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Progressive Apoptosis Resistance Prior to Senescence and Control by the Anti-apoptotic Protein BCL-xL

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Abstract

Senescent cells are known to be resistant to apoptosis induced by genotoxic stress. Here we examine apoptosis in human diploid fibroblasts that are old but not yet senescent. We found that as cells aged, they became progressively more resistant to UV-induced apoptosis with an eventual apoptosis reduction of 10–20 fold. This behavior tracked a progressive disruption of the normal balance between pro- and anti-apoptotic proteins. In young cells, the level of anti-apoptotic protein BCL-xL quickly fell after UV irradiation while pro-apoptotic protein BAX rose. The increase in BAX tracked the level of P53, a transcriptional regulator of BAX. In older cells, the scenario was quite different. Instead of decreasing, the level of BCL-xL increased dramatically after UV stress so that the ratio of pro-apoptotic BAX to anti-apoptotic BCL-xL remained low. RNAi against BCL-xL restored the UV-sensitivity of old cells, indicating that BCL-xL is itself responsible for the pre-senescence decline in the ability of a genotoxic stress to induce apoptosis.

INTRODUCTION

Human cells stop dividing in culture at a point termed "replicative senescence" (Hayflick, 1992; Hayflick et al., 1961). Replicative senescence has been found to be accompanied by a resistance to apoptosis (Seluanov et al., 2001; Wang, 1995; Yeo et al., 2000), although it is not clear whether these two events need to be tightly linked. Loss of apoptosis, in turn, abrogates one of the protection mechanisms against neoplasia. For example, apoptosis-deficient mice accumulate pre-cancerous mutations in the epidermis when they are exposed to UV (Guzman et al., 2003; Hill et al., 1999; Zhang et al., 2005).

How cells become apoptosis resistant during *in vitro* senescence is incompletely understood. One contributor may be that senescent human fibroblasts fail to upregulate P53 after genotoxic stresses such as UV, actinomycin, cisplatin, or etoposide (Seluanov et al., 2001). Under normal conditions, P53 protein is post-translationally stabilized in response to a variety of stress signals. This stabilization can then initiate different programs such as cell cycle arrest, senescence, or apoptosis (Jin et al., 2001). In the case of apoptosis, the process requires both transcription-dependent and transcription-independent activities of P53 (reviewed in (Schuler

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et al., 2005)). It has also been shown that, in senescent human fibroblasts, P53 is preferentially recruited to the promoter of genes for cell cycle arrest (P21 and GADD45) but not those for apoptosis regulators (TNFRSF10b, TNFRSF6, and PUMA) (Jackson et al., 2006). Another mechanism postulated for the apoptosis resistance in senescent cells is a high level of the anti-apoptotic protein BCL-2 in senescent human fibroblasts (Wang, 1995). BCL-2 represses apoptosis by forming heterodimers with pro-apoptotic members of the BCL-2 family such as BAX (reviewed in (Burlacu, 2003)).

However, cells *in vitro* are not young one week and senescent the next. Many events occur between establishment of a primary fibroblast culture and eventual senescence. Fibroblasts accumulate mutations (Fulder et al., 1975; Morley, 1995), telomeres progressively shorten (Harley et al., 1990), and cells produce less collagen and secrete more matrix-degrading enzymes as passage number increases (Campisi, 1998). It is not known exactly when cells become apoptosis resistant. Is this property acquired gradually during aging of cells or does it arise with the senescent state?

In this paper, we examine apoptosis in pre-senescent primary human diploid foreskin fibroblasts. We first report that passaging cells results in progressive acquisition of resistance to ultraviolet-induced apoptosis. Next, we show that BCL-2 family proteins are involved in this UV-induced apoptosis resistance.

MATERIALS AND METHODS

Cells and irradiation

Primary human fibroblasts were derived from breast reduction tissue from a healthy 25-year old female. Cells were grown in high-glucose DMEM (Gibco Invitrogen) supplemented with 10% FBS and 1% penicillin/streptomycin. Fibroblasts were consecutively passaged at a 1:3 ratio to obtain the indicated passage number. Cells were UVB-irradiated at room temperature after replacing the medium with cold sterile phosphate buffered saline (PBS). The UVB source consisted of three fluorescent tubes (FS20T12/UVB/BP, Philips) filtered through a sheet of cellulose acetate to eliminate wavelengths below 290 nm (Kodacel TA-407 clear 0.015 inch; Eastman-Kodak Co.). This source delivered 72.6% UVB, 27.4% UVA, and 0.01% UVC as measured by an IL1700/790 spectroradiometer with double monochromator (International Light, Inc., Newburyport, MA) at a UVB (290–320 nm) dose rate of 2.39 J/m²/sec.

Immunoblots

Cells were plated at 60–70% confluency 24 hr before irradiating with 2000 J/m² UVB. They were harvested at different time points 0 to 24 hrs post-UVB and resuspended in RIPA buffer (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS in PBS, pH 7.4) containing protease inhibitor cocktail (Roche Applied Science). Lysed cells were centrifuged at 16,000g for 30min at 4°C and the cleared supernatant containing total soluble protein was applied on a 5–15% denaturing acrylamide gel (Bio-Rad, Hercules, CA). Following transfer to a nitrocellulose membrane, proteins were immunostained according to standard procedure. Primary antibodies (Santa Cruz Biotech, Santa Cruz CA) used were: P53 (clone DO-1), BCL-2 (clone C-2), BCL-xL (clone H-5), BAX (clone P-19), BAK (clone G-23) and actin (clone I-19). Autoradiograms were scanned and analyzed using ImageQuant 5.0 software (Molecular Dynamics).

Apoptosis

Human diploid fibroblasts were plated 24 hr prior to UVB irradiation. Sixteen hr post-irradiation cells were harvested and assessed for apoptosis using the Vybrant 3 Annexin V/propidium iodide apoptosis kit (Molecular Probes, Eugene, OR). This assay monitors the externalization of phosphatidylserine (PS) by annexin-FITC. In apoptotic cells, PS is

translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment (van Engeland et al., 1998). Necrosis was monitored by staining nucleic acid using propidium iodide (PI). PI is impermeant to live cells and apoptotic cells, but stains necrotic cells with red fluorescence, binding tightly to the nucleic acids in the cell. Apoptotic cells are stained in green by annexin-FITC and necrotic cells are stained both in red by PI and in green by annexin-FITC. Normal living cells show little or no fluorescence. The Annexin / propidium iodide stained cells were analysed using a Becton-Dickinson FACS Calibur flow cytometer on a two-color setting.

In BCL-xL RNAi experiments, many cells lysed due to the pro-apoptotic RNAi even prior to UV; because it was not known whether this death was necrotic or apoptotic, we instead measured survival using trypan blue to identify dead cells.

Senescence-associated β -galactosidase assay

The senescence-associated β -galactosidase assay was performed as published previously (Dimri et al., 1995). Briefly, human diploid fibroblasts at different passages (9, 36 and 49) were fixed in 3% formaldehyde for 5 min and incubated in freshly prepared staining solution (1 mg/mL of 5-bromo-4-chloro-3-indolyl β -D-galactoside (X-Gal), 40 mM citric acid/sodium phosphate buffer pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl and 2 mM MgCl₂) for 14 h at 37°C (without CO₂).

Terminal restriction fragment assay (TRF)

Terminal restriction fragment (TRF) length measurements were obtained using the Telo TTAGGG telomere length assay kit (Roche Molecular Biochemicals, Indianapolis, IN). Briefly, two micrograms of *HinfI/RsaI*-digested genomic DNA were separated on 0.8% agarose gels and southern blotted onto a Hybond-N+ nylon membrane (Amersham Biosciences, Piscataway, NJ). After UV-fixation of DNA fragments onto the membrane, membranes were hybridized with digoxigenin-labeled telomere-specific probe (TTAGGG)₄. After washing out non-bound probe, membranes were incubated with a telomere-specific antibody covalently coupled to alkaline phosphatase. Finally, the telomere fragments were visualized by a chemiluminescent substrate (CDP-star). TRF lengths were determined by comparing the signals relative to a standard molecular weight using ImageQuant 5.0 software (Molecular Dynamics). All lanes were divided into 75 intervals, and the mean TRF length was defined as $\Sigma(\text{OD}_i)/\Sigma(\text{OD}_i/L_i)$, in which OD_i is the chemiluminescent signal and L_i is the length of the TRF fragment at position *i*.

siRNA

Bcl-xL siRNA (Ambion, Austin, TX) and Luciferase siRNA (Dharmacon, Lafayette, CO) were used at a final concentration of 50 nM. Briefly, cells were plated at $\approx$ 50% confluency (400,000 cells / 60mm Petri dish) 24 hrs prior to the transfection in penicillin-streptomycin-free medium. The transfection was performed with oligofectamine (Invitrogen, Carlsbad, CA) at a ratio of 6:1 (vol:vol) with siRNA and incubated with cells in serum-free medium (Invitrogen Gibco, Carlsbad CA) for 4 hr. After the 4 hr incubation, serum was added at a final concentration of 10%. Bcl-xL inactivation occurred in 24–48 hr.

RESULTS

Human diploid fibroblasts become resistant to UVB-induced apoptosis as passage level increases

A UVB dose of 2000 J/m² induced 32% lethality (apoptosis plus necrosis) in young human fibroblasts (passage 9) at 16 hr post-UVB (Figure 1). As fibroblasts grew older, they became

less efficient at dying following a UVB stress. Lethality was 13% and 6% at passages 19 and 36, respectively. The apoptosis portion of this cell death followed a similar pattern: 19%, 10% and 1% at passage 9, 19 and 36, respectively. Similar results were obtained using 1000 J/m² (data not shown). An increase in apoptosis resistance with passage level was also observed in primary mouse fibroblasts (data not shown).

Human diploid fibroblasts are not senescent at these passage levels

Human diploid fibroblasts typically enter replicative senescence at approximately passage 50, so the apoptosis resistance we observe is presumably unrelated to senescence. To check the replicative senescence status of late passage cells, we used several techniques. First, early and late passage cells proliferated at the same rate (Figure 2A). Second, the senescence-associated β -galactosidase activity of cells at passage 36 was undetectable, as it was in cells at passage 9 (Figure 2B). Third, the basal level of P21, an indicator of growth arrest, was the same in early and late passage fibroblasts (Figure 2C). These results indicate that late passage cells are not in replicative senescence.

Telomere length decreases during these passages

It is well-documented that, with increasing passage level, the telomeres in primary human fibroblasts undergo telomere shortening (Harley et al., 1990). However, once human fibroblasts are immortalized (by ablating P53, using viral genes like SV40 large T antigen or HPV E6/E7, or viruses like HPV types 16/18), telomerase is reactivated and telomere length returns to that of young cells (Forsyth et al., 2004). Although our human fibroblasts were not senescent at passage 36, it remained possible that they had spontaneously immortalized. This would mean that they would never enter senescence. Telomere length was measured using the terminal restriction fragment (TRF) technique (see Materials and Methods). In our hands, human fibroblasts passaged 9 times had a mean telomere length of 10 kb, whereas the same fibroblast strain passaged 19 or 36 times had mean telomere lengths of 8.2 and 7.1 kb, respectively (Figure 3). The continual shortening of telomeres, even at passage 36, shows that these skin fibroblasts have not immortalized.

Acquired apoptosis resistance at late passage is associated with altered UVB-regulation of BCL family members

Apoptosis and apoptosis resistance are governed by a network of pro- and anti-apoptotic proteins (reviewed in (Mohamad et al., 2005; Twomey et al., 2005)). To determine the mechanism of passage-level dependent downregulation of UVB-induced apoptosis, we examined key members of this network.

P53—Figure 4, upper left panel shows that P53 accumulated as early as 4 hr after UVB irradiation in both early passage and late passage fibroblasts. However, this accumulation plateaued at 8 hr in early passage cells (passage 10) but in late passage cells (passage 37), the plateau appeared at 4 hr and the maximal level of induction was 40% lower.

BCL family—BCL family proteins are a large family of pro-apoptotic and anti-apoptotic proteins that regulate apoptosis at peri-mitochondrial sites. A cellular stress such as UVB is known to trigger induction of pro-apoptotic members like BAX and degradation of anti-apoptotic proteins like BCL-2 and BCL-xL (reviewed in Burlacu, 2003)).

Because P53 is a direct transcriptional activator of the pro-apoptotic protein BAX, we anticipated that regulation of BAX after UVB would track that of P53 (Maxwell et al., 1997; Miyashita et al., 1995). Accordingly, we found UV-induction of BAX in early passage fibroblasts but not in late passage cells, where the level remained unchanged after UV (Figure 4, upper right panel). The difference between the NoUV and the 24h lane in older cells is not

statistically significant (p value > 0.05 by two-tailed heteroscedastic Student's t -test). However, the basal level of BAX ("No" lane) was higher in older cells, in fact equal to the UVB-induced level in the young cells. Therefore, this result alone would not explain the apoptosis-resistance of older fibroblasts. Another key pro-apoptotic protein, BAK, was not upregulated by lethal UVB doses in either early or late passage cells (Figure 4, middle left panel). BAK, unlike BAX, is not known to be a transcriptional target of P53.

We then examined anti-apoptotic proteins. BCL-2 was downregulated by UVB in both early and late passage cells at 24 hr post-irradiation (Figure 4, middle left panel). No visible difference was seen between passage levels. The scenario was quite different for BCL-xL. As expected (Nakagawa et al., 2002), BCL-xL was rapidly downregulated in young fibroblasts beginning at 4 hr post-UVB. Strikingly, the basal BCL-xL level in old fibroblasts was instead rapidly upregulated after UVB and reached a plateau at 4 hr (Figure 4, middle right panel). BCL-xL acts by antagonistically binding to pro-apoptotic partners such as BAX. We therefore quantitated the change in BAX/BCL-xL ratio between low and high passage levels (passage 10 vs 37, respectively) (Figure 4, lower panel). In young cells (passage 10), this ratio increased 29 fold 24 hr after UVB but was unchanged in the old cells (passage 37) (p value at 8, 12 and 24 hr < 0.05 by two-tailed heteroscedastic Student's t -test; actin normalization had only a minor effect on these ratios). This result shows that control of UVB-induced apoptosis by BCL family members is dysregulated in older – but not senescent – human diploid fibroblasts.

Bcl-xL is a major contributor to UV-induced apoptosis resistance in older cells

An siRNA directed against Bcl-xL reduced the amount of basal BCL-xL protein by 75% in old human fibroblasts (Figure 5, top). This reduction in anti-apoptotic BCL-xL itself led to spontaneous death of 65% of the cells (Figure 5, bottom). Crucially, inactivating Bcl-xL with siRNA restored the UV-inducibility of death in old cells. A UVB dose of 1000 J/m² to Bcl-xL-inactivated old cells induced death in 26% of the original number of cells (i.e., the difference between the Bcl-xL siRNA NoUV bar (55% dead) and the Bcl-xL siRNA UV lane (81% dead). This 26% cell death is comparable to the 32% cell death found in UVB-irradiated young cells without Bcl-xL inactivation (Figure 1). If only the cells that survived siBcl-xL treatment are considered as the starting point, the fraction of UV-induced cell death is even greater (58% of the survivors are killed by UV). In contrast, UVB induced only ~ 5% cell death in aged cells not treated with siBcl-xL (Figure 1 and Figure 5). Thus, siBcl-xL allows a 5-fold increase in the level of UV-induced cell killing, restoring the youthful value.

DISCUSSION

Apoptosis resistance

The well documented apoptosis resistance of senescent cells (Seluanov et al., 2001; Wang, 1995; Yeo et al., 2000) has been assumed to arise with the senescent state. Data presented in this paper clearly show that human cells that are "old" but non-senescent (Figure 2) are also apoptosis resistant, with a decrease of as much as 20-fold. This apoptosis resistance is acquired gradually as cells are passaged in culture (Figure 1). One practical consequence of this finding is that it is no longer possible to assume that cells at different passage numbers have the same apoptosis phenotype.

The 20-fold decline in apoptosis in old diploid fibroblasts (passage 37) is unlikely to result from the 40% difference in P53 induction. Nor can the apoptosis-resistance phenotype be explained by a decrease in the paradigmatic pro-apoptotic protein BAX, which was in fact constitutively elevated in older cells. The other pro-apoptotic BCL family member known to be required for UV-induced apoptosis, BAK, was uninduced by UV at both high and low

passage. The paradigmatic anti-apoptotic protein BCL-2 cannot account for the acquired apoptosis resistance because its induction by UV did not change with passage level.

In contrast, the anti-apoptotic protein BCL-xL was relatively specific in demonstrating a strikingly aberrant behavior with increasing age. Instead of declining rapidly after UV stress as in younger cells (Nakagawa et al., 2002), BCL-xL protein level rose rapidly in UVB-irradiated older cells (Figure 4, middle right panel). BCL-xL has been shown to inhibit cell death induced by a number of apoptotic stimuli (Boise et al., 1993; Chao et al., 1995). Regulation of BCL-xL occurs at several levels. At the transcriptional level, the promoter of the BCL-x gene (which encodes for both BCL-xL and BCL-xS) contains consensus motifs for a large number of transcription factors (Sp1, AP-1, Oct-1, Ets, Rel/NF- κ B, STTS and GATA-1) (Grillot et al, 1997). The STAT, Rel/NF- κ B, and Ets transcription factor families have been reported to regulate BCL-x directly (Grad et al, 2000). At the post-translational level, BCL-xL is phosphorylated by SAPK/JNK after exposure to microtubule-damaging drugs (Poruchynsky et al, 1998; Basu et al, 2003). Which of these or other regulatory molecules are responsible for the progressive aberration in Bcl-xL regulation with cell age, and the cause of their own aberration, could be a fertile line of inquiry.

As expected, downregulating the basal level of BCL-xL by RNA interference itself induces apoptosis in aged human fibroblasts without further stress (Figure 5). This result indicates that Bcl-xL is an important factor in cell-death control even in old fibroblasts. This phenomenon has been described previously in other cell types (Lei et al., 2006; Liu et al., 2005; Xie et al., 2006; Yamanaka et al., 2006). Importantly, in addition to this spontaneous apoptosis, we were able to induce apoptosis by UVB in the Bcl-xL downregulated old cells at a level comparable to that seen in young cells. This result means that downregulating BCL-xL is sufficient to restore the apoptosis that the aged fibroblasts have lost.

The individual elements of the BCL-family apoptosis pathway are well understood and the expected subsequent behavior is clear. Evidence indicates that the ratio between anti-apoptotic and pro-apoptotic BCL-family proteins is essential to determining apoptosis after a lethal stress (reviewed in (Bouillet et al., 2002)). In a human leukemic cell line defective for Bcl-2 and P53, the ratio BAX/BCL-xL was constitutively low and the apoptosis could not be performed after stress stimuli. Transfecting BAX into these cells led to an increase in the BAX/BCL-xL ratio, increased homo-multimerization of BAX, and an increase in apoptosis after various stresses, including UV (Liu et al., 2004). In the present experiments, the protein level of BAX increased and BCL-xL decreased after UV in young fibroblasts, causing a profound increase in the ratio BAX/BCL-xL. This ratio is favourable to apoptosis (Figure 1). In older cells, however, this ratio remains low after UV (Figure 4, lower panel) and is unfavourable for apoptosis (Figure 1).

Adding to this behavior of BCL-family partners is the diminished P53 response. P53 has been reported to bind to BCL-xL and BCL-2 (Mihara et al., 2003). Binding to BCL-xL releases BAX from its partnership with BCL-xL (Chipuk et al., 2004). In our old cells, the low level of P53 in conjunction with the high level of BCL-xL after UV stress would lead to inefficient sequestration of BCL-xL and diminished release of BAX. Moreover, the known ability of P53 to bind BCL-xL leads us to hypothesize that the high level of BCLxL in late passage fibroblasts sequesters P53, preventing its action as a transcription factor for BAX. This effect could explain why the BAX level does not increase post-UVB in older fibroblasts.

Consequences of apoptosis resistance

The prevalence of fibroblasts in cell-senescence experiments tends to obscure the fact that fibroblasts have *in vivo* functions that can be impaired by deficits such as defective apoptosis.

First, apoptosis protects against the accumulation of pre-cancerous mutations by eliminating cells harbouring excessive DNA damage (Guzman et al., 2003; Hill et al., 1999; Zhang et al., 2005). Because non-senescent old cells are still dividing (Figure 2), their apoptosis deficiency will result in DNA replication past DNA lesions. A higher mutation rate can be expected and, in fact, mutations accumulate with age both in fibroblasts *in vitro* and *in vivo* (reviewed in (Vijg et al., 2005)). Clinically, older individuals may be less susceptible to sun induced apoptosis and therefore more susceptible to mutation that can lead to cancer. We attempted to study the mutation susceptibility of our older cells versus younger cells by using a 6-thioguanine selection assay. Mutations in the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene lead to 6-thioguanine resistance (Chiou et al., 1995; Kolman et al., 2002). Surprisingly, and perhaps interestingly, our late passage cells were 6-thioguanine resistant compared to low-passage cells (data not shown), preventing mutation induction experiments.

Apoptosis is also essential to wound repair (reviewed in (Rai et al., 2005)). The inflammation process is curtailed by apoptosis of inflammatory cells (neutrophils and macrophages). During wound maturation, fibroblasts need to be eliminated to reduce the production of collagen and concomitant vascularity (reviewed in (Rai et al., 2005)). Wound repair is impaired with aging (reviewed in (Gosain et al., 2004)). It has also been noted that apoptotic fibroblasts are less abundant in the dermal granulation tissue (the replacement tissue of a wound) of older rats than in younger rats (Ballas et al., 2001). It is possible that delayed skin wound repair of older individuals is due, at least in part, to an apoptosis defect in older fibroblasts.

Moreover, in Fisher-344 rats, aging is associated with a decrease in apoptosis in the colonic mucosa (Xiao et al., 2001). This apoptosis resistance is in part associated with the stimulation of anti-apoptotic Bcl-xL levels. It has been shown that apoptosis plays an important role in the development and progression of colon cancer (Green et al., 1994). This change in apoptosis is suspected by the authors to explain, at least in part, the increased incidence of colon cancer associated with advancing age.

Human skin fibroblasts are widely used in research. They are straightforward to obtain, easy to culture, and never spontaneously immortalize *in vitro* (McCormick et al., 1988). In this paper, we show that passaging fibroblasts in culture leads to pronounced apoptosis resistance. It is a reasonable hypothesis that a similar effect would occur in other cell types and it would, of course, be important to know whether proliferating cells *in vivo* acquire apoptosis resistance during the lifetime of the animal.

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Figure 1. UVB induced cell death decreases in late passage human diploid fibroblasts

Human fibroblasts from same donor but at different passage numbers (9 to 36) were UVB-irradiated (2000 J/m²). After 16 hr, apoptosis and necrosis were monitored by measuring the externalization of phosphatidylserine using annexin-FITC and by staining of nucleic acid using propidium iodide, respectively (see Materials and Methods). Each point is a mean of 3 different experiments with standard deviation error bars. The two-tailed heteroscedastic Student's t-test showed a significant difference ($p < 0.05$) for cell death between fibroblasts at passage 9 vs 19 and 9 vs 36. The same test showed a significant difference for apoptosis between cells at passage 9 and 36.

Figure 2. Human diploid fibroblasts at passage 36 are not senescent

Senescence status of human fibroblasts from same donor was measured at different passage numbers by several techniques. (A) Cells were plated and counted every 12 hr using a hemacytometer. The resulting growth curves show no significant difference in growth rate between low passage (passage 9) and late passage (passage 36) fibroblasts. The population doubling time was ~ 15.8 and 17.2 hours for passages 9 and 36, respectively. (B) Senescence-associated β -galactosidase activity is almost undetectable (<5%) in cells at passage 9 (x9) or at passage 36 (x36). However when same cells are at passage 49, they clearly show the senescence-associated β -galactosidase activity phenotype in about 60% of the cells (blue staining). (C) Basal P21 protein level was monitored by immunoblot. When normalized to

actin, no significant difference is seen between the basal P21 level in early and late passage cells (see the graphical representation of the quantification under the immunoblot).

Figure 3. Telomere shortening induced by passaging human fibroblasts

Telomere length of human diploid fibroblasts from the same donor at different passage numbers (9, 19 or 36). Telomere length was measured using the TRF technique. The quantification of the autoradiogram is shown in the lower part of the figure.

Figure 4. UVB induction of apoptosis-regulatory proteins is dysregulated during *in vitro* aging of non-senescent fibroblasts

Human diploid fibroblasts at passage 10 or 37 were irradiated with 2000 J/m² UVB and harvested for protein extraction at different time points 0–24 hr later. The “No” lane represents the basal level of the corresponding protein in the non-irradiated control. The quantitation of P53, BAX and BCL-xL westerns is shown below the corresponding panel. Actin is used as a protein loading control. Blots are a representative example of at least three experiments. The lower panel shows BCL-xL deregulation expressed as the normalized BAX/BCL-xL ratio.

Figure 5. Inactivating Bcl-xL in old cells restores UV-induced apoptosis

Bcl-xL siRNA at 50 nM reduced BCL-xL protein by 75% in late-passage cells (passage 37) (top panel). Approximately 64% of the initial cells were dead at 36 hr post-Bcl-xL-siRNA transfection, without UVB irradiation (lower panel). BCL-xL inactivation combined with UVB irradiation (1000 J/m²) killed more than 80% of old cells, an increase of 26% that is comparable to the UV-induced killing in young cells (Fig. 1). The same UVB dose killed only 5% of non-siRNA-treated old cells (lower panel), so siBcl-xL conferred a 5-fold increase in UVB sensitivity and restored the youthful level.