene, and embedded in Paraplast (Oxford Labware). Four-micrometer serial sections of skin were made, deparaffinized, rehydrated with water, and used for regular hematoxylin–eosin staining or immunohistochemical staining. The characterization of tumors was done as described previously (Lu et al., 2002). Unless otherwise specified, all immunohistochemical determinations were made with 400× magnification with a light microscope.

p53 and phospho-Chk1 (Ser317) immunostaining

Polyclonal rabbit NCL-p53-CM5p antibody purchased from Novocastra Laboratories Ltd. (Newcastle upon Tyne, United Kingdom) reacts with mouse wild type or mutated p53 proteins (Berg et al., 1996; Lu et al., 1997; Midgley et al., 1995). Skin sections were stained by the Biotin-Streptavidin Amplified System (alkaline phosphatase-conjugated streptavidin) using StrAviGen Super Sensitive Universal immunostaining kit purchased from Biogenex (San Ramon, CA) with some modifications. Antigen retrieval was carried out using 0.01 M sodium citrate buffer (pH 6.0) for p53 staining. The sections were incubated with p53 antibody (1:500 dilution) for 1 h at room temperature. The samples were then incubated with a biotinylated anti-rabbit secondary antibody for 5 min at 37 °C, followed by incubation with conjugated streptavidin solution for 5 min at 37 °C. Color development was achieved by incubation with New Fuchsin Substrate Pack (containing 0.6 mg/ml levamisole solution) for 20 min at room temperature. The slides were then counterstained with hematoxylin and dehydrated, and coverslips were added for permanent mounting. A positive reaction was shown as a pink to red precipitate in the nuclei of the cells.

Skin sections used for the measurement of phospho-Chk1 (Ser317) were stained by the horseradish peroxidase-conjugated avidin method (Lu et al., 2011). Briefly, endogenous peroxidase was blocked by incubating the tissue sections in 3% hydrogen peroxide in methanol for 30 min at room temperature. Sections were then treated with 0.01 mol/L sodium citrate buffer (pH 6.0) in a microwave oven at a high setting for 10 min. The sections were incubated with a protein block (normal goat serum) for 10 min, followed by avidin D for 15 min and biotin blocking solution for 15 min (Avidin-Biotin blocking kit from Vector Laboratory) at room temperature. Sections were incubated with phospho-Chk1 (Ser317) primary antibody (Cell Signaling Technology Inc.) for 30 min (1:50 dilution) at room temperature followed by incubation with a biotinylated anti-rabbit secondary antibody for 30 min and incubation with conjugated avidin solution (ABC Elite Kit purchased from Vector Laboratory) for 30 min. Color development was achieved by incubation with 0.02% 3,3'-diaminobenzidine tetrahydrochloride containing 0.02% hydrogen peroxide for 10 min at

room temperature. The sections were then counterstained with hematoxylin, dehydrated, and coverslips were added for permanent mounting. A positive reaction is shown as a light brown to dark brown precipitate in the cytoplasmic and/or perinuclear portion of the cells.

Western blot analysis

Dorsal skin samples were removed and immediately placed in a buffer solution containing 75 mmol/L dibasic sodium phosphate and monobasic potassium phosphate (pH 7.7) at 52 °C for 20 s. The samples were submerged immediately in an ice bath containing the same buffer for 1 min. The epidermis was scraped from the dermis and placed in 1.2 mL of lysis buffer containing 20 mmol/L Tris-HCl, 150 mmol/L NaCl, 1 mmol/L Na₂EDTA, 1 mmol/L EGTA, 1% Triton, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na₃VO₄, 1 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride (Cell Signaling Technology, Inc.). Epidermal tissue (100 mg) was sonicated in 1.2 mL lysis buffer (Cell Signaling Technology, Danvers, MA) supplemented with phenylmethanesulfonylfluoride and incubated on a rotator for 30 min at 4 °C. Cells were incubated with lysis buffer supplemented with phenylmethanesulfonylfluoride on ice. Samples were clarified by centrifugation at 22,000 ×g for 5 min at 4 °C. Lysates containing equal amounts of total protein (20 μg) were separated in Bio-Rad mini Protean gels (Hercules, CA). Gels were run and transferred onto polyvinylidene difluoride transfer membranes (Millipore, Bedford, MA). Membranes were blocked with the Odyssey infrared imaging system blocking buffer (LI-COR, Lincoln, NE) probed with primary antibodies to phospho-Chk1 (Ser317) (NB100-92499 purchased from Novus Biologicals, Littleton, CO) or Phospho-p53 (Ser15) (NB100-1913 purchased from Novus Biologicals) overnight at 4 °C. Membranes were washed and incubated with goat anti-rabbit IRDye 800 (Rockland Immunochemicals, Gilbertsville, PA) for 30 min at room temperature. Membranes were washed and fluorescence was detected using the Odyssey infrared imaging system (LICOR).

Results

Inverse relationship between the levels of p53 and phospho-Chk1 (Ser317) in tumors and areas away from tumors in UVB-pretreated mice

In the present study, mice were irradiated with 30 mJ/cm² UVB twice a week for 20 weeks and the UVB-treatment was stopped. Twenty-one weeks later, mice were sacrificed and tumors were classified and stained for phospho-Chk1 (Ser317) and p53. Unexpectedly, we observed an inverse relationship between expression levels of these two proteins in all tumors examined. Tumors expressing high levels and large areas of p53 protein had no detectable phospho-Chk1 (Ser317), whereas tumors expressing high levels and large areas of phospho-Chk1 (Ser317) protein had no detectable p53. A representative keratoacanthoma that showed appreciable p53 was negative for phospho-Chk1 (Ser317) immunostaining (Fig. 1A). A representative squamous cell carcinoma that showed focal islands of phospho-Chk1 (Ser317) was negative for p53 immunostaining (Fig. 1B). Similar results were observed with additional tumors.

It has been well-established that mutations and the accumulation of p53 are common changes associated with UVB-induced development of malignancy (Brash et al., 1991;

Moles et al., 1993; Somers et al., 1992; Ziegler et al., 1993). In accordance with previous studies reporting the accumulation of p53 mutations in UVB-induced tumors, several UVB-induced squamous cell carcinomas demonstrated heterogeneous p53 and phospho-Chk1 (Ser317) protein expression within the same tumor. Areas within the tumor expressing p53 were negative for phospho-Chk1 (Ser317) immunostaining and areas expressing phospho-Chk1 (Ser317) were negative for p53 (Fig. 1C). Similar inverse patterns of expression were observed for UVB-induced keratoacanthomas. A representative keratoacanthoma examined showed that areas expressing p53 were negative for phospho-Chk1 (Ser317) immunostaining and areas expressing phospho-Chk1 (Ser317) were negative for p53 (Fig. 1D). This inverse expression between p53 and phospho-Chk1 (Ser317) was also observed in tumors that showed mixtures of single cells either positive or negative for p53 (Fig. 1E). The carcinoma pictured in Fig. 1E showed cells positive for p53 and negative for phospho-Chk1 (Ser317) immunostaining directly adjacent to cells negative for p53 and positive for phospho-Chk1 (Ser317). Normal appearing epithelium distant from tumors demonstrated no detectable phospho-Chk1 (Ser317), but appreciable p53 protein present in the basal layer (Fig. 1F). While phospho-Chk1 (Ser345) protein was analyzed (not shown), this inverse relationship with p53 was exclusive for phospho-Chk1 (Ser317).

Congenetic hairless p53 knockout mice have elevated levels of phospho-Chk1 (Ser317)

Phospho-Chk1 (Ser317) and p53 in UVB-induced tumors from congenic hairless SKH-1 p53 knockout mice and SKH-1 controls were subsequently examined by Western blotting using an antibody that recognizes phospho-p53 at serine 15 (wild-type p53). Tumors from SKH-1 controls had either no detectable p53 or no detectable phospho-Chk1 (Ser317) (Fig. 2A, lane 1), detectable p53 and detectable phospho-Chk1 (Ser317) (Fig. 2A, lane 2), or detectable p53 and no detectable phospho-Chk1 (Ser317) (Fig. 2A, lane 3). As expected, the inverse protein expression between p53 and phospho-Chk1 (Ser317) could not be observed by Western blotting tumors expressing both proteins since tumors have differential and aberrant p53. However, phospho-Chk1 (Ser317) protein was induced in the epidermal tumors of a p53 knockout mouse compared with that of SKH-1 controls (Fig. 2A). Next, we determined the effect of a single exposure of UVB on phospho-Chk1 (Ser317) protein expression in the normal epidermis of p53 knockout mice and their wild-type littermates. Mice were sacrificed 16 h after a single dose of UVB (180 mJ/cm²), which is the peak time for UVB-induced p53 expression (Lu et al., 2000). Irradiation with UVB increased the level of phospho-Chk1 (Ser317) in the WT mice, but to a lesser extent than in the p53 knockout mice (Fig. 2B). The presence of p53 was associated with reduced phospho-Chk1 (Ser317) induction in tumors or in UVB-exposed epidermis, suggesting that p53 may down-regulate ATR activation of Chk1. Collectively, the Western blot data support the immunocytochemical evidence that phospho-Chk1 (Ser317) levels are inversely correlated with the level of p53 protein.

Discussion

This study provides the first evidence of an inverse relationship between p53 and phospho-Chk1 (Ser317) protein expression. We began these studies to investigate the mechanism by which caffeine protected against UVB-induced tumor formation (Lu et al., 2002; Lu et al.,

2004). The topical application of caffeine immediately after UVB exposure increased the number of mitotic epidermal cells with cyclin B1 and increased apoptosis compared with untreated controls (Lou et al., 2010). Interestingly, these effects of caffeine in p53 knockout mice were substantially greater than in their p53 wild-type littermates, suggesting that modest doses of caffeine in vivo could selectively sensitize p53-deficient cells to apoptosis after UVB (Lou et al., 2010). Furthermore, we demonstrated that caffeine stimulated apoptosis in p53-deficient cells by inhibiting the phosphorylation of Chk1 on Serine 317, attenuating G2/M cell cycle arrest and pushing the cells to proceed into mitosis prematurely (Lu et al., 2011). These data suggested that caffeine stimulated apoptosis in DNA damaged cells by inhibiting ATR activation through Chk1 inhibition and stimulating mitotic catastrophe. Therefore, we next wanted to investigate the role of p53 in caffeine-induced apoptosis. We hypothesized that elevated levels of phospho-Chk1 (Ser317) in response to UVB-exposure sensitized p53 knockout mice to caffeine-induced cell death. Interestingly, during our investigations, we unexpectedly found an inverse relationship between p53 and phospho-Chk1 (Ser317) protein expression in UVB-induced tumors and in epidermal areas distant from tumors.

Immunohistochemistry on stored paraffin sections of normal epidermis, keratoacanthomas and squamous cell carcinomas showed that areas or cells expressing p53 protein were negative for phospho-Chk1 (Ser317) immunostaining. Overall, in serial tissue sections from 42 keratoacanthomas and 11 carcinomas expressing both phospho-Chk1 (Ser317) and p53, less than 3% of p53 stained areas expressed phospho-Chk1 (Ser317), and less than 3% of phospho-Chk1 stained areas expressed p53. This inverse expression was not apparent for p53 and phospho-Chk1 (Ser345) (not shown). As expected, the inverse protein expression between p53 and phospho-Chk1 (Ser317) could not be observed by Western blotting tumors expressing both proteins since tumors have differential and aberrant p53. However, phospho-Chk1 (Ser317) protein was induced in the epidermal tumors of a p53 knockout mouse compared with that of SKH-1 controls (Fig. 2A). A similar pattern was observed in normal mouse epidermis exposed to a single irradiation of UVB. After a single exposure to UVB, normal epidermal cells from a p53 knockout mouse expressed a relatively high level of phospho-Chk1 (Ser317) whereas epidermal cells from a p53 wild-type littermate induced p53 protein and expressed a relatively low level of phospho-Chk1 (Ser317) (Fig. 2B). Because the antibody used for immunohistochemistry (polyclonal rabbit NCL-p53-CM5p recognizes both wild-type and mutant p53) is designated for paraffin sections and not for Western blotting, we used an alternative antibody, NB100-1913 [recognizes phospho-p53 (Ser15)], for our Western blot analysis. We observed a similar relationship between p53 and phosphochk1 (Ser317) by both techniques. To our knowledge, this striking phenotypic inverse expression between proteins in skin tumors has not been reported previously.

Response to DNA damage is a complex, highly orchestrated signaling network which includes cell cycle checkpoints and DNA repair, damage tolerance, and apoptotic pathways. UVB-induced DNA damage is sensed by the transducer protein ATR that relays signals to effector proteins such as Chk1 and Chk2. The major downstream effector of Chk2 activation is p53. In normal cells, p53-dependent signaling results in G1 growth arrest to repair damage. For example, following cisplatin-induced DNA damage, apoptosis is initiated through the activation of p53 by phospho-Chk2 (threonine 68) and Chk1 is phosphorylated

at serine 317 and degraded after a few hours (Pabla et al., 2008), suggesting that the cells rely on DNA repair and apoptosis through Chk2 activation and G1 arrest and not G2/M arrest. In p53-deficient cancer cells, growth arrest is facilitated by Chk1 activation and cells are arrested in the G2/M phase. This correlates with our previous and current data in skin that suggests that cells lacking p53 rely on their G2 checkpoint for survival and that DNA damage recognition signaling may be shunted to the Chk1 pathway specifically at phosphorylation on serine 317 (Fig. 3). We have previously demonstrated that p53 knockout epidermal cells defective in G1 arrest and had an increased number of mitotic epidermal cells with cyclin B1 compared with p53 wild-type epidermal cells (Lou et al., 2010). However, the downstream effects of elevated phospho-Chk1 (Ser317) are unknown.

We have found no direct correlation between p53 and phospho-Chk1 (Ser345). We have also found no direct or only partial correlation between phospho-Chk1 (Ser317) and markers for wild-type p53 [phospho-p53 (Ser15)], proliferation (bromodeoxyuridine incorporation into DNA and PCNA), apoptosis (active caspase-3), DNA damage response (histone H2AX phosphorylation at Ser10), ATR/Chk1 signaling pathway [CDC25C, 14-3-3 α + β (y62) and Cyclin B1] and cell stress response pathway (PPM1D). However, the loss of p53 expression in tumors and the increase of phospho-Chk1 (Ser317) may have biological relevance for the treatment of UVB-induced tumors with Chk1 inhibitors. Chk1 inhibitors are attractive chemotherapeutic agents because when the G2 or S checkpoint is abrogated by the inhibition of Chk1, p53-deficient cancer cells undergo mitotic catastrophe and eventually apoptosis, whereas normal cells are still arrested in the G1 phase. Further studies to determine the biological functions of the regulation of the Chk1 pathway by p53 are warranted and may be critical for the design of future anticancer agents.

Conclusion

In summary, immunohistochemistry data demonstrated an inverse relationship between p53 and phospho-Chk1 (Ser317) protein expression. Skin tumors expressing p53 protein had no detectable phospho-Chk1 (Ser317), whereas skin tumors expressing phospho-Chk1 (Ser317) protein had no detectable p53. These findings were also observed in the epidermal areas distant from tumors. Epidermal tissue from p53 knockout mice had elevated levels of phospho-Chk1 (Ser317) in UVB-induced tumors and in normal epidermis. These data illustrate the dynamic regulation of checkpoint function, suggesting that phosphorylation of Chk1 on Serine 317 is regulated by p53 status and that p53 may act as a molecular on/off switch for the phosphorylation of Chk1 on Serine 317.

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References

- Berg RJ, et al. Early p53 alterations in mouse skin carcinogenesis by UVB radiation: immunohistochemical detection of mutant p53 protein in clusters of preneoplastic epidermal cells. *Proc. Natl. Acad. Sci. U. S. A.* 1996; 93(1):274–278. [PubMed: 8552621]
- Brash DE, et al. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc. Natl. Acad. Sci. U. S. A.* 1991; 88(22):10124–10128. [PubMed: 1946433]
- Huang MT, et al. Effects of tea, decaffeinated tea, caffeine on UVB light-induced complete carcinogenesis in SKH-1 mice: demonstration of caffeine as a biologically important constituent of tea. *Cancer Res.* 1997; 57(13):2623–2629. [PubMed: 9205068]
- Kripke ML. Sunlight and skin cancer. *Tex. Med.* 1986; 82(12):52–53. [PubMed: 3810503]
- Lou Y, Peng Q, Nolan B, Wagner GC, Lu Y. Oral administration of caffeine during voluntary exercise markedly decreases tissue fat and stimulates apoptosis and cyclin B1 in UVB-treated skin of hairless p53-knockout mice. *Carcinogenesis.* 2010; 31(4):671–678. [PubMed: 19926639]
- Lu YP, et al. Enhanced skin carcinogenesis in transgenic mice with high expression of glutathione peroxidase or both glutathione peroxidase and superoxide dismutase. *Cancer Res.* 1997; 57(8):1468–1474. [PubMed: 9108447]
- Lu YP, et al. Stimulatory effect of oral administration of green tea or caffeine on ultraviolet light-induced increases in epidermal wild-type p53, p21(WAF1/CIP1), and apoptotic sunburn cells in SKH-1 mice. *Cancer Res.* 2000; 60(17):4785–4791. [PubMed: 10987287]
- Lu YP, et al. Topical applications of caffeine or (–)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. *Proc. Natl. Acad. Sci. U. S. A.* 2002; 99(19):12455–12460. [PubMed: 12205293]
- Lu YP, Lou YR, Peng QY, Xie JG, Conney AH. Stimulatory effect of topical application of caffeine on UVB-induced apoptosis in the epidermis of p53 and Bax knockout mice. *Cancer Res.* 2004; 64(14):5020–5027. [PubMed: 15256477]
- Lu YP, Lou YR, Peng QY, Nghiem P, Conney AH. Caffeine decreases phospho-Chk1 (Ser317) and increases mitotic cells with cyclin B1 and caspase 3 in tumors from UVB-treated mice. *Cancer. Prev. Res. (Phila).* 2011; 4(7):1118–1125. [PubMed: 21505179]
- Midgley CA, et al. Coupling between gamma irradiation, p53 induction and the apoptotic response depends upon cell type *in vivo*. *J. Cell Sci.* 1995; 108(Pt 5):1843–1848. [PubMed: 7657708]
- Moles JP, et al. p53 gene mutations in human epithelial skin cancers. *Oncogene.* 1993; 8(3):583–588. [PubMed: 8437842]
- Pabla N, Huang S, Mi QS, Daniel R, Dong Z. ATR-Chk2 signaling in p53 activation and DNA damage response during cisplatin-induced apoptosis. *J. Biol. Chem.* 2008; 283(10):6572–6583. [PubMed: 18162465]
- Rogers HW, et al. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. *Arch. Dermatol.* 2010; 146(3):283–287. [PubMed: 20231499]
- Somers KD, et al. Frequent p53 mutations in head and neck cancer. *Cancer Res.* 1992; 52(21):5997–6000. [PubMed: 1394225]
- Ziegler A, et al. Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers. *Proc. Natl. Acad. Sci. U. S. A.* 1993; 90(9):4216–4220. [PubMed: 8483937]

Fig. 1.

Inverse relationship of p53 and phospho-Chk1 (Ser317). Female SKH-1 mice (7–8 weeks old) were irradiated with UVB (30 mJ/cm²) twice a week for 20 weeks and UVB treatment was stopped. Mice were sacrificed 21 weeks later. A: A representative keratoacanthoma or B: A squamous cell carcinoma was stained with an antibody to phospho(P)-Chk1 (Ser317) and p53 (microscope magnification = 40× for left panel and 100× for right panel). The keratoacanthoma is positive for p53 and negative for phospho(P)-Chk1 (Ser317), and the squamous cell carcinoma is negative for p53 and positive for phospho(P)-Chk1 (Ser317).

Phospho(P)-chk1 (Ser317) staining for B was previously published (Lu et al., 2011). C: Three squamous cell carcinomas were stained with an antibody to phospho(P)-Chk1 (Ser317) and p53 and demonstrate focal areas of inverse phospho(P)-Chk1 (Ser317) and p53 positive staining (microscope magnification = 40×). D: A keratoacanthoma stained with an antibody to phospho(P)-Chk1 (Ser317) and p53 shows mixtures of areas of inverse phospho(P)-Chk1 (Ser317) and p53 positive staining (microscope magnification = 40× left panel and 100-× right panel). Box shows field magnified in the right panel. Phospho(P)-Chk1 (Ser317) staining was previously published (Lu et al., 2011). E: The squamous cell carcinoma demonstrates a strictly inverse relationship between phospho(P)-Chk1 (Ser317) and p53 staining. Cells that express phospho-Chk1 (Ser317) are negative for p53 and cells that express p53 are negative for phospho(P)-Chk1 (Ser317) (microscope magnification = 100× left panel and 400-× right panel). F: Phospho(P)-Chk1 (Ser317) and p53 were determined immunohistochemically (microscope magnification = 100× for “normal” epidermis away from tumors). No phospho(P)-Chk1 (Ser317) was observed in areas away from tumors that showed positive staining for p53. Phospho(P)-Chk1 (Ser317) was previously published (Lu et al., 2011).

Fig. 2.

Western blot analysis for phospho-p53 (Ser15) and phospho-Chk1 (Ser317) in UVB-induced tumors and UVB-exposed epidermis from SKH-1 and congenic hairless p53 knockout mice. A: Three UVB-induced carcinomas from SKH-1 mice and a carcinoma from congenic hairless p53 knockout mouse. Skin tumors were isolated and subjected to Western blot analysis. B: Congenic SKH-1 hairless p53 knockout mice and their wild-type littermates were treated with UVB (180 mJ/cm²) once and killed at 16 h after UVB. Skin epidermal tissue was isolated and subjected to Western blot analysis.

Fig. 3.

Ultraviolet B (UVB)-induced cell cycle signaling and the relationship between p53 and phospho-Chk1 (Ser317). A: In cells that have p53, UVB-induced DNA damage activates cell cycle checkpoints through ATR activation of Chk1 and Chk2. Chk2 activates p53 leading to G1 arrest with subsequent DNA repair (main pathway indicated by red arrows). If DNA is not repaired properly, these cells undergo apoptosis. Chk1 inhibits Cdc25 activation of cyclin B1 and G2-M progression is prevented (G2 arrest). Our data demonstrate that in the presence of p53, phospho-Chk1 (Ser317) is inhibited. This may lead to an increase in lethal mitosis. B: In cell that lacks p53, DNA damage recognition signaling may be shunted to the Chk1 pathway (main pathway indicated by red). p53 deficient cells have elevated levels of phospho-Chk1 at serine 317 (red arrows) and rely on their G2 checkpoint for survival. The downstream effects of elevated phospho-Chk1 (Ser317) are unknown.