Carcinogenesis vol.26 no.10 pp.1804–1810, 2005
doi:10.1093/carcin/bgi140
Advance Access publication May 25, 2005

Loss of p21$^{WAF1/Cip1}$ in Gadd45-deficient keratinocytes restores DNA repair capacity

Tomoko Maeda, Robin A.Espino, Eugene G.Chomey, Le Luong, Ather Bano, Diana Meakins and Victor A.Tron*

Department of Laboratory Medicine and Pathology, University of Alberta, Faculty of Medicine, 4B1 Walter C Mackenzie Health Science Centre, 8440-112th Street, Edmonton, Alberta, Canada T6G 2R7

*To whom correspondence should be addressed
Email: vtron@cha.ab.ca

Ultraviolet light (UV)-induced DNA damage is repaired primarily by the nucleotide excision repair (NER) pathway. Gadd45 is a multifunctional protein that regulates NER. Gadd45-deficient keratinocytes fail to repair UV-induced DNA damage, but the mechanism by which Gadd45 stimulates repair of UV-induced DNA damage is unknown. p21$^{WAF1/Cip1}$ (p21) is a well-characterized downstream target of p53 that binds to Gadd45 and proliferating cell nuclear antigen (PCNA). The role of p21 in NER is somewhat controversial, however, recent studies appear to suggest that it inhibits DNA repair by inhibiting PCNA activity. Since a physical interplay exists between p21, Gadd45 and PCNA, we hypothesized that Gadd45 promoted DNA repair via p21. Initially, we examined p21 protein expression in Gadd45-deficient and proficient mice and found a higher base level of p21 protein in Gadd45-deficient keratinocytes and in most other tissues. With these results, we next speculated on the role played by p21 in Gadd45 regulated NER, by exposing keratinocytes from wild-type, single and double knockout (Gadd45 and p21) mice to UV, and measuring the responses. We confirmed that Gadd45-deficient keratinocytes were defective in UV-induced NER, but interestingly Gadd45/p21-null keratinocytes had normal NER in response to UV. Furthermore, Gadd45/p21-null keratinocytes were more resistant to UV-induced cell death than Gadd45-deficient keratinocytes. These results support the hypothesis that Gadd45 enhances NER by negatively regulating basal p21 expression in keratinocytes.

Introduction

Exposure to sunlight is a primary risk factor for development of skin cancer. The level of sunlight exposure in humans may be increasing due to the depletion of stratospheric ozone, which could increase the incidence of skin cancer (1). Ultraviolet B (UVB; 290–320 nm), the most damaging component of sunlight, has been shown to be much more carcinogenic than UVA (2). The ability to repair DNA is an important cellular mechanism that protects cells from the deleterious effects of DNA damage and maintains genetic stability (3).

It is quite clear that p53 protein also plays a critical role in regulating DNA repair after exposure to UV light (4–12). Numerous potential mechanisms have been described as to how p53 functions to regulate DNA repair. p53 directly associates with TFIIH, a nucleotide excision repair (NER) component (13), and up-regulates genes implicated in DNA repair such as p48 (14), Gadd45a (15,16), XPC (17) and p21$^{WAF1/Cip1}$ (designated as p21) (18,19).

The p53 regulated molecule, Gadd45α (designated as Gadd45) is rapidly induced at the transcriptional level in UV-irradiated mammalian cells (15,16). Gadd45 may be regulated by both p53-dependent and p53-independent mechanisms in response to genotoxic stress such as UV and alkylating agents (20). Gadd45 is also involved in the G_2/M checkpoint mediated by Cdc2 kinase activity (21,22). Gadd45 has been shown to physically interact with p21 (23) and proliferating cell nuclear antigen (PCNA) (24,25). Mice targeted for homologous deletion of Gadd45 demonstrated genomic instability, in a manner similar to p53-deficient mice (26). Using primary cells, mouse embryonic fibroblasts (27) and mouse keratinocytes (22), Gadd45 has been shown to regulate DNA repair post-UV.

p21, another p53-dependent protein, is a cyclin-dependent kinase inhibitor specifically associated with a cell cycle associated protein, cyclin D1, that may be involved in neoplastic transformation and progression (18). p21 was later proven to inhibit both the kinase activity of cyclin-dependent kinase 2 (Cdk2) and the phosphorylation of retinoblastoma (Rb) protein, acting as a possible negative regulator of the cell cycle (19). The role of p21 in DNA repair is somewhat controversial. Post-UV, this molecule has been shown to inhibit repair (28–31) or have no apparent effect (32,33), have a marginal effect (34) or a positive effect (35,36). However, it now appears that the bulk of the studies appear to favor a negative or no role for p21 in UV-induced NER.

Our laboratory has focused on primary keratinocytes as the target for UV experiments (6,8,37). It is our belief that these cells are the most physiologically relevant targets for UV. Furthermore, we have not used passaged cells and thus accumulated DNA lesions are less likely to develop in our system.

Since Gadd45 is known to physically interact with p21, we tested the hypothesis that Gadd45 may regulate NER by altering the function or expression of p21. This study focuses on the expression of p21 in Gadd45-deficient keratinocytes, and the role these two molecules may play in NER after UVB (295 nm UV-radiation) in a physiologically relevant system.

Abbreviations: MEF, mouse embryo fibroblast; NER, nucleotide excision repair; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; PI, propidium iodide; TCR, transcription-coupled repair; UV, ultraviolet.

Materials and methods

Cells and cell culture

Gadd45-transgenic mice were kindly provided by Dr A.Fornace (26). p21-transgenic mice were purchased from Jackson Laboratory (Bar Harbor,

Maine). To produce Gadd45/p21 double knockout mice, at least six crosses were performed using the following breeding strategy: Gadd45$^{-/-}$ and p21$^{-/-}$ mice were crossbred to generate Gadd45$^{+/-}$p21$^{+/-}$ progeny. The double heterozygote was bred with a p21$^{-/-}$ mouse to produce Gadd45$^{+/-}$p21$^{-/-}$ mice. Finally, Gadd45$^{+/-}$p21$^{-/-}$ littermates were crossbred to produce the following genotypes: Gadd45$^{+/+}$p21$^{-/-}$, Gadd45$^{+/-}$p21$^{-/-}$ and double knockout Gadd45$^{-/-}$p21$^{-/-}$ animals.

Newborn mice were killed and tails were used for genotyping by PCR. Skin was sterilized by 7.5% povidone-iodine (Betadine) and 100% ethanol and rinsed in phosphate-buffered saline (PBS). Skin was then removed and soaked in 0.2% dispase overnight at 4°C. The epidermal layer was separated from the dermis and trypsinized for 5 min, neutralized with fetal bovine serum (FBS) and filtered. Cells were centrifuged and the cell pellet was resuspended with keratinocytes-SFM (Life Technologies, Burlington, Ontario). Keratinocytes were plated in collagen-I coated dishes (Biocoat®, Becton Dickinson, Franklin Lakes, NJ) at 3–4 × 10^4 cells/cm^2. Cells were incubated at 37°C until 80% confluency and used within 2 weeks after plating.

UVB irradiation

Keratinocytes were rinsed twice with PBS and irradiated with a bank of four unfiltered sun lamps (FS20T12/UVB-BP, UBL, USA). To avoid any UVC contamination, lids of the culture dishes were kept on during irradiation. The intensity of the UV light was measured using an IL1700 radiometer connected to a SED 240 detector with a UVB-1 filter and W diffuser (International Light, Newburyport, MA). The light peak was at 295 nm and no UVC contamination was observed when using the culture dish lid (38).

Flow cytometry

Cells were stained with propidium iodide (PI) and sorted by flow cytometry (39). Keratinocytes were trypsinized and combined with floating cells at 8, 24 and 48 h after irradiation. Unirradiated control cells were also harvested and analyzed. Cells were neutralized with DMEM containing 10% FBS, centrifuged and washed twice in 3 ml PBS supplemented 1% glucose (PBS+). The cell pellet was suspended in 1 ml ice-cold 70% ethanol with gentle vortexing. Cells were incubated overnight on a rocker at 4°C, centrifuged and suspended in 0.5 ml PI staining solution (50 μg/ml PI, 100 U/ml RNase in PBS+) and incubated at room temperature for 1 h. PI staining was quantified by FACScan (Becton Dickinson, San Jose, CA). FACScan data were acquired and analyzed by CELLQuest.

DNA repair slot-blot assay

Cells were harvested 0, 24, 48 or 72 h after UV irradiation. Unirradiated control cells were also harvested. Genomic DNA was isolated using the DNeasy kit (Qiagen Mississauga, Ontario) according to the manufacturer's protocol. DNA (100 ng in 0.5 M NaOH and 10 mM EDTA) was denatured by boiling for 10 min. Ice-cold ammonium acetate (2 M) was added to a final concentration of 1 M. Denatured DNA was spotted onto a nitrocellulose membrane pre-wet with 6× SSC using a slot-blot apparatus (Bio-Dot SF, Bio-Rad, Mississauga, Ontario). The filter was baked at 80°C for 2 h. Thymine dimers were quantified using monoclonal antibody MC-062 (clone KTM53, Kamiya Biomedical, Seattle, WA). Bound antibody was detected by ECL plus (Amersham, Baie d'Urfè, Québec), and quantified by autoradiography. The membrane was re-probed with radiolabeled mouse genomic DNA to quantify the amount of the sample DNA per slot. The antibody signal was normalized to the amount of DNA per lane, and the rate of lesion removal was calculated (40).

Western blot analysis

Irradiated mouse keratinocytes were harvested 8, 24 and 48 h after irradiation, and unirradiated cells were harvested as control. Cells were rinsed twice with PBS and harvested by scraping into ice-cold RIPA buffer (150 mM NaCl, 1.0% NP-40, 0.5% deoxycholate, 0.1% SDS and 50 mM Tris–HCl, pH 8.0) with 1 mM phenylmethylsulfonyl fluoride and 1 mM orthovandate. Cell lysates were sonicated and microcentrifuged at 10,000 r.p.m. for 10 min at 4°C. The supernatant was removed and protein concentration was determined by Lowry Assay. Total protein (10 or 20 μg) was analyzed by 15% SDS–PAGE and transferred to a nitrocellulose membrane in 20 mM Tris, 154 mM glycine and 20% methanol at 4°C. Non-specific binding was blocked with 1% non-fat milk in 0.1% Tween/PBS. The membranes were incubated for 1–2 h at room temperature or overnight at 4°C with mouse monoclonal anti-p21 antibody (SXM, BD PharMingen, Mississauga, ON). Unbound antibody was removed by washing 3–4 times for 10–20 min. Membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody in 0.1% Tween/PBS. After additional washing, bound antibody was detected by ECL plus (Amersham, Baie d'Urfè, Québec) according to manufacturer's instruction. The membranes were exposed to X-ray film (Fuji, Japan).

Six-week-old Gadd45$^{+/+}$ or Gadd45$^{-/-}$ mice were killed and organs were homogenized in harvesting buffer. Tissues were sonicated, centrifuged and supernatants were used for western blot: 20 μg of total protein was loaded per lane.

Results

Gadd45-deficient keratinocytes express more p21 than wild-type keratinocytes

p21 protein expression was compared in Gadd45-deficient and proficient keratinocytes. p21 was quantified using western blots as described in Materials and methods. A higher level of p21 protein was detected in Gadd45-deficient than in Gadd45-proficient cells (Figure 1).

Characterization of Gadd45/p21 double knockout mice

Gadd45$^{-/-}$p21$^{-/-}$ mice were crossbred and females produced pups without obvious developmental anomalies. The pups remained healthy and have no obvious spontaneous pathology as adults.

Gadd45-deficient keratinocytes are defective in UV-induced NER but Gadd45/p21-null keratinocytes show normal NER in response to UV

DNA repair capacity was tested in wild-type and mutant keratinocytes using 125 J/m^2. Slot blot analysis using thymine dimer antibody showed delayed repair of thymine dimers in Gadd45-null cells, but not in wild-type, p21-null or Gadd45/p21-null cells (Figure 2a and b). Differences were noted beginning at 24 h, and were maximal at 72 h. Figure 2c is an example of a slot blot used to quantify DNA repair. The top panel shows the loading control re-probed with radiolabeled mouse DNA, and the lower panel shows slot blot probing with thymine dimer antibody. Clearly, the bands remain present in Gadd45 null cells, but disappear in the other genotypes at 48 and 72 h.

Gadd45/p21 double knockout keratinocytes do not undergo UV-induced apoptosis

UV-exposed Gadd45$^{-/-}$ and Gadd45$^{-/-}$p21$^{-/-}$ keratinocytes were analyzed by flow cytometry to identify apoptotic cells in the sub G_0G_1 population. Gadd45$^{-/-}$ keratinocytes underwent UV-induced apoptosis resulting in a large increase in the subG_0G_1 population 48 h after UV exposure; in contrast, the subG_0G_1 population did not increase in UV-exposed Gadd45/p21 double knockout keratinocytes (Table I).

Fig. 1. Expression of p21$^{WAF1/Cip1}$ in wild-type and Gadd45-deficient cultured keratinocytes. Total protein (20 μg) from wild-type and Gadd45-deficient keratinocytes was analyzed by western blot. Actin served as an internal loading control.

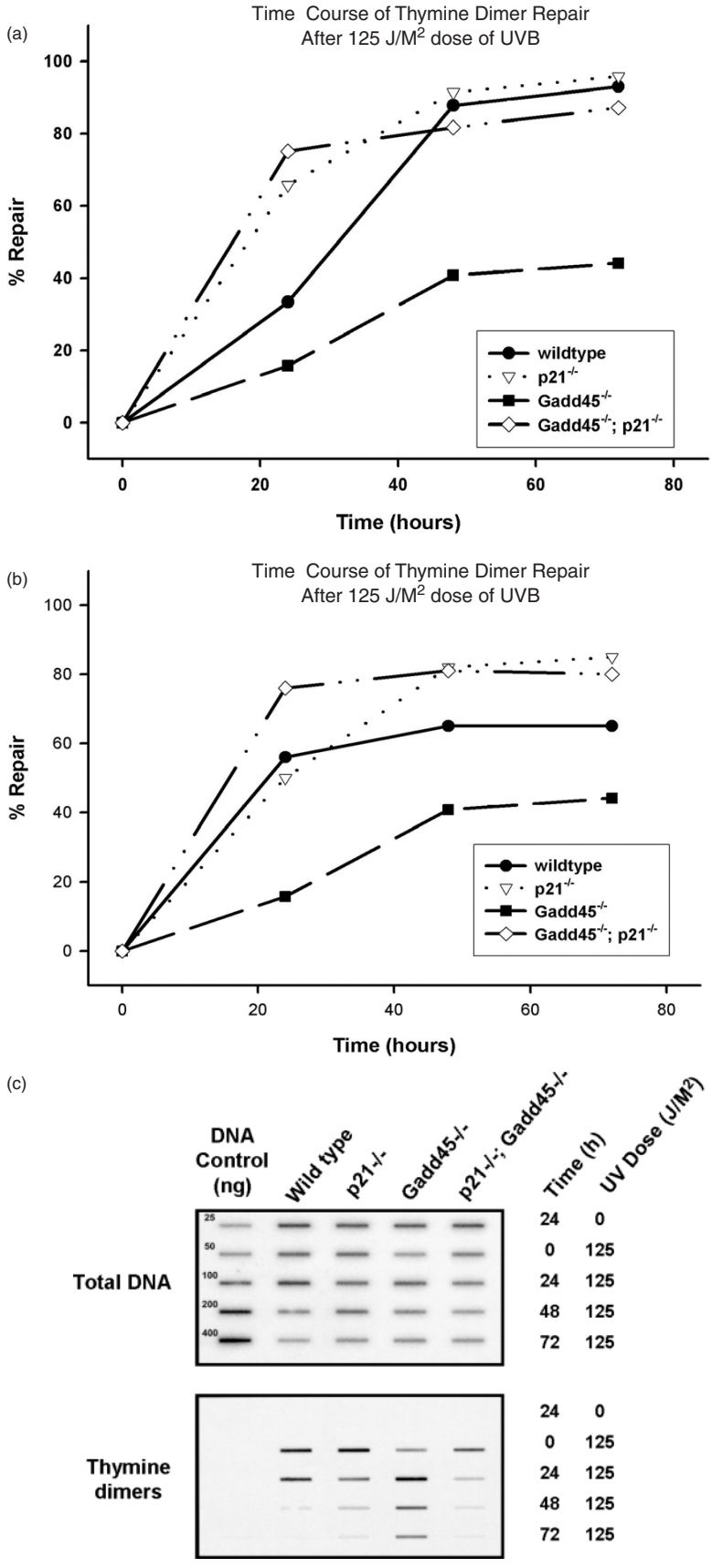

Fig. 2. Slot blot DNA repair assay of wild-type and mutant keratinocytes. DNA was analyzed by slot blot. Thymine dimers were detected using a monoclonal antibody. Assays were carried out with wild-type, Gadd45-null, p21-null or Gadd45/p21-null cells (**a**). This experiment was repeated and almost identical results were obtained (**b**). The slot blots pertaining to experiment (b) are shown in (**c**). The lower blot shows thymine dimer levels as detected with the monoclonal antibody, while the upper blot was probed with radiolabeled mouse DNA to control for loading differences.

UV-induced up-regulation of p21 requires Gadd45

Recent studies in our laboratory indicate that steady-state expression of p21 in keratinocytes is p53-independent (33). Figure 1 examined constitutive expression of p21 and demonstrates that Gadd45-deficient keratinocytes express more p21 protein than Gadd45-proficient cells, a finding confirmed in Figures 3. Previous studies show that UV-induces p21 (33,41–44). This is confirmed in Figure 3. p21 protein expression increases in UV-exposed Gadd45$^{+/+}$ keratinocytes; however, this pattern is not present in UV-exposed Gadd45$^{-/-}$ cells. At all three doses, p21 is markedly induced in Gadd45$^{+/+}$ cells, and then gradually falls at 48 h. Whereas in the Gadd45$^{-/-}$ cells, p21 may rise slightly at 8 h in the lowest dose, but indeed is lower in the intermediate and high doses.

Gadd45-deficient tissues express more p21 than wild-type tissues

p21 protein expression was compared in Gadd45-deficient and proficient tissues. Mice were killed at 6 weeks of age and total protein was extracted from brain, heart, kidney, liver, lung and spleen. p21 was quantified using western blots as described in Materials and methods. In most tissues, a higher level of p21 protein was also detected in Gadd45-deficient than in Gadd45-proficient mice (Figure 4). p21 was expressed at a very low level in brain in both Gadd45-deficient and -proficient animals.

Discussion

We have sought to determine molecular mechanisms regulating UV responses in keratinocytes, specifically p53-dependent pathways. Gadd45 is an important downstream regulatory protein of p53. We have previously shown that Gadd45 regulates DNA repair in keratinocytes (22), but the mechanism responsible for this effect is under investigation. It has been suggested that Gadd45 may promote recruitment of DNA repair proteins, specifically PCNA, to sites of UV damage (27) (Tron,V.A.). Since Gadd45 is known to physically interact with p21 (23), and both of these molecules have been linked to NER, we sought to examine whether Gadd45 regulates DNA repair via p21.

Table I. Comparison of apoptotic population

	No UV	24 h	48 h
Wild-type (%)	9.4	8.0	10.7
Gadd45$^{-/-}$ (%)	12.4	14.4	42.1
Gadd45$^{-/-}$p21$^{-/-}$ (%)	16.3	13.0	13.3

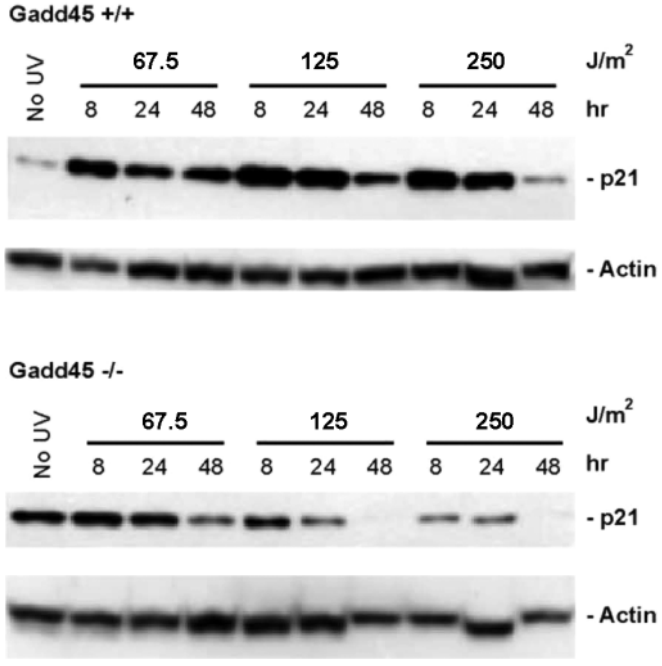

Fig. 3. UV-induction of p21 expression effectively in Gadd45-proficient and -deficient cells. Gadd45$^{+/+}$ and Gadd45$^{-/-}$ cells were treated with UVB. Western blot was performed and p21 expression was quantified.

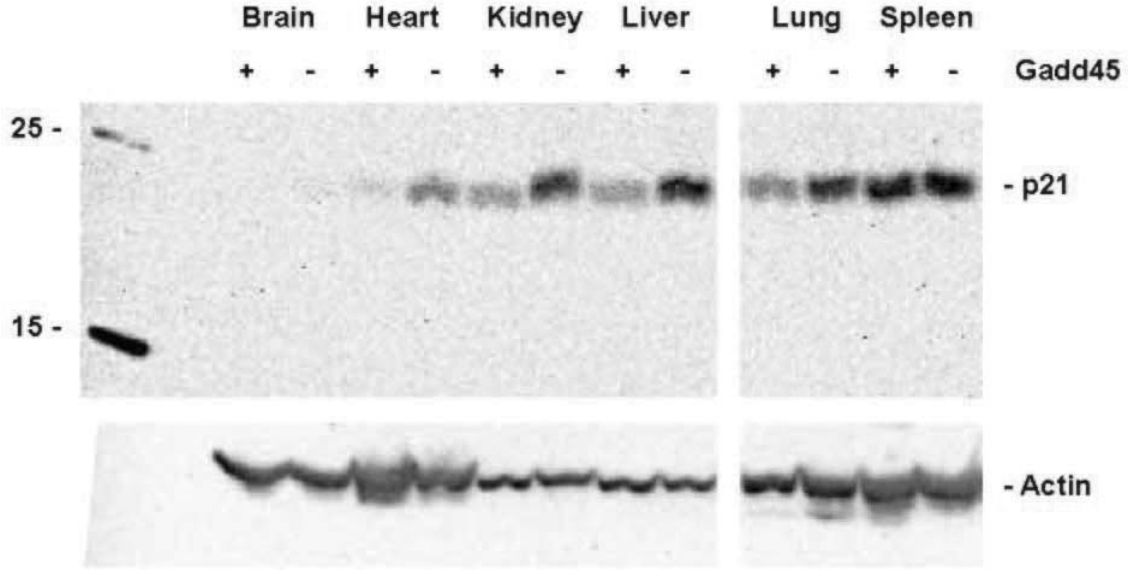

Fig. 4. Expression of p21 in wild-type and Gadd45-deficient tissues. Total protein (20 μg) from wild-type and Gadd45-deficient tissues was analyzed by western blot. Actin served as an internal loading control.

Using a global genomic DNA repair assay (40), which measures the removal of thymine dimers after UV exposure, we confirmed our previous finding that Gadd45-deficient keratinocytes are deficient in NER, and are more sensitive to UV-induced cell death (22). However, the more interesting finding occurred when p21 was also knocked out (Gadd45/p21 double knockout keratinocytes), NER returned to normal levels. In support of this DNA repair finding, the double KO cells also demonstrated less cell death as compared with the single Gadd45 KO cells. Thus, the removal of p21 in Gadd45-deficient keratinocytes was effective in restoring NER. Furthermore, Gadd45 appears to negatively regulate steady-state p21 protein levels, most clearly evident in keratinocytes, as evidenced via western blot analysis. Since most studies now support the notion that p21 functions to inhibit NER, our data point to the conclusion that Gadd45 is regulating NER by inhibiting the steady-state levels of p21 in keratinocytes.

Critical to our conclusion is the observation that the C-terminal region of p21 has been shown to inhibit NER by 50% when a 5-fold excess of p21 to PCNA is present (30). Previous work has shown that p21 and PCNA must interact with each other in the absence of NER proteins in order to observe subsequent inhibition of NER by p21/PCNA (29). Thus, in the event preloading does not occur, p21 will not inhibit DNA polymerases delta or epsilon during PCNA-dependent gap filling, even when p21 is present in great excess (30).

We feel that our current data provide mechanistic support for the earlier observations that Gadd45 promotes recruitment of PCNA to sites of damage (34). We confirm this observation by observing very rapid PCNA DNA binding in Gadd45$^{+/+}$ cells (Tron,V.A.). If p21 is available before DNA damage occurs, NER is inhibited by the lack of DNA polymerase and PCNA binding to areas of damage (30). Thus, the presence of p21 in our Gadd45-deficient cells could interfere with the loading of PCNA onto sites of DNA damage, and thus interfering with DNA repair. Conversely, low levels of p21 in wild-type cells would allow for the binding of PCNA, and allow for the important resynthesis step of NER.

Our data show that p21 is up-regulated in Gadd45-proficient cells after UVB exposure. Since p21 is known to inhibit NER, this could potentially contradict our conclusion. However, as noted earlier, after UV exposure p21 is unable to inhibit NER, since NER proteins have preloaded (29,30). Then, what exactly is p21 doing? It is our belief that p21 functions primarily to inhibit the cell cycle after UV exposure and not inhibit NER. It would not be in a cell's best interest to have a molecule inhibiting DNA repair (a deleterious function), while at the same time, inhibiting the cell cycle (a protective function). *In vivo*, our previous study confirmed p21's primary role was to regulate the cell cycle and not to regulate NER (33).

What role is p53 playing in this pathway? As noted previously, our work demonstrated that p21 appears to be negatively regulated in a Gadd45-dependent manner in keratinocytes in the absence of UV. Work from our group has previously shown that p21 is regulated in a p53-independent manner, in growing steady-state keratinocytes (33). This conclusion is based on a previous study of ours, showing no difference in p21 expression in keratinocytes from p53$^{+/+}$ and p53$^{-/-}$ keratinocytes. In support of this thinking, a p53-response-element transfected into non-irradiated Gadd45a$^{+/+}$ and Gadd45a$^{-/-}$ keratinocytes, demonstrated no difference in activity between the two cell types (45). The same group also showed p21 induction within Gadd45a$^{+/+}$ epidermis, after UV exposure, but not Gadd45a$^{-/-}$ keratinocytes, in a p53-dependent manner (45). In summary, Gadd45 appears to negatively regulate steady-state p21 protein in a p53-independent manner, but after UV, Gadd45 up-regulates p21 via a p53-dependent pathway.

In this current study, we used primary keratinocyte cultures to examine the role of Gadd45 in NER. This experimental design was chosen because it is based on a physiologically relevant system. In particular, non-transformed well-characterized cells and tissues were used whose genotype has been studied. Transformed or passaged cells tend to have uncharacterized genetic changes that can influence experimental results. For example, when normal keratinocytes were compared with a keratinocyte cell line (SCC12B2) in a DNA microarray analysis, major differences were noted (46). While UVB up-regulated key DNA damage response genes and DNA repair genes, there was little or no effect noted in the transformed cell line. Furthermore, many investigators, when using a primary cell line, choose fibroblasts such as mouse embryonic fibroblasts (MEFs). For example, damaged fibroblasts arrest primarily at G_1, and damaged keratinocytes arrest predominately at G_2 (47). Furthermore, DNA repair in fibroblasts occurs via the transcription-coupled repair (TCR) pathway, while in keratinocytes, NER is largely TCR-independent (48). Fibroblasts are more convenient to work with than keratinocytes, but keratinocytes are a more physiologically relevant cell type which has significantly different properties than fibroblasts.

A somewhat similar observation was made by Therrien *et al*. (49) demonstrating that an NER repair defect in p53-deficient tumor cells can be reversed by deleting p21. In order to link this observation with our study, it would be important to compare p21 protein levels with a comparable p53$^{+/+}$p21$^{+/+}$ cell line. The Therrien paper did not include such a genotype, however if one was added, it would be tempting to speculate that basal p21 protein levels in a p53$^{+/+}$p21$^{+/+}$ genotype might be lower than their p53$^{-/-}$p21$^{+/+}$ cells.

In contrast to our findings, Smith *et al*. (34) also studied Gadd45/p21 double knockout MEFs, and they observed that Gadd45/p21 double knockout MEFs repair UV damage less efficiently than Gadd45$^{-/-}$ MEFs. A number of differences between the experimental protocol are important to take into account. First, UVC was the wavelength used, but this may not be relevant since UVC never reaches the earth's surface. Second, MEFs are more rapidly growing than keratinocytes, and are not a major important target of UV. As noted above, fibroblasts do respond differently to keratinocytes. Finally, we have recently examined the basal p21 protein levels in murine fibroblasts, with and without Gadd45. In these cells, as contrasted to keratinocytes, basal p21 protein levels were Gadd45 independent (Tron,V.A.).

In summary, we describe a novel mechanism by which Gadd45 regulates NER in keratinocytes, by negatively regulating steady-state p21 levels.

Acknowledgements

This research was funded by the Canadian Institutes of Health Research (VAT) and Alberta Heritage Foundation for Medical Research (VAT).

Conflict of Interest Statement: None declared.

References

1. Jankowski,J. and Cader,A.B. (1997) The effect of depletion of the earth ozone layer on the human health condition. *Int. J. Occup. Med. Environ. Health*, **10**, 349–364.
2. de Laat,A., van der Leun,J.C. and de Gruijl,F.R. (1997) Carcinogenesis induced by UVA (365-nm) radiation: the dose–time dependence of tumor formation in hairless mice. *Carcinogenesis*, **18**, 1013–1020.
3. Kaufmann,W.K. and Kaufman,D.G. (1993) Cell cycle control, DNA repair and initiation of carcinogenesis. *FASEB J.*, **7**, 1188–1191.
4. Ford,J.M., Lommel,L. and Hanawalt,P.C. (1994) Preferential repair of ultraviolet light-induced DNA damage in the transcribed strand of the human p53 gene. *Mol. Carcinog.*, **10**, 105–109.
5. Ford,J.M. and Hanawalt,P.C. (1995) Li–Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. *Proc. Natl Acad. Sci. USA*, **92**, 8876–8880.
6. Li,G., Mitchell,D.L., Ho,V.C., Reed,J.C. and Tron,V.A. (1996) Decreased DNA repair but normal apoptosis in ultraviolet-irradiated skin of p53-transgenic mice. *Am. J. Pathol.*, **148**, 1113–1123.
7. Ford,J.M. and Hanawalt,P.C. (1997) Expression of wild-type p53 is required for efficient global genomic nucleotide excision repair in UV-irradiated human fibroblasts. *J. Biol. Chem.*, **272**, 28073–28080.
8. Li,G., Ho,V.C., Mitchell,D.L., Trotter,M.J. and Tron,V.A. (1997) Differentiation-dependent p53 regulation of nucleotide excision repair in keratinocytes. *Am. J. Pathol.*, **150**, 1457–1464.
9. Tron,V.A., Trotter,M.J., Ishikawa,T., Ho,V.C. and Li,G. (1998) p53-dependent regulation of nucleotide excision repair in murine epidermis *in vivo*. *J. Cutan. Med. Surg.*, **3**, 16–20.
10. Smith,M.L., Ford,J.M., Hollander,M.C., Bortnick,R.A., Amundson,S.A., Seo,Y.R., Deng,C.X., Hanawalt,P.C. and Fornace,A.J.,Jr. (2000) p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol. Cell. Biol.*, **20**, 3705–3714.
11. Mathonnet,G., Leger,C., Desnoyers,J., Drouin,R., Therrien,J.P. and Drobetsky,E.A. (2003) UV wavelength-dependent regulation of transcription-coupled nucleotide excision repair in p53-deficient human cells. *Proc. Natl Acad. Sci. USA*, **100**, 7219–7224.
12. Therrien,J.P., Drouin,R., Baril,C. and Drobetsky,E.A. (1999) Human cells compromised for p53 function exhibit defective global and transcription-coupled nucleotide excision repair, whereas cells compromised for pRb function are defective only in global repair. *Proc. Natl Acad. Sci. USA*, **96**, 15038–15043.
13. Wang,X.W., Yeh,H., Schaeffer,L., Roy,R., Moncollin,V., Egly,J.M., Wang,Z., Freidberg,E.C., Evans,M.K. and Taffe,B.G. (1995) p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nat. Genet.*, **10**, 188–195.
14. Fitch,M.E., Cross,I.V., Turner,S.J., Adimoolam,S., Lin,C.X., Williams,K.G. and Ford,J.M. (2003) The DDB2 nucleotide excision repair gene product p48 enhances global genomic repair in p53 deficient human fibroblasts. *DNA Repair (Amst.)*, **2**, 819–826.
15. Fornace,A.J.Jr, Nebert,D.W., Hollander,M.C., Luethy,J.D., Papathanasiou,M., Fargnoli,J. and Holbrook,N.J. (1989) Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. *Mol. Cell. Biol.*, **9**, 4196–4203.
16. Hollander,M.C., Alamo,I., Jackman,J., Wang,M.G., McBride,O.W. and Fornace,A.J.Jr. (1993) Analysis of the mammalian gadd45 gene and its response to DNA damage. *J. Biol. Chem.*, **268**, 24385–24393.
17. Adimoolam,S. and Ford,J.M. (2002) p53 and DNA damage-inducible expression of the *Xeroderma pigmentosum* group C gene. *Proc. Natl Acad. Sci. USA*, **99**, 12985–12990.
18. el-Deiry,W.S., Tokino,T., Velculescu,V.E., Levy,D.B., Parsons,R., Trent,J.M., Lin,D., Mercer,W.E., Kinzler,K.W. and Vogelstein,B. (1993) WAF1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817–825.
19. Harper,J.W., Adami,G.R., Wei,N., Keyomarsi,K. and Elledge,S.J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G_1 cyclin-dependent kinases. *Cell*, **75**, 805–816.
20. Smith,M.L. and Fornace,A.J.Jr (1996) Mammalian DNA damage-inducible genes associated with growth arrest and apoptosis. *Mutat. Res.*, **340**, 109–124.
21. Yang,Q., Manicone,A., Coursen,J.D., Linke,S.P., Nagashima,M., Forgues,M. and Wang,X.W. (2000) Identification of a functional domain in a GADD45-mediated G_2/M checkpoint. *J. Biol. Chem.*, **275**, 36892–36898.
22. Maeda,T., Hanna,A.N., Sim,A.B., Chua,P.P., Chong,M.T. and Tron,V.A. (2002) GADD45 regulates G_2/M arrest, DNA repair, and cell death in keratinocytes following ultraviolet exposure. *J. Invest. Dermatol.*, **119**, 22–26.
23. Kearsey,J.M., Coates,P.J., Prescott,A.R., Warbrick,E. and Hall,P.A. (1995) Gadd45 is a nuclear cell cycle regulated protein which interacts with p21Cip1. *Oncogene*, **11**, 1675–1683.
24. Smith,M.L., Chen,I.T., Zhan,Q., Bae,I., Chen,C.Y., Gilmer,T.M., Kastan,M.B., O'Connor,P.M. and Fornace,A.J.Jr (1994) Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science*, **266**, 1376–1380.
25. Chen,I.T., Smith,M.L., O'Connor,P.M. and Fornace,A.J.Jr (1995) Direct interaction of Gadd45 with PCNA and evidence for competitive interaction of Gadd45 and p21Waf1/Cip1 with PCNA. *Oncogene*, **11**, 1931–1937.
26. Hollander,M.C., Sheikh,M.S., Bulavin,D.V. *et al.* (1999) Genomic instability in Gadd45a-deficient mice. *Nat. Genet.*, **23**, 176–184.
27. Smith,M.L., Ford,J.M., Hollander,M.C., Bortnick,R.A., Amundson,S.A., Seo,Y.R., Deng,C.X., Hanawalt,P.C. and Fornace,A.J.Jr (2000) p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol. Cell. Biol.*, **20**, 3705–3714.
28. Pan,Z.Q., Reardon,J.T., Li,L., Flores-Rozas,H., Legerski,R., Sancar,A. and Hurwitz,J. (1995) Inhibition of nucleotide excision repair by the cyclin-dependent kinase inhibitor p21. *J. Biol. Chem.*, **270**, 22008–22016.
29. Shivji,M.K., Ferrari,E., Ball,K., Hubscher,U. and Wood,R.D. (1998) Resistance of human nucleotide excision repair synthesis *in vitro* to p21Cdn1. *Oncogene*, **17**, 2827–2838.
30. Cooper,M.P., Balajee,A.S. and Bohr,V.A. (1999) The C-terminal domain of p21 inhibits nucleotide excision repair *in vitro* and *in vivo*. *Mol. Biol. Cell*, **10**, 2119–2129.
31. Bendjennat,M., Boulaire,J., Jascur,T., Brickner,H., Barbier,V., Sarasin,A., Fotedar,A. and Fotedar,R. (2003) UV irradiation triggers ubiquitin-dependent degradation of p21(WAF1) to promote DNA repair. *Cell*, **114**, 599–610.
32. Adimoolam,S., Lin,C.X. and Ford,J.M. (2001) The p53-regulated cyclin-dependent kinase inhibitor, p21 (cip1, waf1, sdi1), is not required for global genomic and transcription-coupled nucleotide excision repair of UV-induced DNA photoproducts. *J. Biol. Chem.*, **276**, 25813–25822.
33. Maeda,T., Chong,M.T., Espino,R.A., Chua,P.P., Cao,J.Q., Chomey,E.G., Luong,L. and Tron,V.A. (2002) Role of p21(Waf-1) in regulating the G_1 and G_2/M checkpoints in ultraviolet-irradiated keratinocytes. *J. Invest. Dermatol.*, **119**, 513–521.
34. Smith,M.L., Ford,J.M., Hollander,M.C., Bortnick,R.A., Amundson,S.A., Seo,Y.R., Deng,C.X., Hanawalt,P.C. and Fornace,A.J.Jr (2000) p53-mediated DNA repair responses to UV radiation: studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol. Cell. Biol.*, **20**, 3705–3714.
35. Stivala,L.A., Riva,F., Cazzalini,O., Savio,M. and Prosperi,E. (2001) p21(waf1/cip1)-null human fibroblasts are deficient in nucleotide excision repair downstream the recruitment of PCNA to DNA repair sites. *Oncogene*, **20**, 563–570.
36. McDonald,E.R.,III, Wu,G.S., Waldman,T. and El-Deiry,W.S. (1996) Repair defect in p21 WAF1/CIP1$^{-/-}$ human cancer cells. *Cancer Res.*, **56**, 2250–2255.
37. Tron,V.A., Trotter,M.J., Tang,L., Krajewska,M., Reed,J.C., Ho,V.C. and Li,G. (1998) p53-regulated apoptosis is differentiation dependent in ultraviolet B-irradiated mouse keratinocytes. *Am. J. Pathol.*, **153**, 579–585.
38. Werninghaus,K., Handjani,R.M. and Gilchrest,B.A. (1991) Protective effect of alpha-tocopherol in carrier liposomes on ultraviolet-mediated human epidermal cell damage *in vitro*. *Photodermatol. Photoimmunol. Photomed.*, **8**, 236–242.
39. Denning,M.F., Wang,Y., Nickoloff,B.J. and Wrone-Smith,T. (1998) Protein kinase Cdelta is activated by caspase-dependent proteolysis during ultraviolet radiation-induced apoptosis of human keratinocytes. *J. Biol. Chem.*, **273**, 29995–30002.
40. Eller,M.S., Maeda,T., Magnoni,C., Atwal,D. and Gilchrest,B.A. (1997) Enhancement of DNA repair in human skin cells by thymidine dinucleotides: evidence for a p53-mediated mammalian SOS response. *Proc. Natl Acad. Sci. USA*, **94**, 12627–12632.
41. Ponten,F., Berne,B., Ren,Z.P., Nister,M. and Ponten,J. (1995) Ultraviolet light induces expression of p53 and p21 in human skin: effect of sunscreen and constitutive p21 expression in skin appendages. *J. Invest. Dermatol.*, **105**, 402–406.
42. Liu,M. and Pelling,J.C. (1995) UV-B/A irradiation of mouse keratinocytes results in p53-mediated WAF1/CIP1 expression. *Oncogene*, **10**, 1955–1960.

43. Lu,Y.P., Lou,Y.R., Yen,P., Mitchell,D., Huang,M.T. and Conney,A.H. (1999) Time course for early adaptive responses to ultraviolet B light in the epidermis of SKH-1 mice. *Cancer Res*., **59**, 4591–4602.
44. Ouhtit,A., Muller,H.K., Davis,D.W., Ullrich,S.E., McConkey,D. and Ananthaswamy,H.N. (2000) Temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. *Am. J. Pathol*., **156**, 201–207.
45. Hildesheim,J., Bulavin,D.V., Anver,M.R., Alvord,W.G., Hollander,M.C., Vardanian,L. and Fornace,A.J.Jr (2002) Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53. *Cancer Res*., **62**, 7305–7315.
46. Sesto,A., Navarro,M., Burslem,F. and Jorcano,J.L. (2002) Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. *Proc. Natl Acad. Sci. USA*, **99**, 2965–2970.
47. Flatt,P.M., Price,J.O., Shaw,A. and Pietenpol,J.A. (1998) Differential cell cycle checkpoint response in normal human keratinocytes and fibroblasts. *Cell Growth Differ*., **9**, 535–543.
48. D'Errico,M., Teson,M., Calcagnile,A., Nardo,T., De Luca,N., Lazzari,C., Soddu,S., Zambruno,G., Stefanini,M. and Dogliotti,E. (2005) Differential role of transcription-coupled repair in UVB-induced response of human fibroblasts and keratinocytes. *Cancer Res*., **65**, 432–438.
49. Therrien,J.P., Loignon,M., Drouin,R. and Drobetsky,E.A. (2001) Ablation of p21waf1cip1 expression enhances the capacity of p53-deficient human tumor cells to repair UVB-induced DNA damage. *Cancer Res*., **61**, 3781–3786.

Received March 1, 2005; revised May 19, 2005; accepted May 22, 2005