Genetic ablation of caspase-7 promotes solar-simulated light-induced mouse skin carcinogenesis: the involvement of keratin-17

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Abstract

Solar ultraviolet irradiation is an environmental carcinogen that causes skin cancer. Caspase-7 is reportedly expressed at reduced levels in many cancers. The present study was designed to examine the role of caspase-7 in solar-simulated light (SSL)-induced skin cancer and to elucidate its underlying molecular mechanisms. Our study revealed that mice with genetic deficiency of caspase-7 are highly susceptible to SSL-induced skin carcinogenesis. Epidermal hyperplasia, tumor volume and the average number of tumors were significantly increased in caspase-7 knockout (KO) mice compared with SKH1 wild-type mice irradiated with SSL. The expression of cell proliferation markers, such as survivin and Ki-67, was elevated in SSL-exposed wild-type SKH1 mouse skin. Moreover, SSL-induced apoptosis was abolished in skin from caspase-7 KO mice. Two-dimensional gel electrophoresis, followed by matrix-assisted laser desorption/ionization-time-of-flight analysis of skin tissue lysates from SSL-irradiated SKH1 wild-type and caspase-7 KO mice revealed an aberrant induction of keratin-17 in caspase-7 KO mice. Immunohistochemical analysis of skin tumors also showed an increase of keratin-17 expression in caspase-7 KO mice compared with SKH1 wild-type mice. The expression of keratin-17 was also elevated in SSL-irradiated caspase-7 KO keratinocytes as well as in human basal cell carcinomas. The in vitro caspase activity assay showed keratin-17 as a substrate of caspase-7, but not caspase-3. Overall, our study demonstrates that genetic loss of caspase-7 promotes SSL-induced skin carcinogenesis by blocking caspase-7-mediated cleavage of keratin-17.
Introduction

Solar ultraviolet (UV) irradiation, an environmental carcinogen, is a risk factor for non-melanoma skin cancers, including basal cell and squamous cell carcinomas (1-3). Solar UV irradiation (100–400 nm) can be divided into UVA (315–400 nm), UVB (280–315 nm) and UVC (100–280 nm) based on wavelength (4). Because UVC irradiation is absorbed by the atmospheric ozone layer, the solar UV radiation that induces skin inflammation and carcinogenesis comprises a combination of UVA and UVB. Solar UV irradiation-induced oxidative DNA damage and genomic instability contribute to skin carcinogenesis but exposure to UV irradiation induces epidermal apoptosis. The induction of apoptosis tends to eliminate epidermal cells that harbor UV-induced genetic lesions and, hence, apoptosis is considered to be a protective mechanism against UV-induced skin carcinogenesis. Apoptosis, also known as programmed cell death, is executed by the activation of a series of proteolytic enzymes, including the cysteine-aspartic acid proteases (i.e. caspases). Cells harbor caspases in an inactive form (pro-caspases), which are activated through cleavage induced by appropriate death stimuli. The induction of apoptotic cell death involves two distinct mechanisms, death-receptor-mediated (i.e. extrinsic) and mitochondria-mediated (i.e. intrinsic) cell death. For the intrinsic-mediated pathway of apoptosis, a mitochondrial oxidative burst results in mitochondrial membrane depolarization and release of cytochrome c, which induces cleavage of pro-caspase-9, and initiates a cascade of cleavage of other pro-caspases, such as pro-caspase-3 and pro-caspase-7 (5). The activation of caspase-7, which is a common denominator of both the extrinsic and intrinsic pathways, leads to the induction of apoptosis. Previous studies have demonstrated that caspase-7 is deficient in several cancer types (6). In the present study, we investigated the impact and underlying mechanism of genetic ablation of caspase-7 on solar-simulated light (SSL)-induced skin carcinogenesis compared with SKH1 wild-type mice. We report that depletion of caspase-7 promotes UV-induced skin tumorigenesis at least partly by increasing the expression of keratin-17, an intermediate filament protein that stimulates proliferation of epidermal cells and promotes skin carcinogenesis.

Materials and methods

Reagents

Antibodies to detect pro and cleaved caspase-3, caspase-7 or caspase-9 were purchased from Cell Signaling Technology (Beverly, MA). The antibody against total β-actin or Ki-67 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) or Thermo Scientific (Fremont, CA), respectively. Antibodies to detect cystatin and keratin-17 were purchased from Epitomics (Burlingame, CA).

Generation of SKH1 caspase-7 knockout mice

Caspase-7 knockout (KO) mice (Casp7tm1Fly: Casp7−/−), which are on the C57BL/6j genetic background, were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained at the Hormel Institute University of Minnesota Animal Facility following the guidelines approved by the Animal Care and Use Committee (IACUC), University of Minnesota. The Casp7−/− mice were mated with SKH1 hairless mice on a Balb/c genetic background. The progenies, SKH1 caspase-7 heterozygotes (Casp7+/-; filtered to 50% genetic background of SKH1 Balb/c hairless), were obtained by genomic PCR analysis using PCR primers, oMR5943, oMR5944, oMR5945 and oMR5946 according to recommended protocols. The Casp7+/- SKH1 caspase-7 heterozygote (Casp7+/-) mice were continuously mated with SKH1 hairless mice until Casp7−/− genetic background was filtered to >95% of the SKH1 Balb/c hairless mice. Male and female Casp7−/− SKH1 mice were mated until homozygote mice (Casp7−/- SKH1) were obtained with 25% of Mendel’s ratio. The Casp7−/- SKH1 mice were propagated and used for a SSL-induced skin tumorigenesis study. The genetic background of mice in each generation was determined by genomic PCR using the same primers described above.

Primary cultures of skin keratinocytes

Skin keratinocytes were isolated from 6- to 8-week-old mice and cultured as described previously (7). Briefly, mice were euthanized and dorsal skin sections were removed and treated with trypsin to detach the keratinocytes. Cells were harvested and seeded in collagen-coated dishes. After 72 h, culture medium was changed and keratinocytes were irradiated with different doses of SSL to determine dose-dependency or were irradiated with one dose of SSL and harvested at various times to determine a time course.

Western blot analysis

Cells were disrupted on ice for 30 min in cell lysis buffer [20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetracetic acid, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium vanadate and 1 mM PMSF (phenylmethylsulfonyl fluoride)] and skin tissue samples were homogenized in lysis buffer [50 mM Tris–HCl pH 7.5, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, 1% NF-40, 0.1% sodium dodecyl sulfate (SDS) and 1 mM PMSF]. After centrifugation at 13000 r.p.m. for 30 min, the supernatant fraction was harvested as the total cellular protein extract. The protein concentration was determined using the Bio-Rad protein assay reagent (Richmond, CA). The total cellular protein extracts were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene fluoride membranes in 20 mM Tris–HCl (pH 8.0), containing 150 mM glycine and 20% (vol/vol) methanol. Membranes were blocked with 5% non-fat dry milk in 1× Tris-buffered saline (TBS) containing 0.05% Tween 20 (TBS-T) and then incubated with antibodies. Blots were washed 3 times in 1× TBS-T buffer, followed by incubation with the appropriate horseradish peroxidase-linked IgG. Specific proteins were visualized using an enhanced chemiluminescence detection reagent.

SSL system

The SSL source comprised UVA-340 lamps purchased from Q-Lab Corporation (Cleveland, OH). The UVA-340 lamps provide the best possible simulation of sunlight in the critical short wavelength region from 365 nm down to the solar cutoff of 295 nm with a peak emission of 340 nm. The percentage of UVA and UVB of UVA-340 lamps was measured by a UV detector at 365 nm (8). The UVA-340 lamps provide the best possible simulation of sunlight in the critical short wavelength region from 365 nm down to the solar cutoff of 295 nm with a peak emission of 340 nm (8). The percentage of UVA and UVB of UVA-340 lamps was measured by a UV meter and was 94.5 and 5.5%, respectively.

Acute exposure of mice to SSL

Adult SKH1 wild-type and caspase-7 KO mice (6-8 weeks old, five per group for a total of three groups) were irradiated with 115 kJ/m2 SSL. Dorsal trunk skin samples were harvested at 0 and 24 h after irradiation.

SSL-induced mouse skin carcinogenesis study

Skin carcinogenesis in mice was induced using UVA-340 lamps (Q-Lab Corporation). SKH1 wild-type and caspase-7 KO hairless mice were maintained at The Hormel Institute, University of Minnesota (Austin, MN).
Mice were maintained under conditions based on the guidelines established by the Institutional Animal Care and Use Committee, University of Minnesota. The mice were divided into 4 groups of 20 animals each (6–8 weeks old, with an average body weight of 25 g). For untreated control groups, SKH1 wild-type and caspase-7 KO mice were not irradiated with SSL. For the experimental SSL-treated groups, SKH1 wild-type and caspase-7 KO mice were initially treated with an SSL dose of 30 kJ UVA/m²–1.8 kJ UVB/m² twice a week. The dose was increased by 10% each week until week 6. At week 6, the dose reached 48 kJ UVA/m²–2.9 kJ UVB/m², and this dose was maintained from weeks 6 to 10. At 10 weeks, SSL exposure was discontinued, and tumor growth was monitored for an additional 15 weeks. A tumor was defined as an outgrowth >1 mm in diameter. Tumor number and volume were recorded every week until the end of the experiment. Tumor volume was calculated according to the following formula: tumor volume (mm³) = length × width × height × 0.52. Skin and tumors were harvested and one-half of the samples were immediately fixed in 10% neutral-buffered formalin and processed for hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC). The other half was frozen for western blot analysis.

Terminal dUTP nick-end labeling, H&E staining and IHC

Skin tissues from mice were embedded in paraffin blocks and subjected to terminal dUTP nick-end labeling (TUNEL), H&E staining and IHC. Tumor tissues were deparaffinized and hydrated and then permeabilized with 0.5% Triton X-100 for 1 hour. The samples were then hybridized with Ki-67 (1:50) and keratin-17 (1:200) as the primary antibody and a biotinylated goat anti-rabbit or mouse IgG antibody was used as the secondary antibody. The ABC kit (Vector Laboratories, Inc., Burlingame, CA) was used to detect the protein targets according to the manufacturer's instructions. After developing with 3, 3′-diaminobenzidine, the sections were counterstained with hematoxylin. All sections were observed by microscope (>200 magnification) and analyzed with the Image-Pro Plus software (v. 6.1) program (Media Cybernetics, Rockville, MD).

Immunohistochemical staining of skin tissue microarrays

A human skin tissue array (SK801) was purchased from US Biomax (Rockville, MD) and tissue analysis was conducted according to the manufacturer's suggested protocols. The tissue array included matched normal tissues (H1–H7), which were biopsied from the adjacent tissue of each cancer tissue (A1–A7) from seven individual patients. A Vectastain Elite ABC Kit (Vector Laboratories) was used for immunohistochemical staining according to the recommended protocol. Briefly, the slide was baked at 60°C for 2 hours, deparaffinized and rehydrated. To expose antigens, the slide was unmasked by submersion into boiling sodium citrate buffer (10 mM, pH 6.0) for 10 minutes and then treated with 3% H₂O₂ for 10 minutes. The slide was blocked with 5% goat serum albumin in 1× PBS in a humidified chamber for 1 hour at room temperature and then with a keratin-17 antibody (1:100 dilution in 3% goat serum with PBS) at 4°C in a humidified chamber overnight. The slide was washed and hybridized with a secondary antibody from Vector Laboratories (anti-rabbit 1:500) for 1 hour at room temperature. Slides were stained using the Vectastain Elite ABC kit. All sections were observed by microscope (>200 magnification). The intensity of IHC staining was quantified by calculating the integrated optical density using the Image Pro-Plus 7.0 software program (Media Cybernetics, Bethesda, MD).

2-Dimensional gel electrophoresis

2-Dimensional gel electrophoresis (2-DE) was conducted essentially as described (9,10). Aliquots in sample buffer (7 M urea, 2 M thiourea, 4.5% [3-cholamidopropyl] dimethyl-ammonio)1-propanesulfonate, 100 mM dithioerythritol and 40 mM Tris, pH 8.8) were applied to immobilized pH 3–10 non-linear gradient strips (Amersham Biosciences, Uppsala, Sweden). ISoelecet focusing was performed at 80 000 Vh. The second dimension was analyzed on 9–16% linear gradient polyacrylamide gels (18 cm × 20 cm × 1.5 mm) at constant 40 mA per gel for ~5h. After protein fixation in 40% methanol and 5% phosphoric acid for 1h, the gels were stained with CBB G-250 for 12h. The gels were destained with H₂O, scanned in a BioRad GS70 densitometer and converted into electronic files, which were then analyzed with Image Master Platinum 5.0 image analysis software (Amersham Biosciences).

Matrix-assisted laser desorption/ionization-time-of-flight analysis

For MALDI-TOF-MS (matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry) analysis, the peptides were concentrated using a PORES R2, Oligo R3 column (Applied Biosystems, Foster City, CA). After washing the column with 70% acetonitrile, 100% acetonitrile and then 50 mM ammonium bicarbonate, samples were applied to the R2, R3 column and eluted with cyano-4-hydroxycinnamic acid (Sigma, St Louis, MO) dissolved in 70% acetonitrile and 2% formic acid onto the MALDI plate (Opti-TOF™ 384-well Insert; Applied Biosystems) (11). MALDI-TOF-MS was performed on a 4800 MALDI-TOF/TOF™ Analyzer (Applied Biosystems) equipped with a 355 nm Nd:YAG laser. The pressure in the TOF analyzer was ~7.6e-07 Torr. The mass spectra were obtained in the reflectron mode with an accelerating voltage of 20kV and summed from either 500 laser pulses and calibrated using the 4700 calibration mixture (Applied Biosystems).

Mascot database search

Database search criteria were: taxonomy, Mus., fixed modification; carbamoylomethyl (-57) at cysteine residues; variable modification; oxidized (+16) at methionine residues, maximum allowed missed cleavage, 1, MS tolerance, 100 p.p.m. Only peptides resulting from trypsin digests were considered.

In vitro cleavage of keratins with recombinant caspases

Cleavage reactions with recombinant caspases-3 or -7 (PromoCell, Heidelberg, Germany) were performed with 20 µl caspase buffer (50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.4, 0.1% [3-cholamidopropyl] dimethyl-ammonio)1-propanesulfonate, 5 mM dithiothreitol, 1 mM PMSF, 50 mM leupeptin, 200 µg/ml aprotinin) at 30°C for 3h using keratin-5 or -17 as a substrate that was transcribed from the pET24-mKeratin-5 or pET3d-mKeratin-17 (a kind gift from Dr Pierre A.Coulombe, Johns Hopkins University, Baltimore, MD) and translated with the TNT Quick Coupled Transcription/Translation System (Promega) in the presence of [³⁵S]methionine. The translation mixture (2 µl) was used for the cleavage reaction with the recombinant caspases described above. Samples were separated by 12% SDS-PAGE. [³⁵S]-labeled proteins were detected by autoradiography.

Statistical analyses

All quantitative results are expressed as mean values ± SD. Statistically significant differences were obtained using the Student’s t-test or one-way analysis of variance. A value of P < 0.05 was considered to be statistically significant.

Results

SSL induces epidermal cell death in normal SKH1 wild-type mice, but not in caspase-7 KO mice

The induction of apoptosis is largely mediated through the activation of a series of caspases including caspase-9, -3 and -7. In an effort to examine the role of caspase-7 in SSL-induced skin carcinogenesis, we first investigated the effect of genetic ablation of caspase-7 on SSL-induced apoptosis in mouse skin. We irradiated normal SKH1 wild-type and caspase-7 KO mouse skin with SSL (115 kJ/m²) and examined epidermal skin thickness and apoptosis at 24h post-irradiation. Results indicated that epidermal thickness in caspase-7 KO
mice was significantly greater than that of SKH1 wild-type mice even without exposure to SSL (Figure 1A). Whereas SSL caused significantly increased epidermal thickness in SKH1 wild-type mouse skin compared to non-irradiated skin, a several-fold increase in epidermal skin thickness was observed in SSL-treated caspase-7 KO mouse skin. In agreement with the increased epidermal skin thickness, TUNEL assay results revealed that SSL-induced apoptosis was markedly attenuated in caspase-7 KO mouse skin compared to that observed in SKH1 wild-type mouse skin with exposure to SSL (Figure 1B).

Genetic ablation of caspase-7 enhances SSL-induced skin carcinogenesis

We then determined whether the deletion of caspase-7 and the resultant SSL-induced increase in epidermal skin thickness and marked inhibition of apoptosis might affect SSL-induced skin carcinogenesis in caspase-7 KO mice. SKH1 wild-type and caspase-7 KO mice were subjected to irradiation with SSL or unexposed for 10 weeks. SSL treated was then discontinued and tumor growth monitored for an additional 15 weeks. Skin tumor volume and number were monitored until the termination of the experiment at week 25. Analyses of tumor data revealed that the volume and the average number of tumors were significantly increased in SSL-treated SKH1 wild-type mice compared to untreated animals, which validated the experimental protocol for SSL-induced skin carcinogenesis. Compared to normal SKH1 wild-type mice, the volume (Figure 2A) and average number (Figure 2B) of tumors in SSL-exposed caspase-7 KO mice were significantly higher, suggesting that the deficiency of caspase-7 sensitizes mouse skin to SSL-induced carcinogenesis. H&E staining of skin

![Figure 1. Skin thickness and induction of apoptosis in acute SSL-treated SKH1 wild-type and caspase-7 KO mice. (A) Epidermal skin thickness in mice treated or not treated with SSL (115 kJ/m²). After H&E staining of skin samples, epidermal skin thickness was analyzed in five separate areas using EZ-C1 FreeViewer Gold software (v. 3.2; Nikon, Melville, NY) and an average of three samples was calculated per group. Data are shown as mean values ± SD. The asterisk (*) indicates a significant (P < 0.05) increase in epidermal skin thickness. (B) The induction of apoptosis in untreated mouse skin or in skin from mice treated with SSL was analyzed by TUNEL assay. Apoptotic cells in skin tissue samples were detected according to the instructions provided by the manufacturer as described in Materials and methods section. Similar results were obtained from three samples per group.](http://carcin.oxfordjournals.org/)

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Effect of SSL irradiation on the cleavage of caspases in SKH1 wild-type or caspase-7 KO keratinocytes

To elucidate the underlying molecular mechanisms of enhanced skin tumorigenesis in SSL-exposed caspase-7 KO mice, we isolated keratinocytes from both SKH1 wild-type and caspase-7 KO mice and optimized the intensity and exposure time of SSL that can cause cleavage of execution caspases. Results revealed that exposure to SSL induced cleavage of caspases in a dose- and time-dependent manner (Figure 4A and B). Irradiation with SSL caused maximal induction of caspase-7 and -3 cleavage at a dose of 48 kJ/m² in keratinocytes isolated from normal SKH1 wild-type mice 24 h post-irradiation (Figure 4A). A similar pattern of SSL-induced caspase-3 cleavage was noted in keratinocytes obtained from caspase-7 KO mice (Figure 4A). A kinetic study revealed that irradiation with SSL (48 kJ/m²) induced the cleavage of caspase-9, -3 or -7 in wild-type keratinocytes and the cleavage of caspase-9 and -3 in caspase-7 KO keratinocytes at 24 h after exposure (Figure 4B).

Involvement of keratin-17 in SSL-induced tumor promotion in caspase-7 KO mice

To identify a molecular target of caspase-7, we irradiated wild-type and caspase-7 KO mice with SSL and prepared skin tissue lysates for 2-DE. By comparing and analyzing 2-DE gel images, we marked spots that were changed by more than 2-fold and identified 71 spots by MALDI-TOF analysis (Figure 5A). A list of 23 proteins (Supplementary Table S1, available at Carcinogenesis Online) that exhibited a marked alteration in their expression pattern between SKH1 wild-type and caspase-7 KO mice after irradiation with SSL were noteworthy for further analysis. One spot identified as cystatin (Figure 5B, arrow), a protein overexpressed in various cancers (12), was induced in both SSL-irradiated SKH1 wild-type and caspase-7 KO mouse skin. The SSL-induced cystatin expression was more pronounced in caspase-7 KO mice than in SKH1 wild-type mice. Likewise, the expression of keratin-17 was 4.7-fold higher in SSL-irradiated caspase-7 KO mice (Figure 5C, arrow). The increased expression of keratin-17 was confirmed by western blotting with each tissue sample (Figure 5D). Bands were quantified and are shown as mean values ± SD (lower panels). To assess the status of keratin-17 expression in skin cancer, we analyzed a tissue microarray of skin cancer. Results showed that keratin-17 was overexpressed in basal cell carcinoma compared to normal tissues. In addition, the expression of keratin-17 was also significantly increased in squamous cell carcinoma and malignant melanoma, but not to the same extent as basal cell carcinoma (Figure 5E; Supplementary Figure S1, available at Carcinogenesis Online). Immunohistochemical analysis of skin sections revealed that the expression of keratin-17 was also increased in acute SSL-irradiated caspase-7 KO mouse skin (Figure 5F).

Keratin-17 is a direct target of caspase-7

To determine whether keratin-17 is a direct target of caspase-7, we performed in vitro enzyme activity assay. Recombinant caspase-3 and -7 were separately incubated with keratin-5 or keratin-17 as substrates (Figure 6; Supplementary Figure S2, available at Carcinogenesis Online). Results showed that incubation with caspase-7, but not with caspase-3, caused cleavage of keratin-17 (Figure 6). While caspase-7 induced cleavage of keratin-17, it did not cause cleavage of keratin-5 (Supplementary Figure S2, available at Carcinogenesis Online). These findings indicate that keratin-17 is a direct target of caspase-7.
Discussion

Apoptosis induced by UV irradiation is one of the physiological phenomena observed in skin carcinogenesis (13–15). Caspase-7, a key player in apoptosis, is reportedly linked to many types of human cancers. Our results indicated that tumor volume and number were more pronounced in SSL-treated caspase-7 KO mice.
KO mice compared with SSL-treated SKH1 wild-type mice (Figure 2A and B). The expression of cleaved caspase-3 was higher in SSL-treated caspase-7 KO keratinocytes and mice compared to wild-type (Fig. 3C, 4A and B). However, this might not be a complementary enhancement but rather an accumulation of caspase-3 caused by a blockade of downstream signaling because caspase-7 is a downstream signaling protein of caspase-3 (16). Thus, a dysfunctional caspase-7 might lead to more inflammation and cancer because of resistance to apoptosis. This suggests that caspase-7 is a critically important protein in SSL-induced apoptosis. Although caspase-7 KO mice are normal in appearance, organ morphology and lymphoid development (17), we found that the epidermal skin thickness of caspase-7 KO mice was thicker than that of SKH1 wild-type mice (Figures 1A and 3A). Our 2-DE gel and MALDI-TOF results indicated that keratin-17 was markedly increased in skin from SSL-treated SKH1 wild-type mice compared to SSL-treated caspase-7 KO mice (see Supplementary Table S1, available at Carcinogenesis Online). We also classified proteins identified by mass spec by function, and keratin-17 is notably involved in molecular functions and biological process and is an important cellular component (see Supplementary Table S2, available at Carcinogenesis Online; (18,19)). Keratin-17 is a type I filament protein that is involved in epithelial proliferation, tumor growth and angiogenesis (20–22). Notably, results of an in vitro caspase enzyme assay indicated that keratin-17 was cleaved by caspase-7 but not by caspase-3. This suggests that keratin-17 is a specific substrate of caspase-7. In the past, caspase-3 and caspase-7 have been widely believed to possess a similar substrate spectrum, including recognition of tetra-peptide (X-X-X-Asp) motifs, cutting the C-terminal of Asp and redundant functionality (23). On the other hand, numerous studies have suggested that caspase-3 and caspase-7 have different functions, given that caspase-3 KO mice are embryonic lethal, whereas caspase-7 KO mice are viable (24–26). These findings led us to determine whether caspase-7 is a specific protease for keratin-17. In the present study, we found...
that keratin-17 is a specific substrate of caspase-7, but not caspase-3. Furthermore, keratin-17 was up-regulated in caspase-7 KO mice compared to wild-type mice. Thus, keratin-17, which is cleaved by caspase-7, but not caspase-3, could be used as a biomarker for skin cancer patients with caspase-7 malfunction. Overall, our results strongly indicate that caspase-7 acts as an executioner protein in the regulation of SSL-induced skin carcinogenesis acting by down-regulating keratin-17.
Figure 6. Enzymatic activity of caspases against keratin-17. Radioactive-labeled keratin-17 was translated using the TNT Quick Coupled Transcription/Translation System in the presence of [35S]-methionine. Cleavage reactions were performed with or without recombinant caspase-3 or -7 as described in Materials and methods. Samples were separated by 12% SDS-PAGE. [35S]-labeled proteins were detected by autoradiography. The upper band indicates intact [35S] keratin-17 and the lower band designates cleaved [35S] keratin-17.

Supplementary material
Supplementary Tables S1 and S2 and Figures S1 and S2 can be found at http://carcin.oxfordjournals.org/

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Conflict of Interest Statement: None declared.

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