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Perspective

Osteoblast Apoptosis and Bone Turnover

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ABSTRACT

With the discoveries of different death mechanisms, an emerging definition of apoptosis is the process of cell death associated with caspase activation or caspase-mediated cell death. This definition accepts that caspases represent the final common mechanistic pathway in apoptosis. Apoptosis may be triggered either by activation events that target mitochondria or endoplasmic reticulum or by activation of cell surface "death receptors," for example, those in the tumor necrosis factor (TNF) superfamily. In the postnatal and adult skeleton, apoptosis is integral to physiological bone turnover, repair, and regeneration. The balance of osteoblast proliferation, differentiation, and apoptosis determines the size of the osteoblast population at any given time. Although apoptosis has been recorded in many studies of bone, the selective mechanisms invoked in the different models studied rarely have been identified. This review offers a broad overview of the current general concepts and controversies in apoptosis research and then considers specific examples of osteoblast apoptosis pertinent to skeletal development and to the regulation of bone turnover. In reviewing selected work on interdigital apoptosis in the developing skeleton, we discuss the putative roles of the bone morphogenetic proteins (BMPs), Msx2, RAR-γ, and death inducer obliterator 1 (DIO-1). In reviewing factors regulating apoptosis in the postnatal skeleton, we discuss roles of cytokines, growth factors, members of the TNF pathway, and the extracellular matrix (ECM). Finally, the paradoxical effects of parathyroid hormone (PTH) on osteoblast apoptosis in vivo are considered in the perspective of a recent hypothesis speculating that this may be a key mechanism to explain the anabolic effects of the hormone. An improved understanding of the apoptotic pathways and their functional outcomes in bone turnover and fracture healing may facilitate development of more targeted therapeutics to control bone balance in patients with osteoporosis and other skeletal diseases. (J Bone Miner Res 2001;16:975–984)

Key words: apoptosis, caspase, osteoblasts

INTRODUCTION

In the past decade, there has been extensive interest in the processes of cell death, and controversy continues to surround the definitions that distinguish apoptosis from other forms of cell death. Because of these various phenomena and the confusion in their interpretation, the definition of apoptosis has changed. It is defined as the process of cell death associated with caspase activation or caspase-mediated cell death and presumes that caspases represent its

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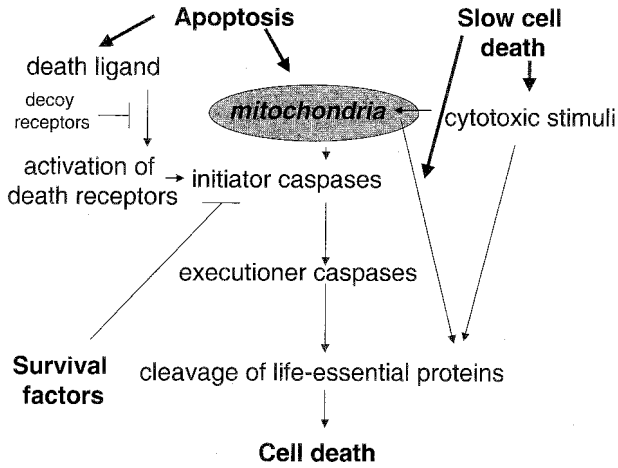

FIG. 1. Diagram to show the different pathways of cell death. Apoptosis may be triggered by activation of cell surface death receptors or of mitochondria and blocked by interventions due to decoy death receptors or pathways activated for cell survival. If caspases are not activated, slow cell death may occur because of cytotoxic stimulus or activation of mitochondria that does not involve the caspase pathways.

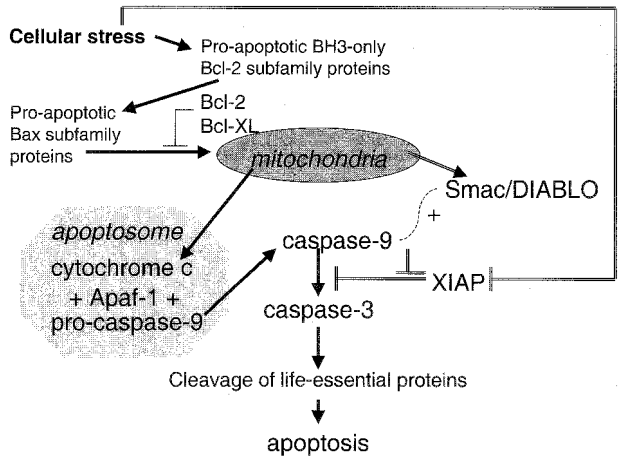

FIG. 2. Diagram to show protein interactions when the mitochondrial pathway of apoptosis is activated. The formation of protein complexes of proteins is required for the apoptosis pathway to progress through activation of initiator and executioner caspases. There are multiple feedback loops and intervention points to control apoptosis progression, and only a few are shown in this diagram.

final common mechanistic pathway (Fig. 1). Until an apoptotic cell is dead, it may continue to exclude trypan blue and exhibit other markers of a living cell. In the presence of caspase inhibitors or in the total absence of caspases death is delayed (Fig. 1).[1,2] "Slow cell death" may exhibit many of the same morphological features originally attributed to apoptosis, suggesting that nuclear fragmentation, DNA degradation, cell shrinkage, membrane blebbing, or chromatin condensation alone are not pathognomic of apoptosis. Unlike either apoptosis or slow cell death, death by necrosis at the tissue level triggers an inflammatory response and may be an ultrafast process activated within hours, before onset of any caspase activity. Given the rapid progression of research in this field, recognition of other forms of programmed cell death is highly likely in the next few years.

THE PROCESS OF APOPTOSIS

Apoptosis is a necessary component of development and a characteristic of all self-renewing tissues, including bone. Apoptosis may occur through one or more key pathways; the two most common are the mitochondria-activated pathway (Fig. 2) and the activation of one of many death receptors in the tumor necrosis factor (TNF) receptor superfamily (Fig. 3). With the discovery of receptor activator of NF-κB (RANK), RANK ligand (RANKL), and osteoprotegerin (OPG) as members of the TNF family of ligands and receptors, regulation of apoptosis in skeletal development, bone turnover by modeling or remodeling, and bone regeneration has become an exciting new area of investigation. This review offers a broad overview of current general concepts and controversies in apoptosis research and then considers specific examples of osteoblast apoptosis pertinent to skeletal development and to the regulation of bone turnover.

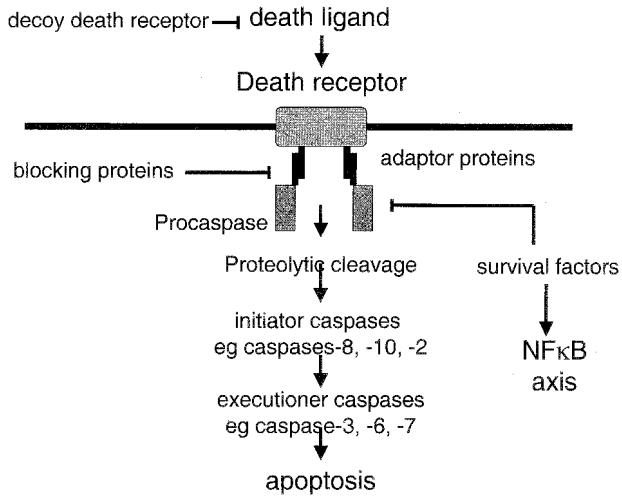

FIG. 3. Diagram to show principles of apoptosis pathway activated by binding of a death ligand to a death receptor. The pathway may be blocked before ligand receptor binding by a decoy receptor or during the intracellular steps by pathways activated to ensure cell survival.

THE CASPASES

Caspases are cysteine proteinases with two aspartate cleavage sites, one of which excises the prodomain and the other that cleaves the large domain from the small domain (Fig. 4). At this time, there are at least 14 caspases. Although it is recognized that these enzymes may have additional functions to their activation during apoptosis, these are not well understood because the predominant research focus has been on their role in cell death. The prodomain has low homology between caspases, whereas the remaining sequence is highly con-

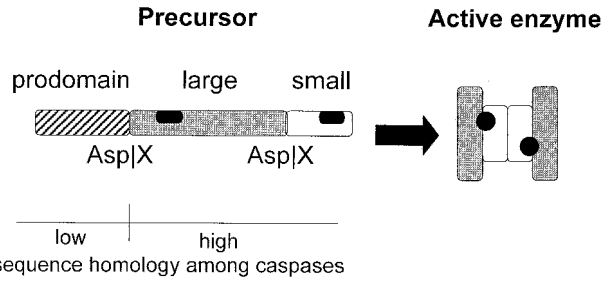

FIG. 4. Diagram to show common structure organization of caspases and their cleavage sites.

served between caspases (Fig. 4). The proforms of these enzymes are inactive; cleavage of the prodomain is prerequisite for activation of the initiator caspases. Initiator caspases (e.g., caspase-2, caspase-8, or caspase-10) cleave a variety of key proteins to irreversibly alter cell function and activate cleavage of the prodomain of the executioner caspases, such as caspase-3, caspase-6, or caspase-7. These then activate noncaspase effectors to process the dying cell in preparation for phagocytosis by neighboring cells. The natural inhibitors of activation of caspases (IAPs) can block the cascade at specific points.[3,4] One of the best studied of the caspase inhibitors is XIAP, which is ubiquitous in normal cells and capable of blocking activation of both caspase-3 and caspase-9 (Fig. 2). What is not well understood is the degree to which differentiated cells can still function if apoptosis is blocked before or during the process of caspase activation and execution. The specific proteins, inhibitors, and caspases activated may vary with species, tissues, and cell types. Although the pathways have been simplified for the purposes of this review, it should be realized that many caspases have overlapping functions and, with the exception of caspase-3, several caspase pathways may be redundant.[5–7]

PATHWAYS OF APOPTOSIS

Apoptosis may be initiated either as a consequence of key proteins being released into the cytosol by the mitochondria or as a consequence of activation of a cell surface death receptor. Either event activates one or more of the initiator caspases (Fig. 1). The most recent research implicates the endoplasmic reticulum as another putative originating site for apoptosis.[7–9]

Mitochondria-activated death pathways

In many tissues, the mitochondrial pathway can be activated directly by apoptogens or indirectly by activation of caspase enzymes, such that mitochondrial membrane permeability transition is induced and profoundly compromises organelle function (Fig. 2). After the combination and insertion into the mitochondrial membrane of a subset of proapoptotic members of the Bcl-2 family of proteins and certain BAX protein family members, cytochrome c is released into the cytosol.[10] This step may be blocked by antiapoptotic members of the Bcl-2 family or by Bcl-XL. Once released, cytochrome c interacts with apoptotic protease activating factor 1 (Apaf-1) and procaspase-9 to form an apoptosome complex, which promotes the processing and cleavage of an initiator caspase, such as procaspase-9. Mice in which the Apaf-1 gene has been ablated show impaired processing of downstream caspases-2, -3, and -8.[11,12] The formation of the apoptosome can be blocked by XIAP, but XIAP itself may be inhibited by Smac (second mitochondrial activator of caspases [SMAC/DIABLO]), which is released from mitochondria.[13,14] The balance in activity between Smac and the apoptosome determines whether a cell lives or dies. Loss of mitochondrial function also may be associated with diversion of oxygen to form short-lived radical intermediates that, in some cell populations, transduce signals activating apoptosis. These multiple levels of regulation suggest a high degree of specificity. For example, certain tissues of Apaf-1 null mice were resistant to a range of apoptotic stimuli; yet some immune cells remained sensitive to Fas-induced apoptosis.[12] The regulators for these steps in osteoblasts have yet to be determined. Recent data showed that when human bone cells were exposed to inorganic phosphate in the culture medium, dose-dependent apoptosis was induced and attributed to activation of the mitochondrial apoptosis pathway, because it could be blocked by inactivation of the plasma membrane Na-Pi transporter.[15]

Death receptor-activated death pathways

Stringent conditions must be in place before the multiple steps activating the apoptotic pathways via the surface death receptor members of the TNF receptor (TNFR) superfamily can be enabled (Fig. 3).[16–18] For the pathway to be activated, there must be critical density of receptors to enable the death domain–associated adaptor proteins, for example, FADD, TRAF, TRADD, RIP, etc., to cluster around the intracellular death domain. This complex may cleave and activate one or more initiator procaspases, which, in turn, cleave and activate executioner caspases (Figs. 1 and 4). Genetic ablation of TRAF6, which blocks TNFR-induced death by links to cell survival pathways (Fig. 3), was associated with failure of osteoclasts and development of osteopetrosis, suggesting this TNF pathway may be nonredundant in osteoclasts (the fate of osteoblasts was not investigated).[19]

APOPTOSIS IN BONE

Apoptosis plays a critical role during embryonic limb development, skeletal maturation, adult bone turnover by modeling and remodeling processes, and during fracture healing and bone regeneration. In humans, increased osteocyte apoptosis has been correlated with sites of rapid bone turnover, but there have been no prospective studies testing this association.[20–22] Programmed cell death of soft tissue between the embryonic digits is necessary for vertebrate limb development and has been used as a classic model for

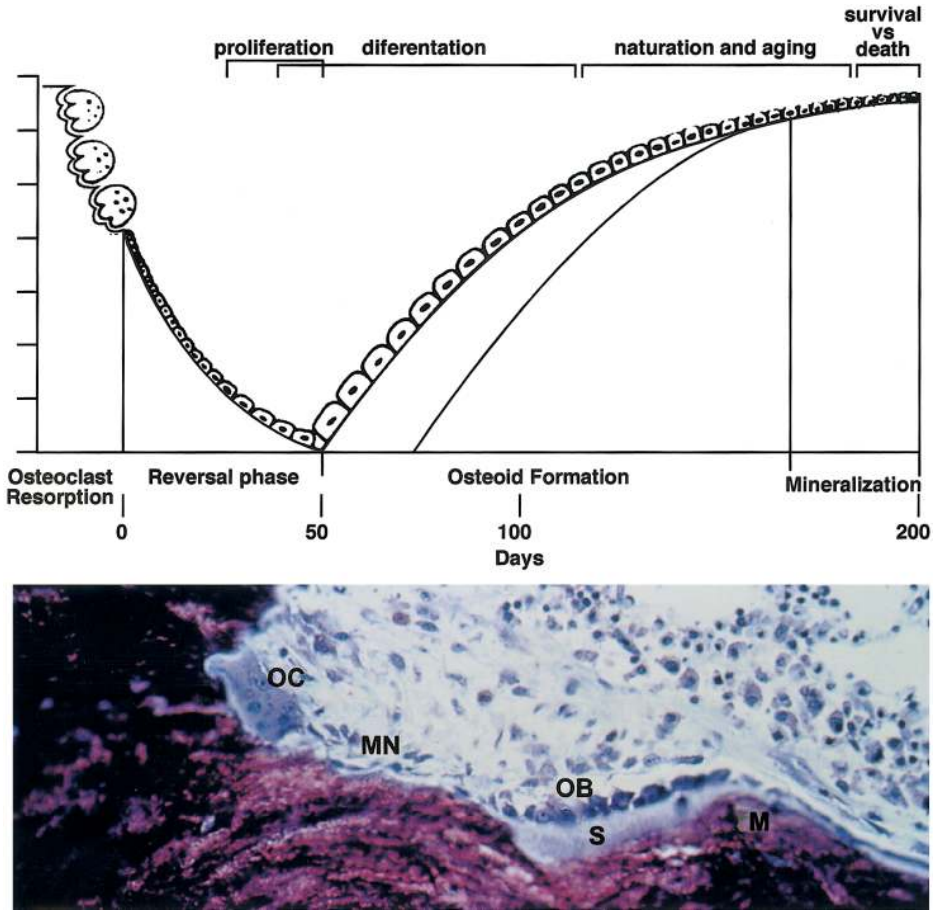

FIG. 5. The top diagram illustrates the remodeling cycle in which osteoclasts precede osteoblast recruitment and function, while the lower photograph of a human trabecula in the iliac crest shows the histological representation. The progression of cell events and their timing is well known, but how the osteoblast numbers are controlled through balanced proliferation-dependent and -independent apoptosis is not understood (photomicrograph was kindly provided by Dr. M. Gunness, Portland DVA, Portland, OR, USA).

studies of apoptosis. During bone formation[23] and fracture healing,[24] osteoblasts and chondrocytes undergo an orderly developmental progression that ultimately ends in apoptosis. During bone remodeling of osteonal or lamellar bone, the sequence of cell events is known (Fig. 5) but control of osteoblast number through the dual effects of proliferation and apoptosis often has not been considered. It is still unclear what signal transduction pathways are used in programmed cell death in osteoblasts. Glucocorticoids and estrogen withdrawal each promote apoptosis in osteoblasts and in osteocytes.[20,21,25] Overexpression of bcl-2 reduces glucocorticoid-induced apoptosis in neonatal murine calvaria,[26] supporting the speculation that increased osteoblast apoptosis may explain the pathogenesis of glucocorticoid-induced osteoporosis.[27] Inhibitors of caspase-3 block apoptosis in neonatal rat calvaria cell cultures.[25] Whether these putative mechanisms are restricted to growth and development of the skull or also can be used as valid models of adult skeletal responses remains to be determined. For the purposes of this review, we have elected to discuss apoptosis associated with embryonic limb development and apoptosis associated with the regulation of bone turnover. Glucocorticoid regulation of bone cell apoptosis will not be examined because it recently has been reviewed thoroughly.[27,28]

Regulation of interdigital apoptosis in limb development

Limb morphogenesis may be initiated as early as 9.5 days postcoitum (dpc) in a mouse embryo. Several pertinent events occur in concert. After establishment of the "zone of polarizing activity" (ZPA) and the formation of an apical ectodermal ridge (AER), the digits form by coordinated proliferation and interdigital apoptosis. The bone morphogenetic proteins (BMPs), specifically BMP-2, BMP-4, and BMP-7, have been identified as key regulators controlling interdigital apoptosis.[29–36] BMP-soaked beads accelerated localized interdigital apoptosis in chick limb buds,[32,33] while overexpression of a dominant negative BMP receptor decreased interdigital apoptosis.[37] Introduction of noggin-overexpressing retroviruses during early chick limb bud development resulted in a severely truncated-limb phenotype, while localized introduction of noggin at the anterior edge of the forelimb in stage 21 chick limb buds inhibited apoptosis in the anterior necrotic zone.[38] These data in chicks have been complemented by findings in mouse models. The *dac* mutation in dactylophasia mice results in development of limbs with missing phalanges in the central digits (ectrodactyly) or monodactyly, depending on the severity of the mutation.[30] In the dac phenotype, BMP-2, BMP-4, and BMP-7 are expressed in the AER at 10.5 dpc

and primarily function as antagonists of proliferative cues induced by fibroblast growth factors (FGFs) in the AER.[30] In a transgenic mouse model, constitutively active BMP-IB receptor located downstream of a Hox-b6 promoter resulted in digit bifurcation and extensive apoptosis at the site of interdigit bifurcation.[39] In these animals, premature and aberrant apoptosis at the AER of the hindlimb bud resulted in loss of metatarsals along with digit bifurcation of the hindlimb.[29] Collectively, these results support a prominent role for BMPs in interdigital apoptosis and in "sculpting" the final shape of the vertebrate limb.

Msx2 may play a key role in mediating BMP-induced interdigital apoptosis.[36,40–42] Msx2 is a member of the homeobox family of transcription factors and is a repressor that exerts tissue-specific actions during craniofacial, skeletal, and neural development.[41,43] In addition to BMP and msx2-dependent pathways of interdigital apoptosis, the retinoid pathways also may mediate apoptosis required for normal limb development. Treatment of hammertoe mice (mutants exhibiting defective apoptosis, including decreased interdigital apoptosis) with all trans retinoic acid (at-RA) at the site of interdigital mesenchyme partially ameliorated the hammertoe phenotype by promoting interdigital apoptosis.[44] Studies of the "webbed" feet phenotype observed in RARγ knockout mice and in the RARγ [$-/-$, RARβ ($+/-$ or $-/-$)] double mutants, implicated retinoid receptors as regulators of interdigital apoptosis.[45] Additional mediators were identified using yet another genetically modified mouse model. The double knockout (RARγ and RARβ $-/-$) mice displayed decreased tissue transglutaminase (tTG) activity and stromelysin-3 expression. Because mediators that promote detachment of a cell from its extracellular matrix (ECM) are known to promote apoptosis, the reduction in tTG activity may be associated with decreased apoptosis. Support for this hypothesis comes from studies showing increased tTG expression at the site of interdigital apoptosis in a 7.5-year-old chick limb bud using in situ hybridization.[46] In humans, expression of tTG colocalized with apoptosis of osteocytes and hypertrophic chondrocytes but not osteoblasts in rib bones of preterm human infants.[22] Putative retinoic acid response elements have been identified in the promoter regions of both tTG and stromelysin-3 genes.[46,47] The absence of retinoid receptors in the RARγ-RARβ knockout mice correlated with the decrease in tTG expression that contributed to the webbed feet phenotype.[46,47] Collectively, these studies suggest that retinoid pathways that regulate apoptosis are independent of the BMP/msx2 pathways.[48] A common final apoptosis pathway during development for all these mediators may be via activation of a recently identified gene named death inducer obliterator 1 (DIO-1).[49] DIO-1 may initiate and trigger caspase-dependent apoptosis, which is reversed by overexpression of Bcl-2.[49]

Apoptosis and the regulation of bone turnover

Bone turnover throughout life occurs by appositional modeling drift and remodeling processes (Fig. 4), which control bone size and geometry. These processes account for the distinctive and different bone architecture of cortical and trabecular bone; the site-specific differences in bone density, mineral size, and composition; and biomechanical properties of bone. Although osteoblast death must be an inevitable component of the bone turnover processes, the selective regulation and mechanistic pathways that initiate apoptosis are still not understood in any depth. Growth factors such as FGF-2 and the FGF-2 receptor may act as survival and apoptotic regulators, depending on the state of differentiation of the test cells in culture.[50] Although growth factors such as insulin-like factor I (IGF-I), IGF-II, FGF2, and platelet-derived growth factor (PDGF) are required for osteoblast survival in vitro, their role in apoptosis of osteoblasts, if any, remains controversial.[51,52] Growth factors were unable to reverse the in vitro inhibition of differentiation of osteoblasts induced by TNF-α.[53] TNF-α has been linked to osteoblasts apoptosis in vitro.[52,54,55] TNF-α regulates the bone turnover and loss associated with ovariectomy,[56] and so likely also has a role in bone cell apoptosis in vivo. The recent discoveries and validation of RANKL, RANK, and OPG, members of the TNFR and TNF ligand families, as powerful influences on bone metabolism have generated an intense interest in the mechanisms of osteoclast differentiation.[57] Because of their relationship to the death receptor family and their synthesis by cells of the osteoblast axis, perhaps it is time to consider a possible role in the osteoblast apoptosis.

Of particular relevance to a discussion of osteoblast survival and death, RANKL and OPG have been implicated as regulators of cell survival. RANKL was independently discovered by two groups during attempts to clone novel genes involved in the regulation of apoptosis and function of dendritic cells.[57,58] RANKL has been shown to promote the survival of T cells, dendritic cells, osteoclasts, and survival/proliferation of mammary epithelia cells.[58–60] RANKL may promote dendritic survival by BCL-X_L–dependent mechanism.[58] The essential role of RANKL in mammary epithelia cell survival comes from recent studies by Fata et al.[60] showing that mice lacking RANKL or its receptor RANK fail to form lobuloalveolar mammary structures during pregnancy, resulting in death of newborns. This effect is specific to mammary epithelial cells, and the pathology can be reversed by recombinant RANKL. Interestingly, OPG may promote or inhibit cell survival. OPG protects endothelia cells from apoptosis induced by serum withdrawal and NF-κB inactivation.[61] In contrast, OPG may block cell survival through its two death domain homologous regions (DDHs).[62] In a chimeric OPG with a transmembrane domain, these DDHs elicited death signals.[63] Another twist may come through TNF-related apoptosis-inducing ligand (TRAIL), which induces apoptosis on binding to a death domain containing receptors DR4 and DR5.[64] OPG can bind TRAIL to inhibit TRAIL-mediated apoptosis of sensitive Jurkat cells.[64] It remains to be seen if a similar mechanism may prevail in cells of the osteoblast axis.

Role of the ECM in osteoblast apoptosis

Little is known about the genes and proteins that control decisions on bone substructure and architecture in the bones

of different species or that differentially activate and regulate bone turnover in modeling and remodeling. Our most common mechanistic models to understand bone physiology are limited to in vitro cultures in which environmental cues are hard to model and to rats and mice. Because the skeleton of larger animals is dominated by osteonal bone structure of lamellar bone and remodeling processes,[65] it becomes important to understand which aspects of their cell and molecular mechanisms can be modeled in mice and by bone cell cultures. Peptide fragments from the ECM may play a role in apoptosis versus cell survival. RGD peptides may not only interfere with cell adhesion to the ECM but also activate caspase-3.[66,67] Preliminary data suggest these mechanisms may be relevant to osteoblast apoptosis.[68] One component of the ECM is fibronectin.[69] In mature osteoblasts isolated from fetal rat calvaria cultures, fibronectin fragments antagonized cell survival effects of intact full-length fibronectin, to induce osteoblast apoptosis.[69] Examples of two recent genetically modified mouse models may offer other clues to the role played in osteoblast homeostasis by the ECM. Young and adult osteonectin null mice showed deficiencies in bone cell balance and bone mass.[70] By 36 weeks, trabecular bone formation was decreased by 85% compared with wild-type mice, resulting in negative bone balance and osteopenia. There was no explanation for the decrease in osteoblast number but apoptosis may contribute if the alterations in ECM perturbed normal adhesion of cells to bone matrix. In a second example, biglycan null mice exhibited osteopenia with decreased bone formation.[71] Apoptosis was not evaluated but may be involved because biglycan has been implicated in neuronal cell survival.

Now, it is widely recognized that osteoblasts synthesize and secrete a variety of matrix metalloproteinases (MMPs) and disintegrin metalloproteinases, when first stimulated by anabolic agents such as PTH, in vivo and in cultured bone cells.[72–78] Although the significance of this phenomenon in bone turnover and osteoblast apoptosis remains speculative, MMPs are able to cleave the proteins in the bone matrix, including fibronectin. The binding elements for Cbfa1/Osf2/Runx2, a key regulator for osteoblast maintenance and survival during a late phase of skeletal development,[79–81] and the AP-1 transcription factors in the promoter for MMP-13 (collagenase-3) are required for induction of MMP-13 by PTH in vitro.[73,75–77] The magnitude of change required for outcomes that would modify osteoblast function and apoptosis has not been investigated. Mutant Cbfa1 mice exhibit decreased bone volume/osteoblast, suggesting a role for Cbfa1 in functional maintenance of osteoblasts. This is consistent with the results that show direct regulation of OPG by this factor.[81] As an antiresorptive, OPG is a "protector" of bone matrix; so it is conceivable that transcription factors like Cbfa1 may maintain OPG, selected MMPs, and other matrix-protecting factors to stabilize the osteoblast environment.

Regulation of apoptosis in bone by PTH

One of the most recent strategies to study regulation of bone cell death has been to use the observations made on the in vivo and in vitro responses of osteoblasts to PTH.[82,83] PTH, when given once daily, increases bone mass and resistance to fracture in rats, mice, rabbits, ovariectomized monkeys, and postmenopausal women,[84–93] whereas continuous PTH infusion decreases bone mass. The anabolic effect is accomplished by (1) increased bone apposition to dominate the modeling process and favor accrual of bone and (2) increased bone turnover to renew the matrix of osteonal bone.[89,90,94–96] The anabolic actions of PTH can be attributed to stimulation of osteoblast differentiation in vivo, as expression of key matrix genes occurs within 6–24 h in young rats[97] and the forming surfaces and apposition rates are increased within 24 h in rats[98] and 14 days in humans.[96] PTH stimulates activation frequency in intact animals, irrespective of whether it is given once daily or continuously.[65] In regulating bone balance by controlling bone turnover, PTH may coordinately regulate osteoblast proliferation, survival, and apoptosis, as well as the signal transduction required to induce osteoclasts in a time-dependent manner.

The ability of the other G protein coupled receptors to stimulate apoptosis[99] suggests that PTH binding to its receptor also may stimulate apoptosis directly. HEK 293 cells transfected with PTH1 receptor induced apoptosis through a calcium sensitive c-*jun*-N-terminal kinase pathway.[100] PTH-induced apoptosis was blocked by caspase inhibitors, but not by Bcl-2 overexpression, and mediated through the $G\alpha_q$ proteins but antagonized by overexpression of RGS4, a selective GTPase-activating protein.[100] However, transfected HEK 293 cells contain higher numbers and density of PTH1 receptors than wild-type bone cells. The c-Jun-kinase–mediated apoptosis likely functions via a caspase/MEKK-1 pathway.[101] Overexpression of mutant G proteins and/or constitutive activation of the cyclic adenosine monophosphate (cAMP) pathway also may diminish osteoblast survival. In, for example, McCune-Albright's fibrous dysplasia of bone, transplanted mutant bone cells failed to thrive in a wild-type environment, implicating an enhanced susceptibility to apoptosis.[102,103]

Apoptosis may be proliferation-dependent or proliferation-independent. Proliferation inevitably is associated with apoptosis through the actions of tumor suppressor genes such as p53, which controls key stages of the cell cycle to ensure that cells in which DNA becomes significantly flawed are eliminated through cell death.[16,104] When actively proliferating cells in young rats were prelabeled with bromodeoxyuridine (BrdUrd) and rats were killed after 3–5 days of PTH treatment (a duration required for them to differentiate into osteoblasts), the percent of BrdUrd-labeled cells increased.[98] In this same time interval, the percent of metaphyseal bone apoptotic cells increased transiently, peaking after 5–7 days of PTH.[83] Although relevant to a role for PTH in the developing skeleton, this alteration of the balance between proliferating and apoptotic cell populations observed in young rats is unlikely to be part of the PTH response in adult rats. In mature rats, PTH did not increase the percent of thymidine-labeled osteoblasts, stimulating the hypothesis that the hormone activated preexisting quiescent bone surface cells.[105] If validated, the absence of stimulated proliferation would exclude the likelihood of proliferation-dependent apoptosis but not

proliferation-independent apoptosis. Paradoxically, PTH inhibited osteoblast apoptosis and decreased caspase activity on enzyme substrates that recognize caspase-3 but did not up-regulate expression of a number of genes implicated in apoptotic pathways in young rats.[83] In vitro, PTH inhibited dexamethasone-induced apoptosis in osteoblasts isolated from neonatal murine calvaria.[26] In a separate study, histomorphometry of the midmetaphyseal region of long bones of mice treated for 4 weeks with PTH showed an inhibition of osteoblast apoptosis, generating the hypothesis that inhibition of cell death could explain the seeming longevity of osteoblasts and favor sustained bone formation and bone accrual.[28,82] A possible role for PTH-regulated intracellular calcium and phosphate fluxes in osteoblast apoptosis has not been explored at the cell and molecular level, although changes in these ions have been associated with apoptosis.[15]

The significance of the PTH-induced inhibition of apoptosis in terms of functional outcomes in bone is not clear. Cell death associated with the absence of PTH in hypoparathyroidism, with continuous infusion of PTH in vivo, or withdrawal from treatment has not been studied. The data on apoptosis in general are not available for us to know if inhibiting apoptosis and prolonging osteoblast longevity will sustain full physiological function in affected cells or, by maintaining adhesion of cells to the ECM, prevent osteoclasts from accessing the bone surface. It is widely recognized that inhibition of apoptosis through effects on caspase activity delays rather than inhibits death of the targeted cell, and death will likely occur through other mechanisms.[2]

SUMMARY

In summary, apoptosis of osteoblasts is unlikely to be indiscriminate. There are numerous molecules that are involved in apoptosis, and the fate of the cell depends on how these molecules interact with each other. Skeletal tissue, during its lifetime, undergoes modeling and remodeling as prerequisites for bone turnover, bone repair, and bone regeneration. These processes not only require generating osteoblasts through proliferation or activation of existing precursors, but also balancing the needs of tissue homeostasis by regulation of bone cell death. During skeletal development, apoptosis and other forms of cell death are required for bones to acquire the appropriate geometry and form. The functional significance of osteoblast death in the postnatal skeleton and adult skeleton for normal bone homeostasis or in response to pharmacologic agents is not well understood at this time. Explorations to determine the selective pathways of apoptosis, which have been identified in other cells and organs, have only just started in the past few years in bone, so we cannot know yet if these can be leveraged into potential targets for new therapeutic strategies.

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