

Keratinocyte Apoptosis in Epidermal Development and Disease

Deepak Raj^{1,2}, Douglas E. Brash^{3,4} and Douglas Grossman^{1,2,5}

Keratinocyte (KC) apoptosis plays a critical role in regulating epidermal development and restraining carcinogenesis. Apoptosis balances proliferation to maintain epidermal thickness, contributes to stratum corneum formation and may eliminate pre-malignant cells. Apart from the normal developmental program, KC apoptosis can be triggered by UV light and other stimuli. Dysfunctional apoptosis occurs in some skin diseases, such as psoriasis and skin cancer. Here we review the current state of knowledge of KC apoptosis, with particular focus on apoptotic signaling pathways and molecular mechanisms of apoptosis control, and discuss new insights into the complex role of apoptosis in skin carcinogenesis that are emerging from mouse models.

Journal of Investigative Dermatology (2006) **126**, 243–257. doi:10.1038/sj.jid.5700008

Perhaps the greatest irony in biology is that life depends on death — programmed cell death, that is. Cell elimination by apoptosis is essential for organ sculpting in development, normal tissue turnover, and efficient

removal of infected or damaged cells and autoreactive lymphocytes. It is estimated that each day, in an average adult, over 50 billion cells undergo apoptosis (Reed, 1999). It has been only 30 years since Olson and Everett (1975)

first described apoptosis in normal skin, shortly after the term was coined in the medical literature in 1972 (Kerr *et al.*, 1972). In the following years, Horvitz, Sulston, and Brenner discovered several *Caenorhabditis elegans* death (ced)

Editor's Note

An organism's or an organ system's ability to grow and renew itself requires both cell proliferation and regulated cell death. A regulated form of cell death has been noted since at least the mid-1800s, and in 1964 Lockshin and Williams coined the term “programmed cell death” to describe developmental events in insect larvae (*J Insect Physiol* 10:643–649, 1964). In 1972 Kerr, Wyllie, and Currie used the term apoptosis to describe morphologic features of dying cells observed in a variety of organs and conditions (*Br J Cancer* 26:239–257, 1972). Early in the story of apoptosis, Kerr and Searle found the prominent shrinkage necrosis of the cells that is characteristic of apoptosis in basal cell carcinomas (*J Pathol* 107:41–44, 1972). In 2002 Brenner, Horvitz, and Sulston were awarded the Nobel Prize in Medicine for their discoveries concerning “genetic regulation of organ development and programmed cell death.” Because skin and hair constantly renew themselves and

are subject to environmental damage, programmed cell death is critical to their normal function. In this issue of the *Journal*, the Perspectives series continues with two articles on apoptosis in keratinocytes and in the hair follicle. Raj, Brash, and Grossman offer insight into the process of apoptosis in development and diseases of the keratinocyte. They provide a special focus on the regulation of apoptosis in the skin and its potential application for our understanding of carcinogenesis in the skin. Botchkareva, Ahluwalia, and Shander extend the focus to include the hair follicle. Understanding how the process of apoptosis applies to the hair cycle provides great opportunity for future investigations regarding the pathogenesis of many hair disorders as well as the hair loss associated with aging. These Perspectives on apoptosis will provide a platform for further investigation of this exciting and important area of the biology of skin and skin diseases.

Russell P. Hall

¹Huntsman Cancer Institute, University of Utah Health Sciences Center, Salt Lake City, Utah, USA; ²Department of Oncological Sciences, University of Utah Health Sciences Center, Salt Lake City, Utah, USA; ³Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut, USA; ⁴Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA; and ⁵Department of Dermatology, University of Utah Health Sciences Center, Salt Lake City, Utah, USA;

Correspondence: Douglas Grossman, Huntsman Cancer Institute, Suite 5243, 2000 Circle of Hope, Salt Lake City, Utah 84112, USA.
Email: doug.grossman@hci.utah.edu

Abbreviations: ASK1, apoptosis signal-regulating kinase-1; ced, *Caenorhabditis elegans* death; DLK, dual leucine zipper-bearing kinase; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; Fas-L, Fas ligand; IAP, inhibitor of apoptosis (gene family); KC, keratinocyte; MAPK, mitogen-activated protein kinase; SCC, squamous-cell carcinoma; TNF, tumor necrosis factor

Manuscript received 5 July 2005; revised 16 August 2005; accepted for publication 2 September 2005

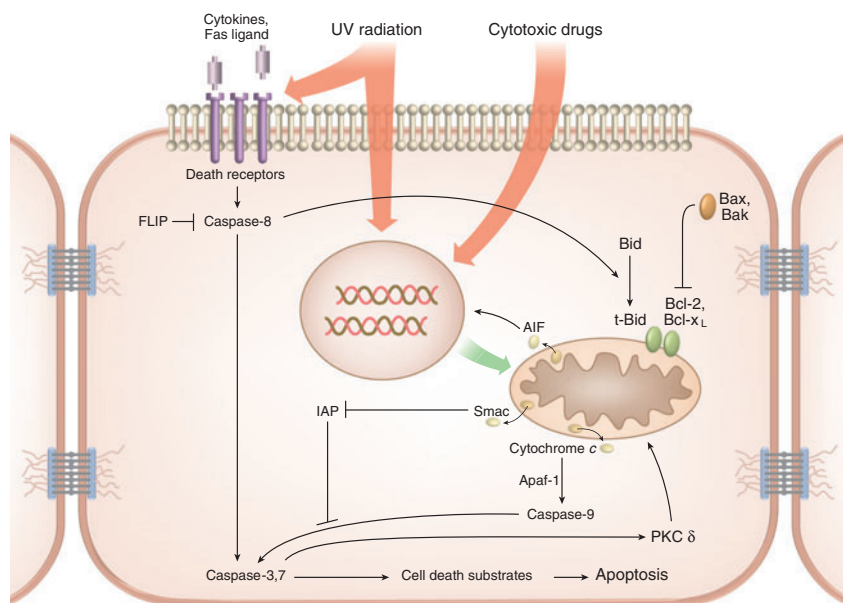


Figure 1. Apoptotic pathways in keratinocytes. The extrinsic pathway is stimulated by UVB or binding of Fas ligand, tumor necrosis factor, or other cytokines to death receptors that results in activation of caspase-8. The intrinsic pathway is also stimulated by UVB, as well as various cytotoxic drugs, and is linked to DNA damage responses. It involves mitochondrial depolarization and release of multiple proapoptotic factors. In addition, UVB results in formation of DNA photo-products and initiation of DNA damage responses. Mitochondrial content release may directly result from these stimuli or may occur indirectly as part of the DNA damage response. Cytochrome c promotes activation of caspase-9, Smac inhibits inhibitor of apoptosis (IAP) caspase inhibitors, and apoptosis-inducing factor (AIF) translocates to the nucleus and mediates caspase-independent apoptosis. The extrinsic and intrinsic pathways are potentially linked by Bid, which is cleaved by caspase-8, and the activated fragment (tBid) causes mitochondrial content release. Activation of upstream caspases (caspase-8 or -9) leads to activation of downstream caspases (caspase-3 or -7), resulting in cleavage of intracellular substrates, cellular condensation, and nuclear fragmentation. Apoptotic inhibitors include caspase inhibitors (FLIP, IAP proteins) and the Bcl-2 family proteins Bcl-2 and Bcl-x_L that prevent mitochondrial membrane permeability.

genes and established that apoptosis represents a genetically encoded pathway in worms, in work for which they were recently awarded a Nobel Prize. The discovery of *bcl-2* translocation in follicular lymphoma in 1984 eventually led to the first link between apoptosis and cancer. In the past decade, studies in mammalian cells have characterized multiple interconnected apoptotic pathways and identified a multitude of additional regulatory factors. A review of our current state of knowledge of keratinocyte (KC) apoptosis, focusing on apoptotic signaling pathways and molecular mechanisms of apoptosis control, is particularly timely because it has been 12 years since the topic of apoptosis in epidermal development and disease was last reviewed in the Journal (Haake and Polakowska, 1993).

In no other organ system does apoptosis play so many vital roles as in the

skin. Apoptotic cell death is critical for balancing of KC proliferation as well as for formation of the stratum corneum. Apoptosis also represents an important cancer defense mechanism, as KCs that may have accumulated mutations or sustained other genetic damage as a consequence of exposure to UV radiation or oxidative damage are eliminated by apoptosis. The development of genetic mouse models in which apoptotic regulatory molecules are deleted or overexpressed in the skin has validated many of these functions of apoptosis. Here, we will first summarize the basic components of the cell death machinery and their integrated function in apoptotic pathways in KCs. Next, we will describe KC apoptosis *in vivo* and consider the evidence that KC cell death in epidermal development represents apoptosis rather than an alternate form of cell death. The essential role

of apoptosis in maintaining epidermal structure and homeostasis will be reviewed, as well as its dysregulation in skin disease and skin cancer. Finally, we will discuss how KC apoptosis serves as a cancer-preventive mechanism by eliminating potentially pre-malignant cells and may modulate early steps in skin carcinogenesis.

General Pathways and Mechanisms of Apoptotic Control

Early work on apoptosis in worms elucidated a sequential genetic pathway consisting of an activator molecule (EGL-1), an inhibitor molecule (ced-9), an adapter molecule (ced-4), and an effector molecule (ced-3). This paradigm of activators, inhibitors, adapters, and effectors is recapitulated in multiple apoptotic pathways in mammalian cells. Apoptotic pathways and regulators potentially important in KCs are depicted in Figure 1, and reviewed extensively elsewhere (Reed, 1999). Cells respond to environmental, extracellular, and internal death signals through multiple sensors that coordinate and integrate apoptotic responses. The 'extrinsic' pathway is stimulated by binding of Fas ligand (Fas-L), tumor necrosis factor (TNF), or related cytokines to extracellular membrane 'death receptors' that recruit adapter molecules and lead to activation of caspase-8. Caspases (ced-3 homologues; cysteine aspartic acid-specific proteases) are constitutive proenzymes that autoactivate or are activated upon cleavage by other caspases, resulting in a proteolytic cascade. Some cells express FLIP, an inhibitor of caspase-8 that blocks death receptor signaling. The 'intrinsic' pathway, triggered by most cytotoxic drugs and DNA damage, involves mitochondrial release of cytochrome c, which combines with the cofactor Apaf-1 (ced-4 homologue) in the formation of an activated caspase-9 'apoptosome.' Mitochondria may also promote apoptosis through release of Smac/DIABLO, which blocks inhibitor of apoptosis (IAP) proteins that function as caspase inhibitors, and apoptosis-inducing factor (AIF), which translocates to the nucleus and mediates caspase-independent apoptosis. Release of these proapoptotic factors

is regulated by Bcl-2 family proteins that form homodimers and heterodimers that control mitochondrial membrane permeability. Some Bcl-2 family proteins (Bcl-2, ced-9 homologue, and Bcl-x_L) may block apoptosis whereas others (Bax, Bak, and Bid) promote apoptosis by interfering with these interactions. The p53 tumor suppressor promotes apoptosis through both transcriptional and non-transcriptional mechanisms, as detailed below. The extrinsic and intrinsic pathways are potentially linked in an amplification loop by Bid, which is cleaved by caspase-8, and the resulting fragment (tBid) translocates to the mitochondria and triggers cytochrome c release and activation of caspase-9. Activation of either of these upstream caspases leads to activation of terminal caspase-3 and caspase-7, which dismantle cells by cleaving proteins involved in nuclear membrane and cytoskeletal structure, DNA repair, and replication systems.

The Apoptotic Program in Keratinocytes

Apoptotic stimulation in keratinocytes.

It is important to note that the characterization of many of these pathways and regulatory networks — indeed, much of our mechanistic knowledge of apoptosis in general — comes largely from *in vitro* studies in HeLa cells or other cell lines. Similarly, many mechanistic studies on KC apoptosis have used the human HaCaT cell line (which harbors two mutant *p53* alleles) as a model for normal KCs. We have actually found HaCaT cells to be more susceptible to apoptosis than normal KCs *in vitro* (Bowen *et al.*, 2003), possibly because of aberrant signaling pathways resulting from long-term culture (Chaturvedi *et al.*, 2001). In addition, most studies examining apoptosis in normal KCs have been performed on cultured cells in the presence of exogenous growth factors. Apoptotic responses *in vivo*, however, necessarily occur in distinct cellular and tissue contexts. Therefore, after a summary of observations made in cultured HaCaT cells and KCs, the remainder of this review will focus on studies using epidermal constructs, *in situ* detection methods, and animal models that may be most relevant to KC apoptosis *in vivo*.

A variety of agents have been shown to induce KC apoptosis *in vitro*. The most intensively studied, and perhaps most physiologically relevant, is UVB (290–320 nm). Although UVB induces expression of multiple genes in KCs, execution of the apoptotic program requires not *de novo* transcription but rather activation of pre-synthesized proteins. Multiple studies have shown that doses ranging from 200 to 700 J/m² induce KC apoptosis over a 24- to 48-hour period that is preceded by mitochondrial depolarization, release of cytochrome c, and activation of multiple caspases (caspase-3, -8, and -9). UVB triggers multimerization of Fas death receptors, leading to activation of caspase-8 (Takahashi *et al.*, 2001a), and caspase-8-mediated cleavage of Bid is important for amplification of the apoptotic response (Assefa *et al.*, 2000). In most KC-based systems, UVB-induced apoptosis appears to be caspase-dependent, as responses can be blocked with caspase inhibitors, although induction of apoptosis-induc-

ing factor-mediated caspase-independent apoptosis has not been rigorously examined. Although caspase-8 and caspase-9 appear to be concomitantly activated in response to UVB, Sitailo *et al.* (2002) showed that upstream caspase-9 is critical for these responses, by blocking with specific peptide or dominant-negative caspase-9 inhibitors. Consistent with this observation, UVB-induced KC apoptosis can be blocked by transfection of Bcl-2 (Takahashi *et al.*, 2001b). One target of activated terminal caspase-3 is protein kinase C δ , which, upon cleavage, translocates to the mitochondria. Blocking protein kinase C δ activation with a kinase-inactive catalytic domain (Denning *et al.*, 2002) or caspase-resistant mutant (D'Costa and Denning, 2005) prevents UVB-induced KC apoptosis. Recent studies by Denning and colleagues (L Sitailo, SS Tibudan and MF Denning, 2005, *J Invest Dermatol* 124:A129) indicate that activated protein kinase C δ phosphorylates the Bcl-2 homologue Mcl-1 *in vitro* and disrupts

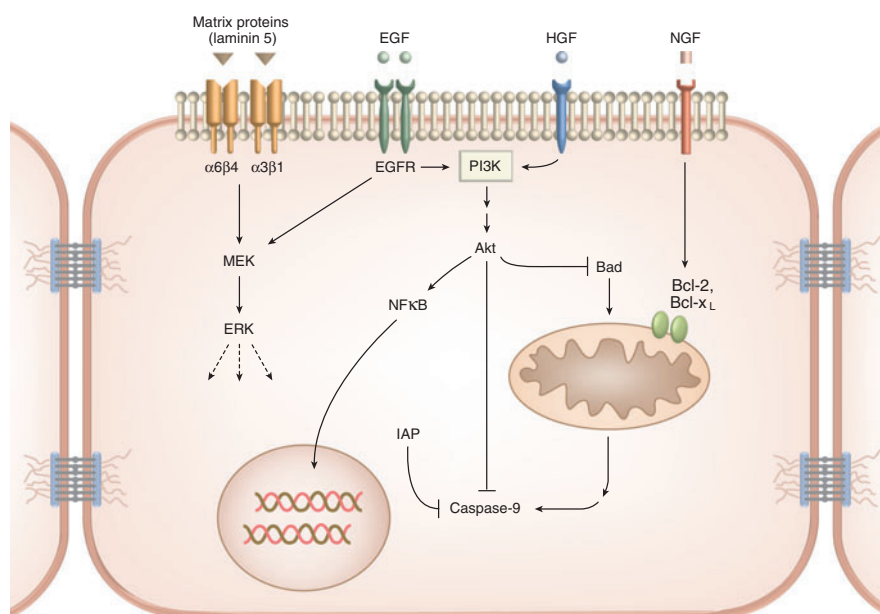


Figure 2. Survival (antiapoptotic) pathways in keratinocytes. The two primary survival pathways involve activation of a mitogen-activated protein kinase (MAPK) cascade and activation of Akt. Integrin-mediated interactions with matrix proteins, particularly laminin 5, as well as signaling through epidermal growth factor receptor (EGFR), lead to sequential phosphorylation and activation of MAPKs. The precise mechanism(s) by which activated MAPK kinases (MEKs) promote cell survival is not known (dotted arrows). Signaling by epidermal growth factor (EGF) or hepatocyte growth factor (HGF) may also stimulate a second survival pathway involving phosphatidylinositol 3-kinase (PI3K), leading to phosphorylation and activation of Akt. Activated Akt promotes survival by activating the transcriptional regulator NF- κ B and counteracts apoptosis by phosphorylating and inhibiting Bad and caspase-9. Caspase-9 is also subject to inhibition by IAP proteins. Finally, signaling by nerve growth factor (NGF) stabilizes levels of the antiapoptotic proteins Bcl-2 and Bcl-x_L. ERK, extracellular signal-regulated kinase.

its interaction with Bax in HaCaT cells. Early apoptotic signaling by UVB in KCs stimulates phosphorylation and stabilization of the p38 mitogen-activated protein kinase (MAPK), which occur within 2 hours of UVB exposure and precedes caspase activation (Shimizu *et al.*, 1999). The function of various MAPK pathways in KCs has been previously reviewed by Eckert and colleagues (Eckert *et al.*, 2002). Inhibition of p38 MAPK activation prevents UVB-induced apoptosis, translocation of Bax to mitochondria, and cytochrome c release (Van Laethem *et al.*, 2004). Lipid peroxidation and generation of oxidative radicals occur in KCs after UVB exposure (Carini *et al.*, 2000), and antioxidants may be protective (Kulms *et al.*, 2002; Mu *et al.*, 2003). Oxidative stress may directly activate p38 MAPK (Peus *et al.*, 1999). As noted below, UVB-induced KC apoptosis *in vivo* involves both membrane-based signaling and DNA damage. Finally, UVB can activate apoptotic pathways through induction of ceramide synthase and *de novo* ceramide synthesis (Uchida *et al.*, 2003).

Other UV wavelengths may also induce KC apoptosis *in vitro*, with apoptotic potency corresponding to increasing energy or decreasing wavelength (Godar, 1999). Although UVC (100–290 nm) damages cellular DNA and is a more potent apoptotic stimulus than UVB, it is largely absorbed in the atmosphere and does not reach the Earth's surface. On the other hand, UVA (320–400 nm), which is less potent than UVB, appears to trigger apoptosis through oxidative damage (Fu *et al.*, 2000). In addition to UV radiation, other apoptotic stimuli reported for KCs include the death receptor agonist TNF-related apoptosis-inducing ligand (TRAIL), rapid detachment (anoikis), growth factor deprivation, ceramide, cytotoxic drugs, 1,25-dihydroxyvitamin D₃, and transforming growth factor β1 (Benassi *et al.*, 1997). Apoptosis induced by these agents generally involves activation of caspases and mitochondrial pathways, but the mechanisms are less well characterized than those involving UVB.

The majority of *in vitro* studies on KC apoptosis have been performed on human KCs. Recently, Chaturvedi and

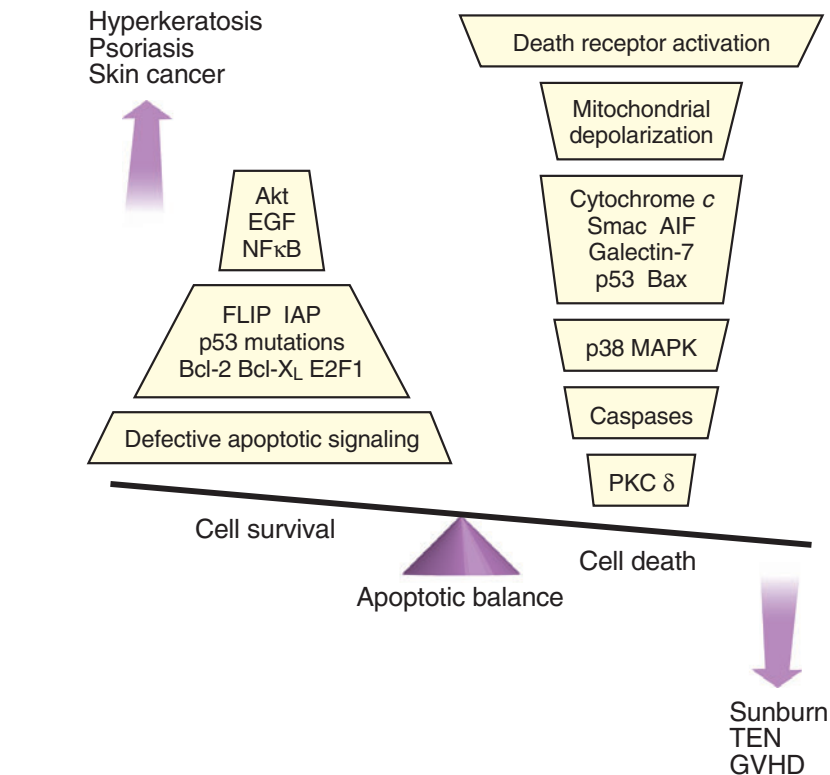


Figure 3. Apoptotic balance in keratinocytes is mediated by multiple factors at multiple levels.

Predominance of cell survival factors is evident in psoriasis, skin cancer, and hyperkeratotic diseases, whereas excessive cell death is characteristic of other diseases, including sunburn, toxic epidermal necrolysis (TEN), and graft-versus-host disease (GVHD). PKCδ, protein kinase Cδ.

colleagues (Chaturvedi *et al.*, 2004) compared apoptotic responses in cultured human versus mouse KCs and reported that human KCs were more susceptible to UV- and drug-induced apoptosis. The relative apoptotic resistance of mouse KCs was associated with decreased constitutive levels of p53 and increased expression of keratin 1, an early marker of KC differentiation. Although further determinants of this differential apoptotic susceptibility remain to be defined, these experiments suggest that mouse KCs maintained *in vitro* may exist in a more differentiated state and be less prone to p53-dependent apoptosis. Nevertheless, p53-dependent apoptosis of mouse KCs *in vivo* can readily be stimulated by UVB, as detailed below.

Antiapoptotic forces in keratinocytes.

As is discussed above, the primary KC signaling pathway leading to cell death is activation of p38 MAPK, followed by mitochondrial depolarization and cas-

pase activation. This cell death pathway is promoted by p53 and Bax, which are minimally expressed in proliferating KCs (Bowen *et al.*, 2003). On the other hand, this pathway may be antagonized in normal KCs through expression of multiple antiapoptotic regulators including Bcl-x_L, FLIP, and various IAP molecules (c-IAP1, c-IAP2, XIAP, and Livin) (Bowen *et al.*, 2003). Two notable cell survival pathways have been partially characterized in KCs. The first is a MAPK pathway (Figure 2) triggered by epidermal growth factor (EGF) and phosphorylation of epidermal growth factor receptor (EGFR). Whereas EGF can protect KCs against UVB-induced apoptosis, EGFR inhibitors enhance UVB-induced apoptosis (Peus *et al.*, 2000). Signaling through EGFR stimulates a MAPK cascade that results in sequential phosphorylation and activation of MAPK kinases (MEKs) and extracellular signal-regulated kinases (ERKs). The mechanism by which activated ERKs promote cell survival in

KCs has not been clearly elucidated. Signaling by EGF may also stimulate a second survival pathway involving phosphatidylinositol 3-kinase-mediated activation of the serine/threonine kinase Akt (protein kinase B; Figure 2). In other cell types, Akt is activated by growth factors and promotes cell survival directly by phosphorylating (and inactivating) Bad and caspase-9 (Datta et al., 1999). Given the important role of caspase-9 in KC apoptosis, noted above, it is not surprising that Akt is a potent KC survival factor (Wang et al., 2003). Akt also promotes survival in KCs by activating NF- κ B, a transcription factor that is repressed by interaction with κ B inhibitor (I κ B). The role of NF- κ B in KC survival has been previously reviewed in the Journal by Nickoloff and colleagues (Nickoloff et al., 2002), who have shown that inhibition of NF- κ B using proteasome or I κ B subunit inhibitors increases KC susceptibility to apoptosis. Activation of NF- κ B through Akt signaling may promote KC survival by modulating expression of IAPs or TRAF death receptor adapters, as has been shown in other cell types.

Finally, in discussing cell death and survival pathways in KCs, it is important to note that apoptotic stimuli may activate multiple pathways simultaneously. For example, several studies have demonstrated that UVB and TNF- α can activate the p38 MAPK death pathway and the MEK/ERK and Akt/NF- κ B survival pathways (Nickoloff et al., 2002). Products of the MAPK pathways may intersect via a p38 MAPK/ERK complex that translocates to the nucleus in KCs in response to protein kinase C δ activation (Efimova et al., 2004). Thus KCs must coordinate a delicate balance of many antiapoptotic and proapoptotic forces in responding to extracellular stimuli, shifting their equilibrium toward cell survival or cell death when one pathway predominates (Figure 3).

Most cell types are more susceptible to apoptosis *in vitro* than *in vivo*, and KCs are no exception. For example, lower doses of UVB are required to induce KC apoptosis in cultured cells compared with normal skin. Multiple survival signals present in tissues are likely to account for this increased KC resistance to apoptosis *in vivo*. Many

of these are derived from interaction with extracellular matrix proteins. Laminin 5 is the primary ligand for epidermal KC adhesion, binding to the α 3 β 1 and α 6 β 4 integrins on KCs. Using KCs derived from α 3 knockout mice, Manohar et al. (Manohar et al., 2004) showed that laminin 5 binding of α 3 β 1, and to a lesser extent α 6 β 4, promotes KC cell survival through activation of a MEK/ERK signaling pathway. This survival pathway can also be activated through EGFR, protecting KCs against anoikis (Jost et al., 2001). In addition to EGF, other growth factors constitute a second class of survival signals that may potentially contribute to apoptosis resistance *in vivo* through Akt. Nerve growth factor and hepatocyte growth (scatter) factor protect KCs against UVB-induced apoptosis by maintaining levels of Bcl-2 and Bcl-x_L (Marconi et al., 1999) and by activating Akt (Mildner et al., 2002), respectively. Nuclear peroxisome proliferator-activated receptors in KCs can also promote Akt signaling through transcriptional effects (Di-Poi et al., 2002). In many of these cases, KC apoptotic responses can be blocked by overexpression of apoptosis inhibitors such as Bcl-2 and the IAP protein Survivin (Grossman et al., 2001). Upregulation of endogenous apoptotic inhibitors of the Bcl-2 and IAP families can promote KC survival *in vivo* and, as discussed below, may play important roles in carcinogenesis.

Evidence for Keratinocyte Apoptosis *In Vivo*

Sunburn cells. After acute UVB exposure, scattered dyskeratotic KCs with condensed or absent nuclei and eosinophilic cytoplasm (termed 'sunburn cells') can be seen in mouse skin by routine hematoxylin and eosin staining. Sunburn cells represent KCs undergoing UVB-induced apoptosis and can be identified by *in situ* end labeling with terminal deoxynucleotidyl transferase (TUNEL technique) (Brash, 1997) and staining for activated caspase-3 (Pentland et al., 2004). Sunburn-cell incidence typically peaks 24 hours after irradiation, then gradually declines over several days (Okamoto et al., 1999). Using p53-deficient mice, Ziegler et al. (1994) demonstrated that sunburn-cell

formation requires p53. Expression of p53 is upregulated in UVB-treated KCs through increased protein stability (Liu et al., 1994). One transcriptional target of p53 is galectin-7 (PIG1), which parallels p53 stabilization and is highly expressed in sunburn cells (Bernerd et al., 1999). Overexpression of galectin-7 in KCs is sufficient to cause apoptosis (Bernerd et al., 1999), through caspase activation and cytochrome c release (Kuwabara et al., 2002). In addition to upregulation of p53, UVB-irradiated skin reveals upregulation of Bax and downregulation of Bcl-2 (Lee et al., 2003). Signaling through the Fas death receptor, which rapidly clusters following UVB exposure (Bang et al., 2003), appears to be involved in sunburn-cell formation, which is reduced in mice deficient in Fas-L (Hill et al., 1999). In addition, UVB-induced DNA damage is also necessary for sunburn-cell formation, which requires generation of cyclobutane pyrimidine dimers (Ley and Applegate, 1985) in actively transcribed genes (Brash et al., 2001). Activation of these apoptotic pathways by UVB is balanced by activation of the E2F1 transcription factor, which is upregulated in response to DNA damage and promotes DNA repair. Transgenic overexpression of E2F1 is associated with reduced UVB-induced apoptosis *in vivo* (Berton et al., 2005), and E2F1-deficient mice demonstrate enhanced susceptibility to sunburn-cell formation (Wikonkal et al., 2003).

Apoptotic versus non-apoptotic cell death. Although it is generally accepted that sunburn cells represent KCs induced to undergo apoptosis *in vivo*, some controversy exists over whether elimination of normal KCs in epidermal development occurs by spontaneous apoptosis or a 'non-apoptotic' form of cell death. The term 'apoptosis' was originally used to describe particular morphologic events observed in isolated cells of normal tissues and tumors (Kerr et al., 1972). Whereas cells undergoing pathologic cell death (necrosis) are characterized by organelle swelling and membrane rupture, with leakage of cell contents, that generates marked inflammation, apoptotic cells, by contrast, exhibit cellular condensa-

to be other granular layer proteins involved in stratum corneum formation. The availability of an inducible caspase-14 knockdown or knockout mouse would be particularly useful in further discriminating the role of caspase-14 from the roles of other caspases in epidermal differentiation and KC apoptotic responses.

Apoptosis in Epidermal Homeostasis and Stratum Corneum Formation

A multigenic developmental program.

Although the rates of KC proliferation and apoptosis are high in newborn skin and decrease with aging (Haake *et al.*, 1998), they remain matched throughout life to maintain epidermal homeostasis. Epidermal development is coordinated at each stage through sequential expression of multiple gene programs, as typified by the keratins (Fuchs, 1993) and summarized in Figure 4. In the basal layer, epidermal stem cells give rise to transient amplifying cells that proliferate and express keratins 5 and 14. In the spinous and granular layers, differentiating KCs terminate expression of keratins 5 and 14 and initiate expression of keratins 1 and 10. Two key signaling molecules activated by the MAPK cascade are expressed in the upper epidermis and appear to trigger the differentiation program: apoptosis signal-regulating kinase-1 (ASK1) and dual leucine zipper-bearing kinase (DLK). Introduction of activated ASK1 (Sayama *et al.*, 2001) or DLK (Robitaille *et al.*, 2005) in KCs is sufficient to induce morphologic changes and expression of the differentiation markers transglutaminase-1, loricrin, and involucrin. In the transitional layer, expression of caspase-14 correlates with the distinct changes seen in KC morphology. The 'apoptotic gene program' — unlike those associated with proliferation and differentiation — is not localized to one region but is manifest throughout the epithelium (Figure 4). For example, basal KCs are protected from apoptosis by expression of Bcl-2, which is absent from suprabasal KCs (Hockenbery *et al.*, 1991), and a gradient of Bak expression increases from lower to upper layers to promote cell death (Krajewski *et al.*, 1996). Similarly, expression of the p53 inhibitor MDM2 increases from the

basal to the suprabasal layer (Ganguli *et al.*, 2000). A more complex view of KC apoptotic control *in vivo* was revealed in experiments by Nickoloff and colleagues (Nickoloff *et al.*, 2002), who fractionated normal epidermal compartments by density gradient and assessed each for expression of various apoptotic components. They found that although some death receptors (TNF receptor 1, death receptor 4) and caspase-8 were expressed more intensely in lower-layer fractions, other molecules (death receptor 5, Decoy R2, caspase-14, Bcl-x_L) were more prevalent in upper-layer fractions.

Similarly, apoptotic control is critical for fetal epidermal development in establishing the final architecture of the epidermis and its appendages. Polakowska *et al.* (1994) showed that transglutaminases in fetal periderm localize to cells with DNA fragmentation that lack Bcl-2. The bone morphogenetic protein signaling pathways are important promoters of apoptosis during skin morphogenesis, as overexpression of the bone morphogenetic protein antagonist noggin is associated with retardation of KC differentiation and with downregulation of death receptors and the KC differentiation markers loricrin and involucrin (Sharov *et al.*, 2003). Although we have limited understanding of all the factors controlling these gene expression programs and directing KC transition to successive stages, these experiments and others noted above suggest that at least some components of the KC apoptotic machinery are involved in normal fetal and adult epidermal development.

Even though apoptotic morphology is not apparent until the transitional layer, it has been suggested that downregulation of $\beta 1$ integrins associated with KC exit from the basal compartment represents 'anoikis *in vivo*' and signals the initiation of apoptosis (Ishida-Yamamoto *et al.*, 1999). Although it is unclear at what point KCs are irreversibly committed to undergo apoptosis, the integration of the apoptotic machinery into the KC developmental program must be highly regulated so that apoptosis does not precede differentiation. Premature apoptosis is prevented by expression

and activation of survival factors including Bcl-2, Akt, and NF- κ B, as discussed above. Equally important, the timing of differentiation must be controlled so that apoptosis is not delayed. Is KC differentiation inextricably linked to cell death? Although forced expression of ASK1 or DLK, as noted above, induces differentiation but not apoptosis, many stimuli that induce KC differentiation *in vitro* do lead to apoptosis (Benassi *et al.*, 1997). Cultured KCs that become senescent, however, are resistant to apoptosis (Qin *et al.*, 2002). Although KC differentiation and apoptosis have been dissociated *in vivo* with caspase inhibitors as noted above and animal models as discussed below, it would also be interesting to examine the converse scenario in which differentiation (but not apoptosis) was blocked. The availability of genetic mouse models deficient in ASK1 or DLK, and one deficient in caspase-14 as suggested above, would be particularly useful in distinguishing the relationship and individual roles of differentiation and apoptosis in epidermal development.

Altered epidermal development in animal models.

The importance of apoptosis in epidermal development and homeostasis has been substantiated in several genetically modified mouse models. Many apoptosis-impaired mouse strains have been constructed by knockout of various apoptotic effector and regulatory molecules. Unfortunately, most of these were not constructed as conditional knockouts or with skin-specific gene deletions. Given the critical role of apoptosis in embryonic development, it is not surprising that many of these animals (particularly those deficient in caspases) exhibit prenatal or perinatal lethality that precludes assessment of skin phenotype. By contrast, most mouse models based on KC-specific transgenic expression or deletion of apoptotic regulators have proven viable and amenable for study.

Mice harboring a Bcl-2 transgene driven by the suprabasal keratin 1 promoter (HK1.bcl-2) display increased resistance to sunburn-cell formation (Rodriguez-Villanueva *et al.*, 1998). Although their skin appears clinically normal, HK1.bcl-2 mice have areas of

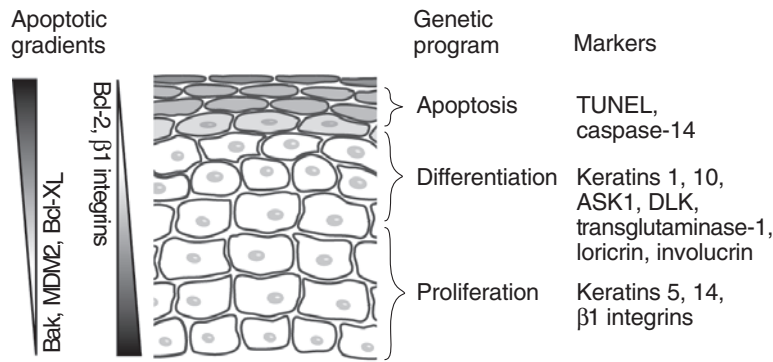


Figure 4. Genetic programs in epidermal development. Apoptosis maintains epidermal homeostasis, balancing cell death in the granular layer with proliferation in the lower layers. The proliferative gene program is characterized by a restricted pattern of keratin gene expression. The differentiation program directs changes in expression of keratin genes, master differentiation genes (apoptosis signal-regulating kinase-1 (ASK1), dual leucine zipper-bearing kinase (DLK)), and genes involved in stratum corneum formation. The apoptotic gene program is manifest throughout the epithelium, maintaining gradients of antiapoptotic and proapoptotic factors that regulate the timing of apoptosis in epidermal development.

tion and nuclear fragmentation and are rapidly cleared by phagocytosis without inflammation. Apoptosis is increasingly defined, however, at a biochemical rather than a cellular level by events such as DNA fragmentation and caspase activation, considered hallmarks of apoptosis. Failure in other systems to demonstrate one or more of these molecular features in the absence of necrosis has led to the suggestion that some cells may be eliminated *in vivo* by non-apoptotic forms of cell death (Leist and Jaattela, 2001).

Many morphologic features of apoptosis have been observed in the transitional layer of the epidermis. Transitional KCs lose their keratohyalin granules and develop condensation of cytoplasm and nuclei, which contain fragmented DNA (Ishida-Yamamoto *et al.*, 1998). There has been some difficulty, however, in detecting specific markers for apoptosis in the upper layers of the epidermis. For example, TUNEL staining often reveals surprisingly few positive cells in normal skin (Haake *et al.*, 1998). Studies in other tissues and tumors have found similarly low rates of spontaneous apoptosis (Staunton and Gaffney, 1995). One possible explanation for the rare detection of apoptotic cells *in vivo* is that they are efficiently removed from tissues. Although rapid clearance by phagocytic cells (presumably Langerhans cells, although cells capable of removing apoptotic cells

from epidermis have not been defined) may explain the decrease in the number of TUNEL-positive cells in basal and spinous layers, we know that most KCs undergoing spontaneous cell death in epidermis are not removed but persist to form the structural component of the stratum corneum. Therefore, failure to identify apoptotic KCs in the upper epidermis may reflect a technical limitation of the TUNEL technique in the granular layer and sub-stratum corneum.

Detection of caspase activation *in situ* initially appeared to be similarly problematic, as early studies identified precursor forms of most caspases, but not their activated fragments. Subsequent experiments demonstrated cytochrome c release and multiple caspase activation in reconstituted human epidermis (Allombert-Blaise *et al.*, 2003) and caspase-3 activity in human epidermal cytosolic extracts (Weil *et al.*, 1999), although other investigators failed to detect caspase-3 activation in mouse skin (Lippens *et al.*, 2000). Recent studies by Nickoloff *et al.* (BJ Nickoloff, B Bodner, L Sitailo, MF Denning, and V Charturvedi, 2005, *J Invest Dermatol* 124:A59) using human epidermal equivalents demonstrated activation of both initiator caspase-8 and -9 and effector caspase-3 during terminal differentiation. Moreover, addition of cell-permeable caspase inhibitors to a developing reconstituted epidermis resulted in

decreased filaggrin staining and retention of KC nuclei in the upper layers (Weil *et al.*, 1999) and prevented stratum corneum formation (Allombert-Blaise *et al.*, 2003). Thus caspase activation appears to be required for normal epidermal development.

Caspase-14, a keratinocyte-specific caspase. Several groups reported in 1998 a newly identified caspase — designated caspase-14 — that, unlike other known caspases that are expressed ubiquitously, is found only in skin (Van de Craen *et al.*, 1998). Caspase-14 expression is detectable in the basal layer and dramatically increases in the granular layer, with both the 28-kilodalton proenzyme and cleavage fragments of 17 and 11 kilodaltons visualized in extracts from whole human epidermis and stratum corneum (Eckhart *et al.*, 2000b). In mouse skin, a 35-kilodalton precursor is cleaved into 20- and 18.5-kilodalton peptides (Lippens *et al.*, 2000). Complementary experiments with HaCaT cells and normal human KCs *in vitro* demonstrated that caspase-14 was only expressed and processed under culture conditions mimicking terminal differentiation and could not be induced by death receptor signaling or UVB treatment (Lippens *et al.*, 2000). Splice variants encoding truncated caspase-14 molecules have been found in both human (Eckhart *et al.*, 2000a) and mouse (Kuechle *et al.*, 2001) skin, but their particular function is unknown. N-terminal sequencing of caspase-14 cleavage fragments suggests that, unlike processing of other caspases, which undergo either autolytic or caspase-mediated cleavage at aspartate residues, processing of caspase-14 occurs by a novel caspase-independent proteolytic mechanism (Chien *et al.*, 2002). The protease calpain is co-expressed with caspase-14 in the granular layer and may mediate its processing, as Kuechle and colleagues (AJ Chien, A Pirrone, and MK Kuechle, 2005, *J Invest Dermatol* 124:A67) recently reported that caspase-14 processing can be blocked in organotypic skin cultures by a calpain inhibitor. The targets of activated caspase-14 have not been defined but are likely

focal epidermal hyperplasia characterized by increased proliferative activity and aberrant expression of keratin 6. Mice deficient in Bcl-2 also have normal skin (Veis *et al.*, 1993) (with the exception of premature hair graying due to the importance of Bcl-2 for melanocyte survival) (Nishimura *et al.*, 2005), but sunburn-cell responses in these mice have not been reported. Spontaneous formation of apoptotic cells was seen in unirradiated mice with KC-specific deletion of Bcl-x_L, and these mice demonstrated a further increase in susceptibility to sunburn-cell formation after UVB exposure (Umeda *et al.*, 2003). Mice transgenic for the p53 inhibitor MDM2 (K14-MDM2) driven by the basal keratin 14 promoter exhibit a more dramatic phenotype, developing scaly skin in the first week of life (Ganguli *et al.*, 2000). Histologic examination of skin in K14-MDM2 mice revealed an epidermis with increased thickness and KC-cell number, and the presence of nucleated cells in the upper compartments. Detection of keratins 5 and 14 in suprabasal KCs provided further evidence that the differentiation program was also altered in K14-MDM2 mice. Mice with KC-specific deletion of the Akt inhibitor PTEN (K5-PTEN-flox/flox) develop epidermal hyperplasia and hyperkeratosis (Suzuki *et al.*, 2003). These examples appear to validate the role of apoptosis in maintaining epider-

mal homeostasis, controlling differentiation, and mediating UVB responses.

There are, however, additional mouse models in which an altered apoptotic state is not associated with perturbation in epidermal development. For example, mice transgenic for Bcl-2 under the control of a keratin 14 promoter have a normal-appearing epidermis despite increased apoptotic resistance in KCs (Rossiter *et al.*, 2001). Similarly, mice with keratin 14-driven transgenes expressing antiapoptotic Bcl-x_L (Pena *et al.*, 1997) and Survivin (Grossman *et al.*, 2001), as well as those deficient in p53 (Grossman *et al.*, 2001; Ziegler *et al.*, 1994), exhibit increased KC resistance to UVB-induced apoptosis yet normal KC differentiation and epidermal maturation. Thus, whereas in some cases, as noted above, manipulation of the KC apoptotic program can significantly affect KC differentiation and epidermal development, in many of these mouse models apoptotic dysregulation (as reflected by altered sunburn-cell formation) does not. Some of the variation in these effects may relate to differences in the levels of transgene expression (that is, promoter effects), as reflected by differences seen between hemizygous and homozygous K14-MDM2 mice (Ganguli *et al.*, 2000). Another factor potentially affected by the particular promoter used is the resulting KC compartment targeted, as promoters active only in stem or basal

cells tend to affect skin phenotype differently from those active in the suprabasal cell layers.

Dysfunctional apoptosis in skin disease

Diseases of increased keratinocyte apoptosis. As sunburn cells represent the histologic manifestation of isolated KC apoptosis, clinical sunburn is the classic example of generalized UVB-induced apoptosis. Although UVB activates both death receptor and mitochondrial apoptotic pathways in KCs, several skin diseases have been ascribed predominantly to excessive death receptor activation, as reviewed previously in the Journal by Wehrli *et al.* (2000). Death receptor signaling is triggered in KCs by cytokines and direct contact with intraepidermal lymphocytes, via TNF and Fas receptors, respectively. These interactions likely play a key role in the pathogenesis of toxic epidermal necrolysis and cutaneous graft-versus-host disease. Apoptotic KCs are also a feature of cutaneous viral infections, alopecia areata, Kindler's syndrome, incontinentia pigmenti, lichen planus, and other lichenoid reactions, but the role of excessive apoptosis in these disorders is poorly understood and hence will not be discussed further. Although apoptosis-based therapies have had limited use in patients, many have been demonstrated in animal models or are in clinical development (Table 1).

Table 1. Summary of skin diseases with dysregulated apoptosis

| Skin disease | Apoptosis | Mechanisms | Potential therapies |
|--|-----------|--|--|
| Sunburn | Increased | UVB-induced apoptosis Cytokine secretion | — |
| Toxic epidermal necrolysis | Increased | Upregulation of Fas ligand TNF production | Antibody blocking (anti-Fas, anti-TNF, intravenous Ig ¹) |
| Graft-versus-host disease | Increased | Fas-mediated lymphocyte-directed killing TNF production | Antibody blocking (anti-Fas, anti-TNF) |
| Psoriasis | Decreased | Keratinocyte senescence Increased TNF (paradoxical) IL-15 Survivin, Bcl-xL expression | TNF inhibitors ¹ Anti-IL-15 antibody |
| Skin cancer (basal-cell and squamous-cell carcinoma) | Decreased | p53 mutation or deletion Decreased death receptors Stat3 activation Survivin, Bcl-2, Bcl-x _L | Introduction of p53 TRAIL Bortezomib Stat3 decoy |

¹Currently used in these patients. Abbreviations: TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand.

In toxic epidermal necrolysis, a drug-induced hypersensitivity reaction causes massive KC apoptosis, resulting in widespread epidermal detachment. French and colleagues demonstrated that Fas-L, which is sequestered by intermediate filaments in normal KCs (Viard-Leveugle *et al.*, 2003), is upregulated and expressed extracellularly in involved skin and capable of mediating cytotoxicity against normal Fas-expressing KCs (Viard *et al.*, 1998). Soluble Fas-L may also be secreted by mononuclear cells in patients with toxic epidermal necrolysis (Abe *et al.*, 2003) and is found in lesional blister fluid (Nassif *et al.*, 2004). Antibody blocking of the Fas-Fas-L interaction appears protective in this disease and represents the rationale for treatment with intravenous Ig, a therapy that has been used with remarkable success in adults (Prins *et al.*, 2003) and children (Tristani-Firouzi *et al.*, 2002). The potential involvement of additional molecules such as TNF, which is also found in lesional blister fluid (Nassif *et al.*, 2004), may explain why efficacy of Ig therapy does not correlate directly with concentration of Fas/Fas-L-blocking antibodies (Prins *et al.*, 2003).

In graft-versus-host disease, a common complication of allogeneic bone marrow transplantation, damage to the skin and other tissues is largely mediated by activated cytotoxic T lymphocytes. The term 'satellite-cell necrosis,' referring to degenerating KCs associated with adjacent lymphocytes, is a misnomer, as the KCs are apoptotic rather than necrotic. These lymphocytes express Fas-L and induce KC apoptosis through direct cell contact and secretion of cytokines, primarily TNF- α . Animal models have revealed that both Fas-L and TNF- α are important in the pathogenesis of graft-versus-host disease. When donor T cells are derived from mice deficient in Fas-L, there is reduced mortality and skin disease (Baker *et al.*, 1996). Although antibodies against TNF- α similarly confer protection, targeting of both Fas-L and TNF- α is required for complete abrogation of graft-versus-host disease (Hattori *et al.*, 1998). TNF- α appears to be more important than Fas-L, however, in mediating beneficial graft-versus-

leukemia reactions that often accompany graft-versus-host disease (Tsukada *et al.*, 1999).

Diseases of decreased keratinocyte apoptosis. Whereas diseases associated with increased KC apoptosis tend to be acute, those associated with decreased apoptosis tend to be chronic. In fact, most skin diseases or cutaneous lesions characterized by epidermal hyperplasia or hyperkeratosis likely involve decreased KC apoptosis. Psoriasis and skin cancer are the most notable examples and will be discussed here. A common feature of these two diseases, along with seborrheic and actinic keratosis, verrucae, and lichen simplex chronicus, is expression of Survivin (Bowen *et al.*, 2004). Unlike other known IAPs and most apoptotic inhibitors, Survivin is generally not expressed in normal skin. Interestingly, we found in psoriasis and lichen simplex chronicus that Survivin expression is localized to the upper third of the epidermis, whereas keratoses, basal-cell carcinoma, and squamous-cell carcinoma (SCC) reveal staining in all epidermal layers (Bowen *et al.*, 2004).

In psoriasis, there is decreased spontaneous KC apoptosis in lesional skin (Laporte *et al.*, 2000), which correlates with decreased levels of caspase-14 (Lippens *et al.*, 2000). KCs in psoriatic plaques exhibit a phenotype reminiscent of that of senescent KCs, characterized by resistance to apoptosis compared with normal KCs and lack of p53 activation (Qin *et al.*, 2002; Wrone-Smith *et al.*, 1997). Multiple factors likely contribute to increased resistance of KCs to apoptosis in this disease. First, it is possible that psoriatic KCs are resistant to TNF signaling, given that TNF- α is paradoxically elevated in psoriatic skin. For unknown reasons, maintenance of disease is dependent on TNF- α , and this represents the basis for multiple novel biologic therapies targeting this cytokine. Other cytokines involved in psoriasis include IL-15, a potent inhibitor of KC apoptosis *in vitro* that is upregulated in psoriatic skin (Ruckert *et al.*, 2000) and has proven to be a viable target in animal models (Villadsen *et al.*, 2003). In addition to

Survivin, multiple studies consistently demonstrated increased levels of Bcl-x_L, which is downregulated in response to topical vitamin D₃ treatment (Fukuya *et al.*, 2002).

Non-melanoma skin cancers demonstrate multiple examples of apoptotic dysregulation in which proapoptotic regulatory molecules are reduced or antiapoptotic molecules are overexpressed. Mutation or deletion of p53 occurs in many skin cancers and is discussed in detail below. Basal-cell carcinomas tend to have reduced expression of Bax (Tomkova *et al.*, 1998) and increased expression of Bcl-2 (Morales-Ducret *et al.*, 1995), and Bcl-x_L is overexpressed in SCC (Wrone-Smith *et al.*, 1999). A relatively higher apoptotic rate in basal-cell carcinoma (Staunton and Gaffney, 1995), however, may account for the slow growth of clinical basal-cell carcinoma lesions. The presence of Survivin in pre-malignant lesions (Bowen *et al.*, 2004) suggests that its expression represents an early step in KC transformation. In SCC, expression of Bcl-2 (Hantschmann and Kurzl, 2000), Bcl-x_L (Matsumoto *et al.*, 2001), and Survivin (Lo Muzio *et al.*, 2001) is associated with metastasis or poor prognosis. Many studies have shown that targeting these molecules in KC cell lines results in apoptosis (Grossman *et al.*, 1999). TNF-related apoptosis-inducing ligand (TRAIL) (Stander and Schwarz, 2005), its receptor (Bachmann *et al.*, 2001), and Fas-L (Bachmann *et al.*, 2001) are reduced in actinic keratosis and SCC. Progression of SCC is also associated with constitutive activation of KC survival signaling pathways. In malignant KCs, signaling through EGFR activates the transcription factor Stat3 (Quadros *et al.*, 2004), which drives expression of multiple antiapoptotic molecules, including Bcl-x_L and Mcl-1 (Buettnner *et al.*, 2002). Activation of the EGFR-Stat3 axis is associated with SCC progression (Bowman *et al.*, 2000; Rubin Grandis *et al.*, 1998), and activation of the Akt/NF- κ B pathway is commonly seen in SCC (Nakayama *et al.*, 2001). The role of some of these regulators and pathways in promoting skin cancer development in experimental models is discussed below.

Apoptosis as a Cancer Defense

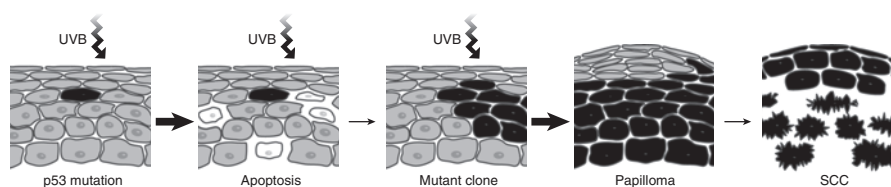
Mechanism

Apoptosis in the skin acts as a defense against cancer at two levels. First, apoptotic elimination of KCs in the upper epidermis results in removal of cells that may have acquired pre-malignant genetic alterations. Second, apoptosis may be directly induced in KCs exposed to carcinogenic stimuli, usually as part of the DNA damage response. This mechanism would be particularly important in eliminating KC stem cells that are long-lived and not destined for apoptotic turnover.

Role of p53. As noted above, KC apoptotic responses *in vivo* are generally dependent on p53, which is activated and regulated in cells by multiple factors (Vogelstein and Kinzler, 1992). Levels of p53 are potentially suppressed in normal KCs by a number of regulators, including PTEN (which inhibits Akt), products of the p38 MAPK pathway, and the Retinoblastoma protein; p53 is activated by ATM-mediated phosphorylation, and p19ARF protects it from degradation by inhibiting MDM2. p53 delays cell cycle progression (in G1 by inducing the Cdk2/cyclin E inhibitor p21Cip1, and in G2 through the Cdc25 inhibitor 14-3-3 σ), presumably until DNA damage can be repaired (Harris and Levine, 2005), or promotes apoptosis through transcriptional and non-transcriptional mechanisms. Transcription of proapoptotic regulators of the mitochondrial pathways (Bax, Noxa, Puma, Apaf-1) and death receptor pathways (Fas, death receptors 4 and 5) may be activated by p53, and that of antiapoptotic molecules (Bcl-2, Bcl-x_L, Survivin) may be repressed (Yu and Zhang, 2005). In addition, p53 can translocate to the mitochondria and interfere with Bcl-2 family proteins, resulting in cytochrome c release (Chipuk et al., 2004).

Exposure of KCs to UVB does not always result in apoptosis but rather may cause mutations in the p53 gene that lead to apoptosis resistance. Compromise of p53 function through UVB-induced mutation conceivably undermines the apoptosis-based defense mechanism, allowing p53-mutant KCs to survive repeated cycles

a Normal apoptosis sensitivity



b Increased apoptotic resistance

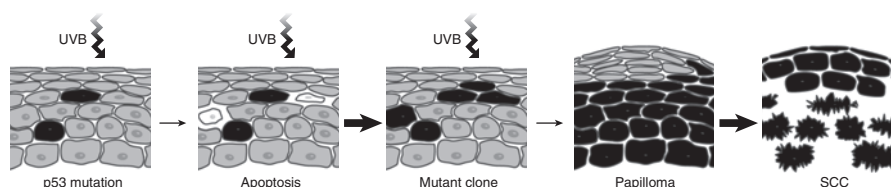


Figure 5. Schematic model depicting multiple roles of apoptosis in the various stages of UVB-induced skin carcinogenesis. Under normal conditions (a), UVB causes a p53 mutation (black cell) that confers apoptosis resistance. However, continued UVB exposure also induces apoptosis in neighboring keratinocytes (white cells, thick arrow), facilitating expansion of the p53-mutant clone. Ultimately, large clones develop into papillomas (thick arrow), and some papillomas that acquire additional mutations will convert to SCC. Under conditions of increased apoptosis resistance (b), as seen in the skin of K14-Survivin mice, creation of initial p53-mutant cells is enhanced (increased number of black cells, thick arrow) but fewer neighboring keratinocytes undergo apoptosis; this results in the formation of smaller but more numerous p53-mutant clones. This reduced clonal expansion leads to formation of fewer papillomas, but the increased apoptosis resistance selects for mutations that facilitate malignant conversion to squamous-cell carcinoma (SCC) (thick arrow).

of UVB exposure. Further impairment of p53 and other genes through additional UVB-induced mutations may then lead to even greater resistance to apoptosis and acquisition of pre-malignant changes. Consistent with this scenario, p53-deficient mice are highly susceptible to UVB-induced SCC (Li et al., 1998).

Clonal model of squamous-cell carcinoma. Given the proapoptotic function of p53 in UVB-treated KCs, it is not surprising that the p53 gene is disabled by mutation in most SCCs (Brash et al., 1991). Commonly, these mutations are C→T and CC→TT transitions, also known as 'UVB signature' mutations (Kress et al., 1992). Most SCCs exhibit loss of heterozygosity with respect to p53 and have point mutations on the remaining allele (Basset-Seguín et al., 1994). With respect to SCC precursors, p53 mutations are found in most actinic keratoses (Ziegler et al., 1994). Different p53 mutations occur in separate keratoses, but all cells within a single precursor lesion tend to have the same mutation (Ziegler et al., 1994). Mutations in

p53 can be detected in KCs from clinically normal sun-exposed skin with a frequency of up to 1% (Nakazawa et al., 1994), and clonal patches of p53-mutated KCs are larger and more frequent in sun-exposed skin (Jonason et al., 1996). In mouse models, the same p53 mutations are present in clones and subsequent SCC (Kramata et al., 2005). Moreover, development of these p53-mutated clones correlates with tumor induction in a mouse model (Rebel et al., 2001). These findings substantiate a clonal basis for SCC and suggest that p53 mutation is an early event in UVB-induced skin carcinogenesis (Figure 5a).

This clonal hypothesis suggests a defined role for p53-mediated apoptosis in suppressing the development and progression of SCC. At the earliest stage, apoptosis would be predicted to act selectively on normal but not on p53-mutant cells, promoting formation of a p53^{+/−} clone. During continuous UVB exposure, there is subsequent clonal expansion in which mutant cells extend beyond the borders of their original stem-cell compartment (Zhang et al.,

2001). Large clones either regress or ultimately form a papilloma. Transition from papilloma to SCC (malignant conversion) involves new mutations (Yuspa, 1998), and thus apoptosis would be expected to exert a negative or protective influence at this stage as well (Figure 5a).

Role of Apoptosis in Animal Models of Skin Cancer

Antitumorigenic effects of apoptosis.

A number of animal models support the notion that KC apoptosis suppresses tumor formation. It is worth noting, however, that in most cases neither forced expression of apoptotic resistance factors nor deletion of proapoptotic molecules is purely oncogenic (that is, sufficient to cause skin tumors). In these models, a mutagenic stimulus such as UVB or a chemical carcinogen like 9,10-dimethyl-1,2-benzanthracene is also needed. For example, whereas p53-deficient animals spontaneously develop lymphoma and sarcoma, topical application of 9,10-dimethyl-1,2-benzanthracene or chronic UVB is required for development of cutaneous SCC. As is noted above, these animals are more prone to UVB-induced SCC (Li *et al.*, 1998). Similarly, K14-MDM2 mice are more susceptible to chemical carcinogenesis (Ganguli *et al.*, 2000). Mice transgenic for Bcl-x_L also demonstrate enhancement of chemical-induced tumors as well as an increased rate of malignant conversion (Pena *et al.*, 1998). Genetic alterations in primary KC survival pathways also predispose to cutaneous tumorigenesis. For example, the K5-PTEN-flox/flox mice with constitutive Akt activation demonstrate accelerated tumor onset with chemical treatment (Suzuki *et al.*, 2003). Treatment with an EGFR inhibitor reduces UVB-induced skin tumorigenesis (El-Abaseri *et al.*, 2005). Similarly, mice deficient in Stat3 are resistant to chemical carcinogenesis (Chan *et al.*, 2004). The HK1.bcl-2 mice demonstrate increased susceptibility to chemical carcinogenesis (Rodriguez-Villanueva *et al.*, 1998). Taken together, these studies support a role for apoptosis resistance in the development of SCC.

Paradoxical role of apoptosis in carcinogenesis.

Other mouse models engineered to modulate KC apoptosis, however, demonstrate unexpected phenotypes. Although p53-deficient mice are more susceptible to UVB-induced tumor formation (Li *et al.*, 1998), paradoxical findings have emerged from studies in mice with reduced (but not absent) p53. Chemical carcinogenesis studies with mice on a p53^{+/-} background demonstrated increased resistance to tumor formation compared with that in wild-type animals (Greenhalgh *et al.*, 1996; Kemp *et al.*, 1993), although the rate of malignant conversion was enhanced (Kemp *et al.*, 1993). These results, contrasting with those in K14-MDM2 mice (Ganguli *et al.*, 2000), may reflect multiple mechanisms of p53 regulation and targets of MDM2. Consistent with these findings, Chaturvedi *et al.* (2005) reported that RNA interference-mediated reduction of p53 levels in KCs enhances rather than inhibits UVB-induced apoptosis.

Although NF-κB is a survival signal in KCs, as is noted above, and is activated in many tumor types, NF-κB blockade in the skin, surprisingly, promotes KC transformation and SCC formation (Dajee *et al.*, 2003). Mice transgenic for IκB demonstrate increased KC apoptosis but also spontaneously develop SCC (van Hogerlinden *et al.*, 1999). Similarly, increased KC apoptosis in mice deficient in Cox-1 is not associated with reduction in UVB-induced tumors (Pentland *et al.*, 2004). In addition, several mouse models with increased KC apoptosis resistance are less susceptible to skin carcinogenesis. For example, K14-Bcl-2 (Rossiter *et al.*, 2001) and K14-Survivin (Allen *et al.*, 2003; Zhang *et al.*, 2005) mice are resistant to sunburn-cell formation but yield reduced numbers of chemical- and UVB-induced papillomas. These mice have normal skin, whereas others with increased susceptibility to tumorigenesis have hyperkeratosis (Ganguli *et al.*, 2000; Rodriguez-Villanueva *et al.*, 1998) — although some mice with such susceptibility, for example, Bcl-x_L transgenic mice, do not have skin hyperkeratosis (Pena *et al.*, 1998). Thus, the lack of correlation between apoptosis resistance and susceptibility to tumor formation and malignant conversion in these studies suggests that apoptosis

may play a more complex role in controlling skin tumorigenesis than previously expected.

Apoptosis promotes early tumorigenic events.

It is curious that agents required for experimental induction of skin cancer — namely 9,10-dimethyl-1,2-benzanthracene and UVB — are also potent apoptotic stimuli in KCs (Allen *et al.*, 2003). This may merely reflect the need to induce mutations in p53 and other genes but, alternatively, may indicate a requirement for apoptosis in some early steps of cutaneous carcinogenesis. Perturbations of KC apoptotic balance might then potentially result in multiple (and unpredictable) effects on carcinogenesis. Zhang *et al.* (2001) examined p53-mutant clones in multi-stage UVB carcinogenesis and found that, when UVB exposure was discontinued, clones ceased expanding and regressed; this suggested that UVB was driving clonal expansion by a physiologic mechanism other than creation of additional mutations. It seemed plausible that this dependence of clonal expansion on sustained UVB exposure might reflect a requirement for apoptosis in adjacent KCs, providing space to accommodate the expanding clone (Figure 5a). Indeed, formation of isolated 'imprisoned' clones, characterized by increased cell number without increased area, was observed following withdrawal of UVB during clonal expansion (Zhang *et al.*, 2001).

To test this possibility, we examined p53-mutant clones in apoptosis-defective K14-Survivin mice at multiple steps during UVB carcinogenesis (Zhang *et al.*, 2005). We found that, under conditions of reduced apoptosis, formation of small p53-mutant clones was enhanced, consistent with a role for apoptosis in suppressing new mutations by removing mutant cells. Formation of medium and large clones, on the other hand, was impaired, suggesting that inhibition of apoptosis impairs clonal expansion; and tumor yield was reduced. Papillomas that formed under these conditions, however, demonstrated an increased tendency for malignant conversion. These results support a revised model, with apoptosis restraining stages that involve new mutations — initiation and malignant conver-

sion — but promoting clonal expansion of existing mutant cells and development of papillomas (Figure 5b). These opposing roles of apoptosis in restraining and promoting early steps in skin carcinogenesis may explain the divergent phenotypes observed in the animal models of dysregulated apoptosis described above.

Future Challenges and Directions

In the 30 years since apoptosis was first described in the skin, we have acquired a vast understanding of the biochemistry and molecular controls involved in programmed cell death and have come to appreciate the role of KC apoptosis in epidermal development (Figure 4) and various skin diseases (Figure 3, Table 1). Future challenges include unraveling the details of how differentiation and apoptosis are controlled in epidermal development, and further understanding the factors that shift apoptotic balance in KCs in response to different stimuli. Findings from experiments with KCs *in vitro* need to be validated *in vivo*. New animal models of apoptotic dysfunction based on inducible or regulated KC gene expression are needed, as well as models that better recapitulate the morphology of human skin. The clinical implications of this new knowledge will be development of new apoptosis-based therapeutics, directed toward protection of KCs from apoptosis in those diseases characterized by excessive KC cell death, or enhancement of KC apoptosis in diseases in which KC cell death is impaired (Table 1). Although a wide range of inflammatory and hyperkeratotic diseases might be amenable to apoptosis-based therapies, there appears to be greatest interest in developing agents for cancer. Several have recently been tested in SCC, including the proteasome inhibitor bortezomib (Velcade) (Fribley *et al.*, 2004), an adenovirus expressing wild-type p53 (Yoo *et al.*, 2004) and a Stat3 decoy (Xi *et al.*, 2005) to shift the apoptotic balance in malignant KCs. The multiple roles of apoptosis in multi-step skin carcinogenesis (Figure 5) suggest that apoptosis-modulating therapies may have varied and unanticipated effects on formation of actinic keratoses and SCC.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

Supported by NIH grants KO8 AR48618 and RO3 AR048953 and by the Huntsman Cancer Foundation. We apologize to those colleagues whose work we neglected to cite, primarily because of limitations on space and the number of references allowed.

REFERENCES

- Abe R, Shimizu T, Shibaki A, Nakamura H, Watanabe H, Shimizu H, *et al.* (2003) Toxic epidermal necrolysis and Stevens-Johnson syndrome are induced by soluble Fas ligand. *Am J Pathol* 162:1515–1520
- Allen SM, Florell SR, Hanks AN, Alexander A, Diedrich MJ, Altieri DC, *et al.* (2003) Survivin expression in mouse skin prevents papilloma regression and promotes chemical-induced tumor progression. *Cancer Res* 63:567–572
- Allombert-Blaise C, Tamiji S, Mortier L, Fauvel H, Tual M, Delaporte E, *et al.* (2003) Terminal differentiation of human epidermal keratinocytes involves mitochondria- and caspase-dependent cell death pathway. *Cell Death Differ* 10:850–852
- Assefa Z, Vantiegheem A, Garmyn M, Declercq W, Vandenabeele P, Vandenheede JR, *et al.* (2000) p38 mitogen-activated protein kinase regulates a novel, caspase-independent pathway for the mitochondrial cytochrome c release in ultraviolet B radiation-induced apoptosis. *J Biol Chem* 275:21416–21421
- Bachmann F, Buechner SA, Wernli M, Strebel S, Erb P (2001) Ultraviolet light downregulates CD95 ligand and TRAIL receptor expression facilitating actinic keratosis and squamous cell carcinoma formation. *J Invest Dermatol* 117:59–66
- Baker MB, Altman NH, Podack ER, Levy RB (1996) The role of cell-mediated cytotoxicity in acute GVHD after MHC-matched allogeneic bone marrow transplantation in mice. *J Exp Med* 183:2645–2656
- Bang B, Gniadecki R, Larsen JK, Baadsgaard O, Skov L (2003) *In vivo* UVB irradiation induces clustering of Fas (CD95) on human epidermal cells. *Exp Dermatol* 12:791–798
- Basset-Seguín N, Moles JP, Mills V, Dereure O, Guilhou JJ (1994) TP53 tumor suppressor gene and skin carcinogenesis. *J Invest Dermatol* 103(Suppl):102S–106S
- Benassi L, Ottani D, Fantini F, Marconi A, Chiodino C, Giannetti A, *et al.* (1997) 1,25-dihydroxyvitamin D₃, transforming growth factor beta1, calcium, and ultraviolet B radiation induce apoptosis in cultured human keratinocytes. *J Invest Dermatol* 109:276–282
- Bernerdt F, Sarasin A, Magnaldo T (1999) Galectin-7 overexpression is associated with the apoptotic process in UVB-induced sunburn keratinocytes. *Proc Natl Acad Sci USA* 96:11329–11334
- Berton TR, Mitchell DL, Guo R, Johnson DG (2005) Regulation of epidermal apoptosis and DNA repair by E2F1 in response to ultraviolet B radiation. *Oncogene* 24:2449–2460
- Bowen AR, Hanks AN, Allen SM, Alexander A, Diedrich MJ, Grossman D (2003) Apoptosis regulators and responses in human melanocytic and keratinocytic cells. *J Invest Dermatol* 120:48–55
- Bowen AR, Hanks AN, Murphy KJ, Florell SR, Grossman D (2004) Proliferation, apoptosis, and survivin expression in keratinocytic neoplasms and hyperplasias. *Am J Dermatopathol* 26:177–181
- Bowman T, Garcia R, Turkson J, Jove R (2000) STATs in oncogenesis. *Oncogene* 19:2474–2488
- Brash DE (1997) Sunlight and the onset of skin cancer. *Trends Genet* 13:410–414
- Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, *et al.* (1991) A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci USA* 88:10124–10128
- Brash DE, Wikonkal NM, Remenyik E, van der Horst GT, Friedberg EC, Cheo DL, *et al.* (2001) The DNA damage signal for Mdm2 regulation, Trp53 induction, and sunburn cell formation *in vivo* originates from actively transcribed genes. *J Invest Dermatol* 117:1234–1240
- Buettner R, Mora LB, Jove R (2002) Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clin Cancer Res* 8:945–954.
- Carini M, Aldini G, Piccone M, Facino RM (2000). Fluorescent probes as markers of oxidative stress in keratinocyte cell lines following UVB exposure. *Farmacol* 55:526–534.
- Chan KS, Sano S, Kiguchi K, Anders J, Komazawa N, Takeda J, *et al.* (2004) Disruption of Stat3 reveals a critical role in both the initiation and the promotion stages of epithelial carcinogenesis. *J Clin Invest* 114:720–728
- Chaturvedi V, Bacon P, Bodner B, Nickoloff BJ (2004) Proliferating cultured human keratinocytes are more susceptible to apoptosis compared with mouse keratinocytes. *J Invest Dermatol* 123:1200–1203
- Chaturvedi V, Qin JZ, Denning MF, Choubey D, Diaz MO, Nickoloff BJ (2001) Abnormal NF-kappaB signaling pathway with enhanced susceptibility to apoptosis in immortalized keratinocytes. *J Dermatol Sci* 26:67–78
- Chaturvedi V, Sitailo LA, Qin JZ, Bodner B, Denning MF, Curry J, *et al.* (2005). Knockdown of p53 levels in human keratinocytes accelerates Mcl-1 and Bcl-x(L) reduction thereby enhancing UV-light induced apoptosis. *Oncogene* 24:5299–5312
- Chien AJ, Presland RB, Kuechle MK (2002) Processing of native caspase-14 occurs at an atypical cleavage site in normal epidermal differentiation. *Biochem Biophys Res Commun* 296:911–917
- Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD *et al.* (2004) Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* 303:1010–1014
- Dajee M, Lazarov M, Zhang JY, Cai T, Green CL, Russell AJ, *et al.* (2003). NF-kappaB blockade and oncogenic Ras trigger invasive human

- epidermal neoplasia. *Nature* 421:639–643
- Datta SR, Brunet A, Greenberg ME (1999) Cellular survival: a play in three Akts. *Genes Dev* 13:2905–2927
- D'Costa AM, Denning MF (2005) A caspase-resistant mutant of PKC-delta protects keratinocytes from UV-induced apoptosis. *Cell Death Differ* 12:224–232
- Denning MF, Wang Y, Tibudan S, Alkan S, Nickoloff BJ, Qin JZ (2002) Caspase activation and disruption of mitochondrial membrane potential during UV radiation-induced apoptosis of human keratinocytes requires activation of protein kinase C. *Cell Death Differ* 9:40–52
- Di-Poi N, Tan NS, Michalik L, Wahli W, Desvergne B (2002) Antiapoptotic role of PPARbeta in keratinocytes via transcriptional control of the Akt1 signaling pathway. *Mol Cell* 10:721–733
- Eckert RL, Efimova T, Dashti SR, Balasubramanian S, Deucher A, Crish JF, et al. (2002). Keratinocyte survival, differentiation, and death: many roads lead to mitogen-activated protein kinase. *J Invest Dermatol Symp Proc* 7:36–40
- Eckhart L, Ban J, Fischer H, Tschachler E (2000a) Caspase-14: analysis of gene structure and mRNA expression during keratinocyte differentiation. *Biochem Biophys Res Commun* 277:655–659
- Eckhart L, Declercq W, Ban J, Rendl M, Lengauer B, Mayer C, et al. (2000b). Terminal differentiation of human keratinocytes and stratum corneum formation is associated with caspase-14 activation. *J Invest Dermatol* 115:1148–1151
- Efimova T, Broome AM, Eckert RL (2004) Protein kinase Cdelta regulates keratinocyte death and survival by regulating activity and subcellular localization of a p38delta-extracellular signal-regulated kinase 1/2 complex. *Mol Cell Biol* 24:8167–8183
- El-Abaseri TB, Fuhrman J, Trempus C, Shendrik I, Tennant RW, Hansen LA (2005) Chemoprevention of UV light-induced skin tumorigenesis by inhibition of the epidermal growth factor receptor. *Cancer Res* 65:3958–3965
- Fribley A, Zeng Q, Wang CY (2004) Proteasome inhibitor PS-341 induces apoptosis through induction of endoplasmic reticulum stress-reactive oxygen species in head and neck squamous cell carcinoma cells. *Mol Cell Biol* 24:9695–9704
- Fu YC, Jin XP, Wei SM, Lin HF, Kacew S (2000) Ultraviolet radiation and reactive oxygen generation as inducers of keratinocyte apoptosis: protective role of tea polyphenols. *J Toxicol Environ Health A* 61:177–188
- Fuchs E (1993) Epidermal differentiation and keratin gene expression. *J Cell Sci Suppl* 17: 197–208
- Fukuya Y, Higaki M, Higaki Y, Kawashima M (2002) Effect of vitamin D3 on the increased expression of Bcl-xL in psoriasis. *Arch Dermatol Res* 293:620–625
- Ganguli G, Abecassis J, Wasyluk B (2000) MDM2 induces hyperplasia and premalignant lesions when expressed in the basal layer of the epidermis. *EMBO J* 19:5135–5147
- Godar DE (1999) Light and death: photons and apoptosis. *J Invest Dermatol Symp Proc* 4:17–23
- Greenhalgh DA, Wang XJ, Donehower LA, Roop DR (1996) Paradoxical tumor inhibitory effect of p53 loss in transgenic mice expressing epidermal-targeted v-rasHa, v-fos, or human transforming growth factor alpha. *Cancer Res* 56:4413–4423
- Grossman D, McNiff JM, Li F, Altieri DC (1999) Expression of the apoptosis inhibitor, survivin, in nonmelanoma skin cancer and gene targeting in a keratinocyte cell line. *Lab Invest* 79:1121–1126
- Grossman D, Kim PJ, Blanc-Brude OP, Brash DE, Tognin S, Marchisio PC et al. (2001) Transgenic expression of survivin in keratinocytes counteracts UVB-induced apoptosis and cooperates with loss of p53. *J Clin Invest* 108:991–999
- Haake AR, Polakowska RR (1993) Cell death by apoptosis in epidermal biology. *J Invest Dermatol* 101:107–112
- Haake AR, Roublevskaia I, Cooklis M (1998) Apoptosis: a role in skin aging? *J Invest Dermatol Symp Proc* 3:28–35
- Hantschmann P, Kurzl R (2000) Regulation of apoptosis in squamous cell carcinoma of the vulva. *J Reprod Med* 45:633–642
- Harris SL, Levine AJ (2005) The p53 pathway: positive and negative feedback loops. *Oncogene* 24:2899–2908
- Hattori K, Hirano T, Miyajima H, Yamakawa N, Tateno M, Oshimi K, et al. (1998). Differential effects of anti-Fas ligand and anti-tumor necrosis factor alpha antibodies on acute graft-versus-host disease pathologies. *Blood* 91:4051–4055
- Hill LL, Ouhtit A, Loughlin SM, Kripke ML, Ananthaswamy HN, Owen-Schaub LB (1999) Fas ligand: a sensor for DNA damage critical in skin cancer etiology. *Science* 285:898–900
- Hockenbery DM, Zutter M, Hickey W, Nahm M, Korsmeyer SJ (1991) BCL2 protein is topographically restricted in tissues characterized by apoptotic cell death. *Proc Natl Acad Sci USA* 88:6961–6965
- Ishida-Yamamoto A, Takahashi H, Presland RB, Dale BA, Iizuka H (1998) Translocation of profilaggrin N-terminal domain into keratinocyte nuclei with fragmented DNA in normal human skin and loricrin keratoderma. *Lab Invest* 78:1245–1253
- Ishida-Yamamoto A, Tanaka H, Nakane H, Takahashi H, Hashimoto Y, Iizuka H (1999) Programmed cell death in normal epidermis and loricrin keratoderma. Multiple functions of profilaggrin in keratinization. *J Invest Dermatol Symp Proc* 4:145–149
- Jonason AS, Kunala S, Price GJ, Restifo RJ, Spinelli HM, Persing JA, et al. (1996). Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci USA* 93:14025–14029
- Jost M, Huggett TM, Kari C, Rodeck U (2001) Matrix-independent survival of human keratinocytes through an EGF receptor/MAPK-kinase-dependent pathway. *Mol Biol Cell* 12:1519–1527
- Kemp CJ, Donehower LA, Bradley A, Balmain A (1993) Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell* 74:813–822
- Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239–257
- Krajewski S, Krajewska M, Reed JC (1996) Immunohistochemical analysis of in vivo patterns of Bak expression, a proapoptotic member of the Bcl-2 protein family. *Cancer Res* 56:2849–2855
- Kramata P, Lu YP, LouYR, Singh RN, Kwon SM., Conney AH (2005) Patches of mutant p53-immunoreactive epidermal cells induced by chronic UVB irradiation harbor the same p53 mutations as squamous cell carcinomas in the skin of hairless SKH-1 mice. *Cancer Res* 65:3577–3585
- Kress S, Sutter C, Strickland PT, Mukhtar H, Schweizer J, Schwarz M (1992) Carcinogen-specific mutational pattern in the p53 gene in ultraviolet B radiation-induced squamous cell carcinomas of mouse skin. *Cancer Res* 52:6400–6403
- Kuehle MK, Predd HM, Fleckman P, Dale BA, Presland RB (2001) Caspase-14, a keratinocyte specific caspase: mRNA splice variants and expression pattern in embryonic and adult mouse. *Cell Death Differ* 8:868–870
- Kulms D, Zeise E, Poppelmann B, Schwarz T (2002) DNA damage, death receptor activation and reactive oxygen species contribute to ultraviolet radiation-induced apoptosis in an essential and independent way. *Oncogene* 21:5844–5851
- Kuwabara I, Kuwabara Y, Yang RY, Schuler M, Green DR, Zuraw BL (2002). Galectin-7 (PLG1) exhibits pro-apoptotic function through JNK activation and mitochondrial cytochrome c release. *J Biol Chem* 277:3487–3497
- Laporte M, Galand P, Fokan D, de Graef C, Heenen M (2000) Apoptosis in established and healing psoriasis. *Dermatology* 200:314–316
- Lee JK, Kim JH, Nam KT, Lee SH (2003) Molecular events associated with apoptosis and proliferation induced by ultraviolet-B radiation in the skin of hairless mice. *J Dermatol Sci* 32:171–179
- Leist M, Jaattela M (2001) Four deaths and a funeral: from caspases to alternative mechanisms. *Nat Rev Mol Cell Biol* 2:589–598
- Ley RD, Applegate LA (1985) Ultraviolet radiation-induced histopathologic changes in the skin of the marsupial *Monodelphis domestica*. II. Quantitative studies of the photoreactivation of induced hyperplasia and sunburn cell formation. *J Invest Dermatol* 85:365–367
- Li G, Tron V, Ho V (1998) Induction of squamous cell carcinoma in p53-deficient mice after ultraviolet irradiation. *J Invest Dermatol* 110:72–75
- Lippens S, Kockx M, Knaepen M, Mortier L, Polakowska R, Verheyen A, et al. (2000).

- Epidermal differentiation does not involve the pro-apoptotic executioner caspases, but is associated with caspase-14 induction and processing. *Cell Death Differ* 7:1218–1224
- Liu M, Dhanwada KR, Birt DF, Hecht S, Pelling JC (1994) Increase in p53 protein half-life in mouse keratinocytes following UV-B irradiation. *Carcinogenesis* 15:1089–1092
- Lo Muzio L, Farina A, Rubini C, Pezzetti F, Stabellini G, Laino G, et al. (2001). Expression of the apoptosis inhibitor survivin in aggressive squamous cell carcinoma. *Exp Mol Pathol* 70:249–254
- Manohar A, Shome SG, Lamar J, Stirling L, Iyer V, Pumiglia K, et al. (2004) Alpha 3 beta 1 integrin promotes keratinocyte cell survival through activation of a MEK/ERK signaling pathway. *J Cell Sci* 117: 4043–4054
- Marconi A, Vaschieri C, Zanolli S, Giannetti A, Pincelli C (1999) Nerve growth factor protects human keratinocytes from ultraviolet-B-induced apoptosis. *J Invest Dermatol* 113:920–927
- Matsumoto M, Natsugoe S, Nakashima S, Okumura H, Sakita H, Baba M, et al. (2001). Clinical significance and prognostic value of apoptosis related proteins in superficial esophageal squamous cell carcinoma. *Ann Surg Oncol* 8:598–604
- Mildner M, Eckhart L, Lengauer B, Tschachler E (2002) Hepatocyte growth factor/scatter factor inhibits UVB-induced apoptosis of human keratinocytes but not of keratinocyte-derived cell lines via the phosphatidylinositol 3-kinase/AKT pathway. *J Biol Chem* 277:14146–14152
- Morales-Ducet CR, van de Rijn M, LeBrun DP, Smoller BR (1995) bcl-2 expression in primary malignancies of the skin. *Arch Dermatol* 131:909–912
- Mu Y, Lv S, Ren X, Jin G, Liu J, Yan G, et al. (2003). UV-B induced keratinocyte apoptosis is blocked by 2-selenium-bridged beta-cyclodextrin, a GPX mimic. *J Photochem Photobiol B* 69:7–12
- Nakayama H, Ikebe T, Beppu M, Shirasuna K (2001) High expression levels of nuclear factor kappaB, IkappaB kinase alpha and Akt kinase in squamous cell carcinoma of the oral cavity. *Cancer* 92:3037–3044
- Nakazawa H, English D, Randell PL, Nakazawa K, Martel N, Armstrong BK, et al. (1994) UV and skin cancer: specific p53 gene mutation in normal skin as a biologically relevant exposure measurement. *Proc Natl Acad Sci USA* 91: 360–364
- Nassif A, Moslehi H, Le Gouvello S, Bagot M, Lyonnnet L, Michel L, et al. (2004). Evaluation of the potential role of cytokines in toxic epidermal necrolysis. *J Invest Dermatol* 123:850–855
- Nickoloff BJ, Qin JZ, Chaturvedi V, Bacon P, Panella J, Denning MF (2002) Life and death signaling pathways contributing to skin cancer. *J Invest Dermatol Symp Proc* 7:27–35
- Nishimura EK, Granter SR, Fisher DE (2005) Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. *Science* 307:720–724
- Okamoto H, Mizuno K, Itoh T, Tanaka K, Horio T (1999) Evaluation of apoptotic cells induced by ultraviolet light B radiation in epidermal sheets stained by the TUNEL technique. *J Invest Dermatol* 113:802–807
- Olson RL, Everett MA (1975) Epidermal apoptosis: cell deletion by phagocytosis. *J Cutan Pathol* 2:53–57
- Pena JC, Fuchs E, Thompson CB (1997) Bcl-x expression influences keratinocyte cell survival but not terminal differentiation. *Cell Growth Differ* 8:619–629
- Pena JC, Rudin CM, Thompson CB (1998) A Bcl-xL transgene promotes malignant conversion of chemically initiated skin papillomas. *Cancer Res* 58:2111–2116
- Pentland AP, Scott G, VanBuskirk J, Tanck C, LaRossa G, Brouxon S (2004) Cyclooxygenase-1 deletion enhances apoptosis but does not protect against ultraviolet light-induced tumors. *Cancer Res* 64:5587–5591
- Peus D, Vasa RA, Meves A, Beyerle A, Pittelkow MR (2000) UVB-induced epidermal growth factor receptor phosphorylation is critical for downstream signaling and keratinocyte survival. *Photochem Photobiol* 72:135–140
- Peus D, Vasa RA, Beyerle A, Meves A, Krautmacher C, Pittelkow MR (1999) UVB activates ERK1/2 and p38 signaling pathways via reactive oxygen species in cultured keratinocytes. *J Invest Dermatol* 112:751–756
- Polakowska RR, Piacentini M, Bartlett R, Goldsmith LA, Haake AR (1994) Apoptosis in human skin development: morphogenesis, periderm, and stem cells. *Dev Dyn* 199:176–188
- Prins C, Kerdell FA, Padilla RS, Hunziker T, Chimenti S, Viard I, et al. (2003). Treatment of toxic epidermal necrolysis with high-dose intravenous immunoglobulins: multicenter retrospective analysis of 48 consecutive cases. *Arch Dermatol* 139:26–32
- Qin JZ, Chaturvedi V, Denning MF, Bacon P, Panella J, Choubey D, et al. (2002) Regulation of apoptosis by p53 in UV-irradiated human epidermis, psoriatic plaques and senescent keratinocytes. *Oncogene* 21:2991–3002
- Quadros MR, Peruzzi F, Kari C, Rodeck U (2004) Complex regulation of signal transducers and activators of transcription 3 activation in normal and malignant keratinocytes. *Cancer Res* 64:3934–3939
- Rebel H, Mosnier LO, Berg RJ, Westerman-de Vries A, van Steeg H, van Kranen HJ, et al. (2001) Early p53-positive foci as indicators of tumor risk in ultraviolet-exposed hairless mice: kinetics of induction, effects of DNA repair deficiency, and p53 heterozygosity. *Cancer Res* 61:977–983
- Reed JC (1999) Dysregulation of apoptosis in cancer. *J Clin Oncol* 17:2941–2953
- Robitaille H, Proulx R, Robitaille K, Blouin R, Germain L (2005) The mitogen-activated protein kinase kinase kinase dual leucine zipper-bearing kinase (DLK) acts as a key regulator of keratinocyte terminal differentiation. *J Biol Chem* 280:12732–12741
- Rodriguez-Villanueva J, Greenhalgh D, Wang XJ, Bundman D, Cho S, Delehedde M, et al. (1998). Human keratin-1.bcl-2 transgenic mice aberrantly express keratin 6, exhibit reduced sensitivity to keratinocyte cell death induction, and are susceptible to skin tumor formation. *Oncogene* 16:853–863
- Rossiter H, Beissert S, Mayer C, Schon MP, Wienrich BG, Tschachler E, et al. (2001) Targeted expression of bcl-2 to murine basal epidermal keratinocytes results in paradoxical retardation of ultraviolet- and chemical-induced tumorigenesis. *Cancer Res* 61:3619–3626
- Rubin Grandis J, Twardy DJ, Melhem MF (1998) Asynchronous modulation of transforming growth factor alpha and epidermal growth factor receptor protein expression in progression of premalignant lesions to head and neck squamous cell carcinoma. *Clin Cancer Res* 4:13–20
- Ruckert R, Asadullah K, Seifert M, Budagian VM, Arnold R, Trombotto C, et al. (2000). Inhibition of keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis? *J Immunol* 165:2240–2250
- Sayama K, Hanakawa Y, Shirakata Y, Yamasaki K, Sawada Y, Sun L, et al. (2001). Apoptosis signal-regulating kinase 1 (ASK1) is an intracellular inducer of keratinocyte differentiation. *J Biol Chem* 276:999–1004
- Sharov AA, Weiner L, Sharova TY, Siebenhaar F, Atoyan R, Reginato AM, et al. (2003). Noggin overexpression inhibits eyelid opening by altering epidermal apoptosis and differentiation. *EMBO J* 22:2992–3003
- Shimizu H, Banno Y, Sumi N, Naganawa T, Kitajima Y, Nozawa Y (1999) Activation of p38 mitogen-activated protein kinase and caspases in UVB-induced apoptosis of human keratinocyte HaCaT cells. *J Invest Dermatol* 112:769–774
- Sitailo LA, Tibudan SS, Denning MF (2002) Activation of caspase-9 is required for UV-induced apoptosis of human keratinocytes. *J Biol Chem* 277:19346–19352
- Stander S, Schwarz T (2005) Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is expressed in normal skin and cutaneous inflammatory diseases, but not in chronically UV-exposed skin and non-melanoma skin cancer. *Am J Dermatopathol* 27:116–121
- Staunton MJ, Gaffney EF (1995) Tumor type is a determinant of susceptibility to apoptosis. *Am J Clin Pathol* 103:300–307
- Suzuki A, Itami S, Ohishi M, Hamada K, Inoue T, Komazawa N, et al. (2003). Keratinocyte-specific Pten deficiency results in epidermal hyperplasia, accelerated hair follicle morphogenesis and tumor formation. *Cancer Res* 63:674–681
- Takahashi H, Ishida-Yamamoto A, Iizuka H (2001a) Ultraviolet B irradiation induces apoptosis of keratinocytes by direct activation of Fas antigen. *J Invest Dermatol Symp Proc* 6:64–68
- Takahashi H, Honma M, Ishida-Yamamoto A, Namikawa K, Miwa A, Okado H, et al. (2001b) *In vitro* and *in vivo* transfer of bcl-2 gene

- into keratinocytes suppresses UVB-induced apoptosis. *Photochem Photobiol* 74:579–586
- Tomkova H, Fujimoto W, Arata J (1998) Expression of the bcl-2 homologue bax in normal human skin, psoriasis vulgaris and non-melanoma skin cancers. *Eur J Dermatol* 8:256–260
- Tristani-Firouzi P, Petersen MJ, Saffle JR, Morris SE, Zone JJ (2002) Treatment of toxic epidermal necrolysis with intravenous immunoglobulin in children. *J Am Acad Dermatol* 47:548–552
- Tsukada N, Kobata T, Aizawa Y, Yagita H, Okumura K (1999) Graft-versus-leukemia effect and graft-versus-host disease can be differentiated by cytotoxic mechanisms in a murine model of allogeneic bone marrow transplantation. *Blood* 93:2738–2747
- Uchida Y, Nardo AD, Collins V, Elias PM, Holleran WM (2003) De novo ceramide synthesis participates in the ultraviolet B irradiation-induced apoptosis in undifferentiated cultured human keratinocytes. *J Invest Dermatol* 120:662–669
- Umeda J, Sano S, Kogawa K, Motoyama N, Yoshikawa K, Itami S, et al. (2003). In vivo cooperation between Bcl-xL and the phosphoinositide 3-kinase-Akt signaling pathway for the protection of epidermal keratinocytes from apoptosis. *FASEB J* 17:610–620
- Van de Craen M, Van Loo G, Pype S, Van Criekeing W, Van den Brande I, Molemans F, et al. (1998). Identification of a new caspase homologue: caspase-14. *Cell Death Differ* 5:838–846
- van Hogerlinden M, Rozell BL, Ahrlund-Richter L, Toftgard R (1999) Squamous cell carcinomas and increased apoptosis in skin with inhibited Rel/nuclear factor-kappaB signaling. *Cancer Res* 59:3299–3303
- Van Laethem A, Van Kelst S, Lippens S, Declercq W, Vandenaabeele P, Janssens S, et al. (2004). Activation of p38 MAPK is required for Bax translocation to mitochondria, cytochrome c release and apoptosis induced by UVB irradiation in human keratinocytes. *FASEB J* 18:1946–1948
- Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ (1993) Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 75:229–240
- Viard I, Wehrli P, Bullani R, Schneider P, Holler N, Salomon D, et al. (1998). Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 282:490–493
- Viard-Leveugle I, Bullani RR, Meda P, Micheau O, Limat A, Saurat JH, et al. (2003). Intracellular localization of keratinocyte Fas ligand explains lack of cytolytic activity under physiological conditions. *J Biol Chem* 278:16183–16188
- Villadsen LS, Schuurman J, Beurskens F, Dam TN, Dagnaes-Hansen F, Skov L, et al. (2003). Resolution of psoriasis upon blockade of IL-15 biological activity in a xenograft mouse model. *J Clin Invest* 112:1571–1580
- Vogelstein B, Kinzler KW (1992). p53 function and dysfunction. *Cell* 70:523–526
- Wang HQ, Quan T, He T, Franke TF, Voorhees J J, Fisher GJ (2003) Epidermal growth factor receptor-dependent, NF-kappaB-independent activation of the phosphatidylinositol 3-kinase/Akt pathway inhibits ultraviolet irradiation-induced caspases-3, -8, and -9 in human keratinocytes. *J Biol Chem* 278:45737–45745
- Wehrli P, Viard I, Bullani R, Tschopp J, French LE (2000) Death receptors in cutaneous biology and disease. *J Invest Dermatol* 115:141–148
- Weil M, Raff MC, Braga VM (1999) Caspase activation in the terminal differentiation of human epidermal keratinocytes. *Curr Biol* 9:361–364
- Wikonkal NM, Remenyik E, Knezevic D, Zhang W, Liu M, Zhao H, et al. (2003). Inactivating E2f1 reverts apoptosis resistance and cancer sensitivity in Trp53-deficient mice. *Nat Cell Biol* 5:655–660
- Wrone-Smith T, Mitra RS, Thompson CB, Jasty R, Castle VP, Nickoloff BJ (1997) Keratinocytes derived from psoriatic plaques are resistant to apoptosis compared with normal skin. *Am J Pathol* 151:1321–1329
- Wrone-Smith T, Bergstrom J., Quevedo ME, Reddy V, Gutierrez-Steil C, Nickoloff BJ (1999) Differential expression of cell survival and cell cycle regulatory proteins in cutaneous squamoproliferative lesions. *J Dermatol Sci* 19:53–67
- Xi S, Gooding WE, Grandis JR (2005) *In vivo* antitumor efficacy of STAT3 blockade using a transcription factor decoy approach: implications for cancer therapy. *Oncogene* 24:970–979
- Yoo GH, Piechocki MP, Oliver J, Lonardo F, Zumstein L, Lin HS, et al. (2004). Enhancement of Ad-p53 therapy with docetaxel in head and neck cancer. *Laryngoscope* 114:1871–1879
- Yu J, Zhang L (2005) The transcriptional targets of p53 in apoptosis control. *Biochem Biophys Res Commun* 331:851–858
- Yuspa SH (1998) The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. *J Dermatol Sci* 17:1–7
- Zhang W, Hanks AN, Boucher K, Florell SR, Allen SM, Alexander A, et al. (2005). UVB-induced apoptosis drives clonal expansion during skin tumor development. *Carcinogenesis* 26:249–257
- Zhang W, Remenyik E, Zelterman D, Brash DE, Wikonkal NM (2001) Escaping the stem cell compartment: sustained UVB exposure allows p53-mutant keratinocytes to colonize adjacent epidermal proliferating units without incurring additional mutations. *Proc Natl Acad Sci USA* 98:13948–13953
- Ziegler A, Jonason AS, Leffell DJ, Simon JA, Sharma HW, Kimmelman J, et al. (1994). Sunburn and p53 in the onset of skin cancer. *Nature* 372:773–776