Annexin-mediated Ca\(^{2+}\) Influx Regulates Growth Plate Chondrocyte Maturation and Apoptosis*

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Maturation of epiphyseal growth plate chondrocytes plays an important role in endochondral bone formation. Previously, we demonstrated that retinoic acid (RA) treatment stimulated annexin-mediated Ca\(^{2+}\) influx into growth plate chondrocytes leading to a significant increase in cytosolic Ca\(^{2+}\), whereas K-201, a specific annexin Ca\(^{2+}\) channel blocker, inhibited this increase markedly. The present study addressed the hypothesis that annexin-mediated Ca\(^{2+}\) influx into growth plate chondrocytes is a major regulator of terminal differentiation, mineralization, and apoptosis of these cells. We found that K-201 significantly reduced up-regulation of expression of terminal differentiation marker genes, such as cbfa1, alkaline phosphatase (APase), osteocalcin, and type I collagen in RA-treated cultures. Furthermore, K-201 inhibited up-regulation of annexin II, V, and VI gene expression in these cells. RA-treated chondrocytes released mineralization-competent matrix vesicles, which contained significantly higher amounts of annexins II, V, and VI as well as APase activity than vesicles isolated from untreated or RA/K-201-treated cultures. Consistently, only RA-treated cultures showed significant mineralization. RA treatment stimulated the whole sequence of terminal differentiation events, including apoptosis as the final event. After a 6-day treatment gene expression of bel-2, an anti-apoptotic protein, was down-regulated, whereas caspase-3 activity and the percentage of TUNEL-positive cells were significantly increased in RA-treated cultures compared with untreated cultures. Interestingly, the cytosolic calcium chelator BAPTA-AM and K-201 protected RA-treated chondrocytes from undergoing apoptotic changes, as indicated by higher bel-2 gene expression, reduced caspase-3 activity, and the percentage of TUNEL-positive cells. In conclusion, annexin-mediated Ca\(^{2+}\) influx into growth plate chondrocytes is a positive regulator of terminal differentiation, mineralization, and apoptosis events in growth plate chondrocytes.

Maturation of epiphyseal growth plate chondrocytes, which plays an important role during endochondral ossification, is accompanied by major changes of chondrocyte morphology, biosynthetic activities, and energy metabolism. These processes involve an ordered progression of various cell differentiation stages, including proliferation, hypertrophic differentiation, terminal differentiation, and ultimately programmed cell death (apoptosis) (1, 2). During normal development these sequential events are under the strict control of local and systemic factors such as hormones and growth factors. If these processes, however, occur during pathological conditions, they can result in serious cartilage or bone defects. Evidence of endochondral ossification is also seen during osteophyte formation in osteoarthritic cartilage (3, 4). Terminal differentiation of growth plate chondrocytes is an essential process, which primes the cartilage skeleton for its subsequent invasion by osteoblasts and its replacement by a bone matrix. Despite the obvious importance of these terminal differentiation events still little is known about mechanisms regulating these processes.

cbfa1, a member of the runt domain family of transcription factors, was originally discovered as a key transcription factor, which controls osteoblast differentiation. In cbfa1-null mice no endochondral and intramembranous bone formation occurs due to an arrest in osteoblast differentiation (5–8). Recent studies have indicated that cbfa1 also plays an important regulative role in terminal chondrocyte maturation. Transgenic mice, which overexpress cbfa1 in non-hypertrophic chondrocytes, display an acceleration of endochondral ossification. Overexpression of cbfa1 in chondrocytes of cbfa1-null mice partially rescued the abnormalities of cbfa1-null mutant mice. In particular, it rescued hypertrophic chondrocyte differentiation in the humerus and femur (9). Thus, cbfa1 seems to play dual functions in endochondral bone formation; it plays a key role in osteoblast differentiation from mesenchymal precursor cells, and it has the ability to stimulate hypertrophic and terminal chondrocyte differentiation.

Chondrocyte hypertrophy and terminal differentiation are accompanied by an increase in cytosolic calcium, [Ca\(^{2+}\)], (10–12). Calcium is recognized as an important regulator of many cellular processes, and it controls a diverse range of cell functions, including adhesion, motility, gene expression, cell differentiation, and proliferation. For example, the amplitude and duration of calcium signals control differential activation of different transcription factors in B lymphocytes (13). Calcium has been shown to play several roles in vesiculation and the formation of vesicles. For example, Iannotti et al. (14) have shown a correlation between increasing [Ca\(^{2+}\)] and the release of matrix vesicles (14). Matrix vesicles are small membrane-enclosed particles, which are released from the plasma membrane of growth plate chondrocytes and which initiate the mineralization process (15). We have previously shown that RA, which stimulates terminal differentiation and mineralization,

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The abbreviations used are: RA, retinoic acid; APase, alkaline phosphatase activity; RAR, retinoic acid receptor; PBS, phosphate-buffered saline; BAPTA/AM, 1,2-bis(O-aminophenoxy)ethane-N,N,N',N"-tetraacetic acid acetoxymethyl ester; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling.

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They also form Ca\(^{2+}\) channels in phospholipid bilayers or in liposomes (20). They also form Ca\(^{2+}\) channels in matrix vesicles enabling Ca\(^{2+}\) influx into these particles as a possible initial step for the formation of the first mineral phase within the vesicle lumen (21).

Careful studies have provided evidence that apoptosis is the final fate of terminally differentiated growth plate chondrocytes. Chondrocyte apoptosis in the growth plate is centered at the site of the transition of cartilage to bone (22, 22). These apoptotic chondrocytes show characteristic hallmarks of apoptosis, including condensed nuclei, DNA fragmentation, activation of caspase cascade, and phosphatidylserine externalization. Previous studies have indicated that elevation of [Ca\(^{2+}\)] is involved in the induction of apoptosis. For example, apoptosis of cultured human endothelial cells was inhibited by chelating extracellular calcium with EGTA or by inhibiting the calcium influx by calcium channel blockers. It has been suggested that elevated [Ca\(^{2+}\)], leads to activation of proteases, lipases, and nuclease.

All these actions can contribute to cell death (23–25).

We have provided evidence that RA promotes annexin channel formation in growth plate chondrocytes and that annexin-mediated Ca\(^{2+}\) influx into these cells controls Ca\(^{2+}\) homeostasis (16). To determine the role of annexin-mediated alteration of Ca\(^{2+}\) homeostasis in terminal differentiation, mineralization, and apoptosis of growth plate chondrocytes, we cotreated growth plate chondrocytes isolated from the hypertrophic zone of day 19 embryonic chicken growth plate cartilage with RA and K-201, a specific annexin channel blocker, or RA and BAPTA-AM, a cytosolic Ca\(^{2+}\) chelator, and analyzed the rate of terminal differentiation, mineralization, and apoptosis in these cells.

EXPERIMENTAL PROCEDURES

Chondrocyte Culture—Chondrocytes were isolated from the hypertrophic zone of day 19 embryonic chick tibia growth plate cartilage as described previously (19). Briefly, sliced growth plate cartilage was digested with 0.25% trypsin and 0.05% collagenase for 5 h at 37 °C. Isolation of Total RNA and Real Time PCR—Total RNA was isolated from untreated, RA-treated, RA/K-201-treated, and RA/BAPTA-treated chondrocyte cultures after 1-, 3-, and 5-day treatments using RNeasy Mini Kit (Qiagen, Stanford, CA). 1 μg of RNA was reverse-transcribed using Ominiscript RT Kit (Qiagen). A 1:10 dilution of the resulting cDNA was used as the template to quantify the relative content of mRNA by real time PCR (ABI PRISM 7700 sequence detection system) using respective primers and SYBR Green. The following primers for real time PCR were designed using Primer Express software. Annexin II: forward primer, 5′-CTCCGCTCTTGGTTGATCCGTTAC-3′; reverse primer, 5′-TACGGCGGCAAGTTTGCTGTTTGA; annexin X: forward primer, 5′-ATGCCGAGGACCTGATTGTTTAGCGA-3′; reverse primer, 5′-CAGCAATGTCGAGGATTTTTCTGA-3′.

For the determination of Ca\(^{2+}\) channels, cultured chondrocytes were cultured in the presence of 1.5 mM phosphate and in the absence or presence of (a) 50 μM BAPTA-AM, (b) 50 μM BAPTA-AM, (c) 50 μM BAPTA-AM, (d) 50 μM BAPTA-AM, and (e) 50 μM BAPTA-AM.

Isolation of Matrix Vesicles—Matrix vesicles were isolated from chondrocyte cultures after a 6-day treatment by enzymatic digestion and ultracentrifugation as described previously (19).

Measurement of Apase Activity and Protein Content—Apase activity was measured using p-nitrophenyl phosphate (Sigma-Aldrich) as a substrate as described previously (19). Protein content was analyzed by the BCA protein assay from Pierce.

SDS-PAGE and Immunoblotting—To determine the amounts of annexin II, V, and VI in matrix vesicles, vesicle fractions (total protein of 30 μg) were subjected to SDS-PAGE and immunoblotted with primary antibodies specific for annexins II, and VI. Samples were dissolved in 4× NuPAGE SDS sample buffer (Invitrogen). Prior to electrophoresis, the reducing reagent was added to the sample solution, denatured at 70 °C for 10 min, and analyzed by electrophoresis in 10% Bis-Tris gels following the NuPAGE electrophoresis protocols. Samples were electroblotted onto nitrocellulose filters after electrophoresis. After blocking with 5% nonfat milk protein, blotted proteins were immobilized with primary antibodies followed by peroxidase-conjugated secondary antibody, and the signal was detected by enhanced chemiluminescence (Pierce).

Alizarin Red S Staining—To determine the degree of mineralization chondrocyte cultures were stained with alizarin red S after a 6-day culture as described previously (19). Chondrocyte cultures were fixed with 70% ethanol and then stained with 0.5% alizarin red S solution, pH 4.0, for 5 min at room temperature. To quantify the intensity of alizarin red S staining, alizarin red S-stained cultures were incubated with 100 mM cetylpyridinium chloride for 1 h to solubilize and release calcium-bound alizarin red S into solution (26). The absorbance of the released alizarin red S staining was measured at 570 nm using a spectrophotometer. Data were expressed as units of alizarin red S released per mg of protein in each culture.

Caspase-3 Activity Assay—Caspase-3 activity was determined using the ApoAlert caspase fluorescent assay kit (Clontech) following the manufacturer’s protocol. Briefly, after a 6-day treatment chondrocyte cultures were washed twice with ice-cold phosphate-buffered saline (PBS), scraped into tubes, and centrifuged at 1500 rpm for 10 min. Cell pellets were washed one more time with ice-cold PBS and centrifuged again. Then air-dried cell pellets were resuspended in 60 μl of chilled cell lysis buffer and incubated on ice for 10 min. Cellular debris was removed by centrifugation, and 50 μl of 2× reaction buffer/dithiothreitol containing caspase-3 substrate (DEVD-AMC) were added to each sample. Samples were then incubated at 37 °C for 1 h. Caspase-3 activity was measured in a fluorescence reader using the excitation wavelength of 480 nm and the emission wavelength of 505 nm. Caspase-3 activity was quantitated using 7-amino-4-trifluoromethyl-7H-coumarin standard and normalized to the protein content in each culture.

In Situ Detection of Apoptotic Chondrocytes by TUNEL Labeling—Apoptotic chondrocytes in day 6 chondrocyte cultures were detected using ApoTag in situ apoptosis detection kit to label apoptotic cells by modifying genomic DNA utilizing terminal deoxynucleotidyltransferase (TdT). Briefly, chondrocyte cultures were incubated twice with PBS and fixed with 1% paraformaldehyde/PBS solution (pH 7.4) for 10 min. Then fixed chondrocytes were incubated with 1% Triton X-100, followed by incubation with a proteinase K solution (20 μg/ml) for 10 min at room temperature. Samples were then incubated in...
RESULTS

3% hydrogen peroxide/PBS for 5 min at room temperature to quench endogenous peroxidases, followed by rinsing with PBS and incubation with equilibration buffer. Samples were incubated with a reaction mixture containing terminal deoxynucleotidyltransferase enzyme and digoxigenin-labeled dNTPs at 37 °C in a humidified chamber. After 1 h, the reaction was stopped, and digoxigenin-labeled nucleotides were detected by peroxidase-conjugated anti-digoxigenin antibodies in a humidified chamber for 30 min at room temperature. The signal was detected using 3,3'-diaminobenzidine as a color substrate. Sections were counterstained with methylene green, mounted, and viewed under an Olympus microscope. To gain insights into the extent of apoptosis in the various chondrocyte cultures, the percentage of stained cells was determined. 500 chondrocytes were counted in 10 randomly chosen areas of three different cultures. Data were expressed as the mean ± S.D. of the percentage of total cells that show TUNEL staining. (n > 4), and statistical significance between groups was identified using the two-tailed Student’s t test (p values are reported in the figure legends).

Treatment of hypertrophic growth plate chondrocytes with RA induced terminal differentiation of these cells, as indicated by up-regulation of terminal differentiation marker genes, including cbfa1, APase, and osteocalcin. Gene expressions were up-regulation of terminal differentiation marker genes, including cbfa1, APase, and osteocalcin. Gene expressions were detected by quantitative real time PCR. Values are mean ± S.D. of the percentage of total cells that show TUNEL staining.

RA treatment also up-regulated annexin II, V, and VI gene expression. K-201 significantly reduced the rate of mineralization compared with the degree of mineralization in RA-treated cultures (Fig. 2).
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It is now well established that the final fate of terminally differentiated chondrocytes is apoptosis (2). Therefore, we addressed the question of whether RA triggers the whole cascade of terminal differentiation events including apoptosis and whether annexin-mediated Ca\(^{2+}\) influx into chondrocytes is also involved in the regulation of apoptotic changes. To determine the degree of apoptosis in the various treated chondrocyte cultures we measured bcl-2 gene expression and caspase-3 activity and performed TUNEL labeling. A 5-day treatment with RA led to a significant decrease in gene expression of bcl-2, an anti-apoptotic protein (27) (Fig. 6). In contrast, caspase-3 activity, an active cell death protease involved in the execution phase of apoptosis (28), was more than 5-fold elevated in cultures treated for 6 days with RA compared with untreated cells (Fig. 7). Cotreatment of cultures with RA and the cytosolic Ca\(^{2+}\) chelator BAPTA-AM abolished the decrease in bcl-2 gene expression (Fig. 6) and the increase in caspase-3 activity (Fig. 7), suggesting that cytosolic calcium is directly involved in the regulation of apoptotic events. Interestingly, bcl-2 gene expression was also higher in RA/K-201-treated cells than in RA-treated cells (Fig. 6), whereas caspase-3 activity was lower (Fig. 7). In addition, TUNEL labeling revealed that in RA-treated cultures more than 10% of cells were TUNEL-positive, whereas only ~2% were TUNEL-positive in untreated and RA/BAPTA-treated cells, and ~4% of cells were TUNEL-positive in RA/K-201-treated cultures (Fig. 8).

**DISCUSSION**

In this study we show that RA triggers a whole series of terminal differentiation events, including up-regulation of terminal differentiation marker genes (APase, cbfa1, osteocalcin), release of mineralization-competent matrix vesicles, subsequent mineralization, and finally apoptosis. RA also induces annexin-mediated Ca\(^{2+}\) influx into growth plate chondrocytes. Blocking annexin Ca\(^{2+}\) channel activities inhibited the whole series of terminal differentiation events, including up-regulation of terminal differentiation marker gene expression, release versus RA/K-201 treatment).

...mediated Ca\(^{2+}\) influx into chondrocytes regulates expression of terminal differentiation marker genes, the release of APase- and annexin II-, V-, and VI-containing matrix vesicles, and subsequent mineralization.

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of mineralization-competent matrix vesicles, extracellular matrix mineralization, and apoptosis. These findings clearly establish the prominent regulatory function of annexin-mediated Ca\textsuperscript{2+} influx into growth plate chondrocytes in terminal differentiation and apoptosis of these cells.

RA is known to regulate transcription after binding to the retinoic acid receptor complex. Three RA receptors have been identified, retinoic acid receptors α, β, and γ. RA binds to one of these receptors, and this receptor complex then dimerizes with another receptor, retinoid X receptor (RXR). These receptor complexes then directly activate gene expression of transcription factors and other genes (29). Our study, however, indicates that RA also uses other mechanisms to regulate cell differentiation. As shown in this and previous studies, RA induces annexin channel formation in the plasma membrane of growth plate chondrocytes leading to an increased cytosolic calcium concentration (16). This increased cytosolic calcium concentration leads to further up-regulation of annexin and other terminal differentiation marker gene expression and causes the release of mineralization-competent matrix vesicles and the induction of apoptotic events.

It is not clear how RA induces annexin channel formation. However, several possibilities can be envisioned. RA may bind to membrane receptors yet to be discovered. This binding activates an initial increase in cytosolic calcium concentration. Annexins require a certain Ca\textsuperscript{2+} concentration to bind to phospholipids (20). The initial increase in cytosolic calcium may then lead to a relocation of annexins from the cytoplasm to the plasma membrane and channel formation causing a further boost in cytosolic calcium. Alternatively, RA might have similar effects on the membrane as vitamin D, which has been shown to increase plasma membrane fluidity (30). Increased membrane fluidity might favor annexin channel formation. A third possibility is that annexins bind directly to RA, and this binding favors annexin channel formation. This possibility is supported by recent findings showing that annexin II binds directly to vitamin D and acts as an alternative vitamin D receptor (31).

RA up-regulates gene expression of cbfa1, APase, and osteo-
calcium. These genes are considered terminal differentiation markers and are expressed by late hypertrophic chondrocytes in the growth plate. cbfa1, a member of the runt-domain family of transcription factors, is expressed in osteoblasts and hypertrophic chondrocytes (6–9). In cbfa1-deficient mice no endochondral and intramembranous ossification occurs because of an arrest of osteoblast differentiation (7, 8). A disturbance of chondrocyte differentiation, especially terminal differentiation, was also observed in these mice (8). Continuous expression of cbfa1 in non-hypertrophic chondrocytes induced hypertrophic differentiation and endochondral ossification (9). Furthermore, overexpression of cbfa1 in chick immature chondrocytes induced type X collagen and MMP-13 expression, APase activity, and extensive matrix mineralization (32). These findings indicate that cbfa1 is an important regulatory factor in chondrocyte terminal differentiation. Our results show that annexin-mediated Ca2+ influx into hypertrophic chondrocytes sequentially activates gene expression of cbfa1, APase, and osteocalcin. Interestingly, cbfa1 directly regulates gene expression of osteocalcin and other genes (33). Thus, it is possible that annexin-mediated alteration of Ca2+ homeostasis might control terminal differentiation and apoptotic events through the regulation of cbfa1 gene expression.

Terminal differentiation is also accompanied by extracellular matrix remodeling and alteration of collagen gene expression. When chondrocytes undergo hypertrophic changes they turn on type X collagen gene expression and down-regulate type II collagen synthesis (34). Furthermore, previous studies in vivo and in vitro have demonstrated that terminal differentiated mineralizing chicken growth plate chondrocytes express type I collagen and other bone-related proteins, including osteocalcin and osteopontin (35, 36). Our study showing that RA treatment leads to down-regulation of type II collagen and up-regulation of type I collagen gene expression confirms these previous findings and indicates that alteration of Ca2+ homeostasis is involved in regulating collagen gene expression during growth plate development. In addition, our findings reveal that RA or RA/K-201 treatment did not affect type X collagen gene expression, indicating that type X collagen expression in growth plate chondrocytes used in this study was already at a high level. These results are consistent with previous findings demonstrating that type X collagen synthesis was greatly up-regulated in immature growth plate chondrocytes after RA treatment but remained unchanged in RA-treated mature hypertrophic growth plate chondrocytes (37, 38).

Annexin-mediated alteration of Ca2+ homeostasis up-regulates annexin II, V, and VI and APase gene expression. The up-regulation of annexin and APase gene expression might be required for the release of mineralization-competent matrix vesicles. These vesicles contain large amounts of annexins II,
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V, and VI and APase activity. Annexins II, V, and VI also form Ca$^{2+}$ channels in matrix vesicles (19, 21). Thus, annexin channel formation seems to play multiple functions in terminal differentiation of growth plate chondrocytes. Firstly, channel formation alters Ca$^{2+}$ homeostasis, which controls terminal differentiation events, and secondly annexin channel formation in matrix vesicles allows Ca$^{2+}$ influx into these particles as a possible initial step of mineral formation.

RA does not only induce mineralization of growth plate chondrocytes, but it triggers the whole cascade of terminal differentiation events, including apoptosis. Apoptosis, or programmed cell death, has been shown to be the final event of chondrocyte differentiation (2, 22). Induction of apoptosis by RA has also been demonstrated in other cell types, including leukemia cells, thymocytes, neuroblastoma cell lines, and articular chondrocytes (39–42). We show that RA down-regulates bcl-2 gene expression and stimulates caspase-3 activity. In addition, the percentage of TUNEL-positive cells was significantly higher in RA-treated cells compared with the number of apoptotic cells in untreated cultures. bcl-2 belongs to a rapidly expanding family of genes implicated in the control of apoptosis. Up-regulation of bcl-2 by PTHrP delays maturation of growth plate chondrocytes toward hypertrophy and subsequent apoptosis (27). In contrast, caspase-3 is an active cell death protease involved in the execution phase of apoptosis (28).

Interestingly, apoptotic changes were significantly reduced in cultures cotreated with RA and BAPTA or RA and K-201, suggesting that annexin-mediated Ca$^{2+}$ influx into growth plate chondrocytes is involved in the regulation of the complete terminal differentiation program, including apoptosis. Thus, RA and RA-mediated annexin Ca$^{2+}$ channel formation appear to stimulate a similar sequence of events as observed in growth plate cartilage. A recent study has demonstrated high amounts of retinoids in the perichondrium and that implantation of beads containing RA antagonist near the humeral anlagen drastically decreased chondrocyte hypertrophy and terminal differentiation. In addition, retinoid receptor $\gamma$ is expressed in hypertrophic and terminally differentiated chondrocytes (43). Thus, it is likely that RA and the resulting annexin Ca$^{2+}$ channel formation play important regulatory roles in the regulation of terminal differentiation events in the growth plate during endochondral ossification.

Annexin V has also been shown to mediate Ca$^{2+}$ influx induced by hydrogen peroxide into B-lymphocytes (44). Thus, annexins not only form Ca$^{2+}$ channels in chondrocytes but also other cell types. In addition, B-lymphocytes lacking annexin V are resistant to apoptosis (45). Ca$^{2+}$ is known to be required for apoptosis, and it is known that Ca$^{2+}$ can cause apoptosis by itself under conditions of Ca$^{2+}$ overload. Our study demonstrates that the cytosolic calcium chelator BAPTA-AM or the annexin channel blocker K-201 significantly inhibits apoptosis of growth plate chondrocytes. These findings suggest that annexin-mediated Ca$^{2+}$ influx or related annexin functions in various cell types can lead to a Ca$^{2+}$ overload and apoptosis resulting from this overload.

Hypertrophic and terminally differentiated growth plate chondrocytes express three annexins (annexins II, V, and VI). Previous studies have shown that all three annexins can form Ca$^{2+}$ channels and that K-201 inhibits Ca$^{2+}$ channel activities of all three annexins (21). However, it is not clear, whether all three annexins form Ca$^{2+}$ channels in growth plate chondrocytes independently and mediate Ca$^{2+}$ influx into these cells. Our previous findings show that each antibody fraction specific for annexin II, V, or VI partially inhibited increases in [Ca$^{2+}$], in growth plate chondrocytes, suggesting that all three annexins contributed to Ca$^{2+}$ influx into these cells (16). However, antibodies specific for annexin V inhibited mineralization of growth plate chondrocytes to a degree similar to K-201 (see Fig. 2). Other studies have shown that peroxide-mediated Ca$^{2+}$ influx was altered only in B cells lacking annexin V but not in cells lacking annexin II (44). In addition, only B cells lacking annexin V but not cells lacking annexin II were resistant to apoptosis (45). Future studies have to establish whether only annexin V modulates Ca$^{2+}$ homeostasis of growth plate chondrocytes and is involved in the regulation of chondrocyte terminal differentiation, whether all three annexins form Ca$^{2+}$ channels independently and regulate Ca$^{2+}$ influx into growth plate chondrocytes, or whether the interactions between the three annexins are required for optimal annexin V channel activities in growth plate chondrocytes.

Previous studies from our and other laboratories have shown the expression of hypertrophic and terminal differentiation markers, including annexins II, V, and VI, APase, osteopontin, osteocalcin, and type X collagen in osteoarthritic cartilage (17, 18, 46–49). In addition, mineralization and apoptosis were detected in osteoarthritic cartilage (17, 50–52). Thus, it is possible that up-regulation of annexin gene expression in osteoarthritic cartilage might lead to annexin-mediated Ca$^{2+}$ influx into articular chondrocytes and subsequent stimulation of terminal differentiation events in these cells. Terminal differentiation events are required for endochondral bone formation during normal development; however, if these events occur under pathological conditions, such as osteoarthritis, they will lead to cartilage destruction. If these annexins are as essential for terminal differentiation in osteoarthritic cartilage as they are in growth plate cartilage during endochondral ossification, they could be promising targets for therapies.

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