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p62 modulates the intrinsic signaling of UVB-induced apoptosis

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ABSTRACT

Background: UVB radiation is the main source of sunburn and skin cancers. Apoptosis eliminates photodamaged cells, and is thus important for preventing epidermal carcinogenesis. The cytoplasmic regulatory protein p62/A170/sequestosome 1 (p62) molecule is involved in a variety of cellular and signaling pathways. p62 is known to be and important in autophagy, but its role in UVB-induced apoptosis remains to be clarified.
Objective: To investigate the role of p62 against UVB-induced apoptotic changes, using mouse embryonic fibroblasts (MEFs) derived from p62 homozygous knockout (p62$^{-/-}$) mice.
Methods: p62$^{-/-}$ and wild-type (p62$^{+/+}$) mice and MEFs were subjected to UVB irradiation, and the resultant apoptosis was analyzed using flow cytometry, quantitative real-time PCR, and western blots.
Results: Apoptosis was decreased in the p62$^{-/-}$ MEFs compared to p62$^{+/+}$ MEFs in response to UVB treatment. Compared with p62$^{+/+}$ MEFs, p62$^{-/-}$ MEFs expressed significantly more Bcl-2 and less Bax, and showed increased Src and Stat3 phosphorylation. Our results show that p62 regulates apoptotic pathways by modifying critical signaling intermediates such as Src and Stat3.
Conclusion: p62 reduces UVB-induced apoptosis by modulating intrinsic apoptotic signaling through Src phosphorylation.

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1. Introduction

Ultraviolet (UV) radiation is one of the most important stimuli affecting the epidermis. UVB (280–315 nm) enter the dermis, which is only partially blocked by clouds or fog. UVB radiation can cause skin redness and is considered the main cause of sunburn and skin cancers. Epidermal cells that are irreparably damaged by excessive UV exposure are cleared by apoptosis to avoid malignant transformation; thus, apoptosis is important in preventing skin cancers [1,2].

UV-induced apoptosis is a complex event that involves several pathways, including the extrinsic and intrinsic apoptosis signaling

Abbreviations: ROS, reactive oxygen species; UV, ultraviolet; PCR, polymerase chain reaction; MEF, mouse embryo fibroblast; RIPA buffer, radioimmunoprecipitation assay buffer; PVDF, polyvinylidene difluoride; PI, propidium iodide; Bcl-2, B-cell lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; Bax, Bcl-2–associated X protein; PUMA, p53 upregulated modulator of apoptosis; Noxa, Phorbol-12-myristate-13-acetate-induced protein 1; ATM, Ataxia telangiectasia mutated; ATR, ATM- and RAD3-related; Stat3, signal transducer and activator of transcription 3; SRC, SRC proto-oncogene; PIKK, phosphatidylinositol 3-kinase-related kinases; UVRAG, UV radiation resistance-associated gene protein; Beclin1, Bcl-2 interacting protein 1.

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pathways. The extrinsic pathway acts through the fibroblast-associated tumor necrosis factor (TNF) receptor and other death receptors, which activate a caspase cascade in response to ligand binding. The intrinsic apoptosis pathway is regulated by the Bcl-2 family of proteins, which includes both anti-apoptotic (Bcl-2, Bcl-xl, Bcl-w) and pro-apoptotic (Bax, Bak, Bid) members. Balance between anti-apoptotic and pro-apoptotic proteins determines whether apoptosis is induced or prevented [1]. These Bcl-2 proteins are crucial regulators of epidermal homeostasis and cell fate in stressed skin, and are involved in skin carcinogenesis and responses to cancer therapy [3].

The cytoplasmic protein p62/A170/sequestosome 1 is a multifunctional signaling molecule that is involved in a variety of cellular pathways [4]. Murine p62, formerly called A170, was cloned as an oxidative-stress-inducible protein by our group [5]. p62 regulates autophagy by interacting directly with LC3 [6]; it also interacts with other key components of various signaling mechanisms, and is required for tumor transformation [7,8]. p62 has important roles in cell growth and cancer, through its functions as a regulator of autophagy, as a key factor in nutrient sensing and in genomic stability, and as an inducer of oxidative detoxifying proteins [4]. Many studies report crosstalk between autophagy and apoptosis [9], and p62 is associated with both of these functions. p62 is also implicated in activating the transcription factor NF-κB and in restraining ROS production and inhibiting tumor-cell death [7,8].

A previous study suggested that p62-deficient cells are highly proliferative, and that p62 inhibits proliferation [10]. In this study, we sought to clarify the role of p62 in UVB-induced apoptosis by focusing on the intrinsic apoptotic signaling pathway.

2. Materials and methods

2.1. Cell culture

All animal experiments were approved by the University of Tsukuba Animal Research Committee. Gene-recombination experiments were authorized by the University of Tsukuba Gene Recombination Experiment Safety Committee (authorization No. 14-369). We obtained $p62^{-/-}$ and $p62^{+/+}$ mouse embryonic fibroblasts (MEFs) as previously described [11]. Briefly, the brain and dark-red (internal) organs were dissected away from the embryos, the remaining tissue was finely minced, and the cells were dissociated using 0.25% trypsin, which was removed by

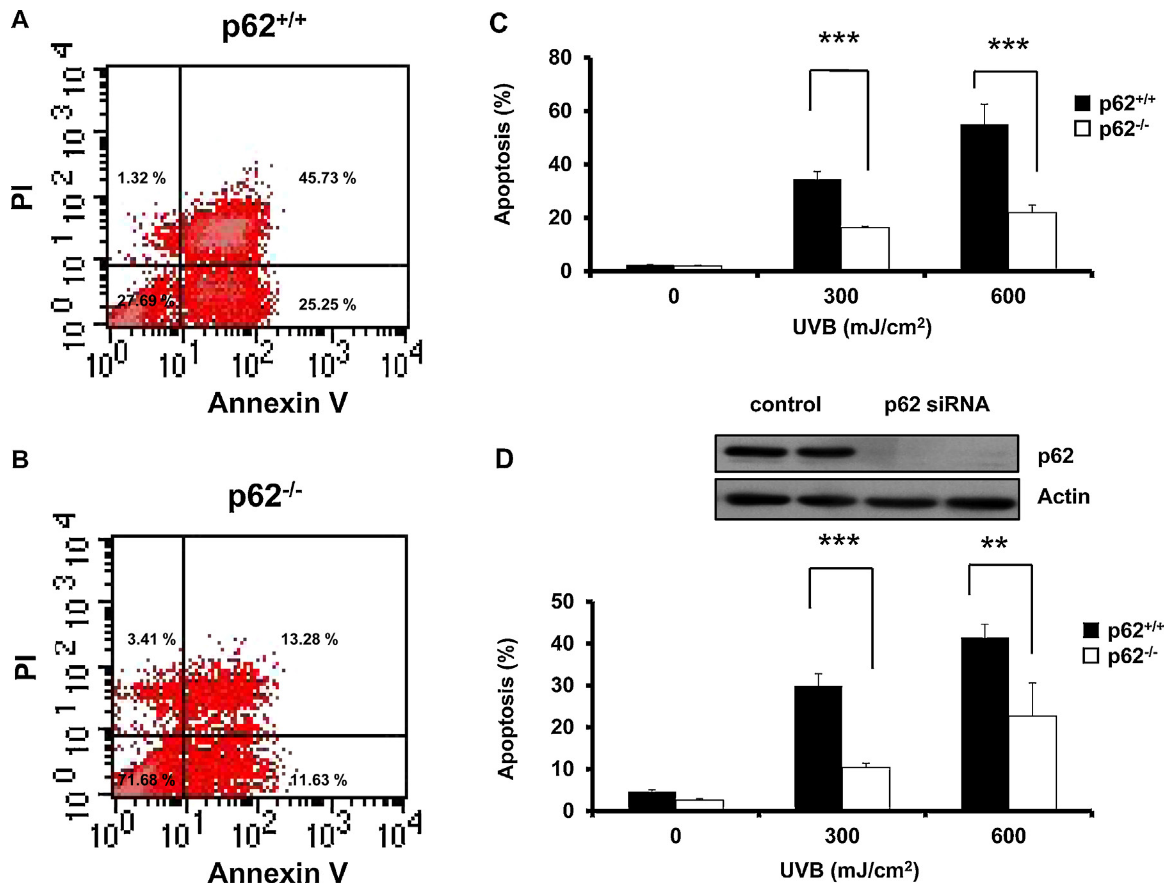

Fig. 1. Apoptosis ratio of p62-deficient cells, analyzed by flow cytometry.
Panels A and B: representative scatterplots of $p62^{+/+}$ and $p62^{-/-}$ MEFs, respectively. The upper-right area indicates cells in late apoptosis and the lower-right area indicates early apoptosis; $p62^{+/+}$ MEFs had more cells in the apoptosis area than did $p62^{-/-}$ MEFs.
Panel C: Apoptosis ratio of $p62^{+/+}$ (black bars) and $p62^{-/-}$ MEFs (white bars) with and without UVB irradiation, determined by flow cytometry analysis (FACS). The percentage of apoptotic cells was significantly lower in $p62^{-/-}$ MEFs than in $p62^{+/+}$ MEFs after UVB exposure at 300 and 600 mJ/cm^2.
The apoptosis ratio of $p62^{+/+}$ MEFs at 0, 300, and 600 mJ/cm^2 was 2.41 ± 0.12%, 34.47 ± 2.86%, and 55.14 ± 7.40%, respectively, and that of $p62^{-/-}$ MEFs was 1.97 ± 0.31%, 16.35 ± 0.46%, and 21.96 ± 2.84%, respectively.
Panel D: Apoptosis ratio of p62-knockdown HaCaT cells with and without UVB irradiation, determined by FACS. Upper panel, immunoblot for p62 demonstrating that p62 was absent in the p62-knockdown cells.
Compared to control cells (black bars), apoptosis was significantly reduced in cells treated with p62 siRNA (knockdown cells, white bars); cells were irradiated at 300 and 600 mJ/cm^2. The apoptosis ratio of control cells at 0, 300, and 600 mJ/cm^2 was 4.58 ± 0.48%, 29.87 ± 2.89%, and 41.41 ± 3.20%, respectively; that of p62-knockdown cells was 2.60 ± 0.31%, 10.37 ± 1.02%, and 22.70 ± 7.87%, respectively. Values are shown as mean ± standard deviation. $^{**}P < 0.01$, $^{***}P < 0.001$.

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