Osteocyte Apoptosis Is Mechanically Regulated and Induces Angiogenesis In Vitro

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ABSTRACT: Osteocyte apoptosis, associated with reduced interstitial fluid flow, precedes osteoclast precursor recruitment and may aid in the delivery of osteoclast precursors to the remodeling site by promoting angiogenesis. To test the association between fluid flow and osteocyte apoptosis, osteocyte-like MLO-Y4 cells were subjected to either oscillatory fluid flow (10 dynes/cm^2, 1 Hz) or no flow conditions with or without TNF-α treatment to induce osteocyte apoptosis chemically. Flow protected osteocytes from apoptosis regardless of whether they were treated with TNF-α ($p < 0.001$) or not ($p < 0.05$). TNF-α-induced apoptotic and nonapoptotic osteocyte conditioned media were used to study the effect of osteocyte apoptosis on angiogenesis. Apoptotic osteocyte conditioned media caused more endothelial cell proliferation ($p < 0.05$) and migration ($p < 0.05$), and tubule networks with longer ($p < 0.01$) and more ($p < 0.001$) branches than nonapoptotic osteocyte conditioned media. Apoptotic osteocyte conditioned media contained more vascular endothelial growth factor (VEGF) than nonapoptotic osteocyte conditioned media ($p < 0.05$). VEGF concentrations found in apoptotic osteocyte conditioned media formed endothelial tubule networks with longer ($p < 0.05$) and more ($p < 0.02$) branches than VEGF concentrations in nonapoptotic osteocyte conditioned media. Blocking VEGF in apoptotic osteocyte conditioned media abolished tubule formation effects ($p < 0.001$). Our results suggest that osteocyte apoptosis is flow-regulated and promotes angiogenesis in a VEGF-mediated manner. © 2010 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 29:523–530, 2011

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Osteocyte apoptosis precedes osteoclast recruitment and activity[1,2] and co-localizes in areas of osteoclast resorption.[3] Recently, Cardoso et al.[4] demonstrated complete abolishment of osteoclast resorption in fatigue-loaded bone when osteocyte apoptosis was inhibited. Together, these studies suggest a role for osteocyte apoptosis in osteoclast precursor recruitment and bone remodeling,[4] although the mechanisms remain unknown.

Osteoclast precursors are transported from the marrow to the remodeling site via circulation.[5] Bone capillaries are about 8 μm in diameter,[6] and human Haversian canals range from 30 to170 μm in diameter,[7] depending on the type of bone. The trafficking of osteoclast precursors from capillaries to the bone surface is a crucial process in osteoclast recruitment, but how osteoclast precursors are recruited and transported over the large distance from the capillary to the bone surface is unclear. Webber et al.[8] found significant angiogenic activity with osteoclast precursors and osteoclasts found at the periphery of an ectopically implanted bone. Furthermore, capillaries were found in close proximity to bone resorption initiation sites in human trabecular bone.[9] These observations suggest that angiogenesis may provide a conduit for osteoclast precursors to be recruited to the bone surface close to the resorption site.

Interestingly, reduced fluid flow causes osteocyte apoptosis in vitro,[10] consistent with in vivo evidence that disuse increases apoptosis.[1] Housed in the lacuna-canalicular system, osteocytes are bathed in interstitial fluid flowing in an oscillatory fashion in response to pressure gradients created by dynamic mechanical loading.[11] Due to their unique location and abundance, osteocytes are thought to sense and translate loading induced interstitial flow into chemical signals that regulate bone remodeling and formation.[12] During normal loading, osteocytes experience between 8 and 30 dynes/cm^2 of shear stress.[12] In cases of disuse, such as weightlessness in space and bed immobilization, osteocytes are deprived of normal mechanical signals, resulting in negligible interstitial fluid flow (0.5 to 2 dynes/cm^2).[13]

Based on this evidence, we hypothesized that osteocyte apoptosis potentiates angiogenesis, which enables delivery of osteoclast precursors directly to the remodeling site. Because of the in vivo association between disuse and apoptosis, we also hypothesized that interstitial fluid flow regulates osteocyte apoptosis. We tested these hypotheses in vitro by investigating the effect of TNF-α-induced osteocyte apoptosis on endothelial cell proliferation, migration, and tubule network formation, and by determining the role of oscillatory fluid flow in regulating osteocyte apoptosis.

MATERIALS AND METHODS

Cell Culture

MLO-Y4 osteocyte-like cells (gift from Dr. Lynda Bonewald, University of Missouri-Kansas City) were cultured on rat tail collagen I (BD Biosciences, Bedford, MA) coated surfaces with α-MEM media (Gibco/Invitrogen, Carlsbad, CA) supplemented with 2.5% calf serum (Hyclone, Logan, UT), 2.5% fetal bovine serum (Hyclone; FBS), and 1% penicillin and streptomycin

Additional supporting information may be found in the online version of this article.

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(Gibco/Invitrogen, PS; α-MEM supplemented media). A model endothelial cell line (D4T cells, gift from Dr. Peter Zandstra, University of Toronto) was cultured with IMDM + L-glutamate media supplemented with 5% certified FBS (Gibco/Invitrogen) and 1% PS.

Fluid Flow Induction

MLO-Y4 cells were seeded at 10,500 cells/cm^2 on rat tail type I collagen coated glass slides (see Supplementary Material) and placed in parallel plate flow chambers that were connected to glass syringes driven by an electromechanical loading device.[14] Cells were exposed to a peak sinusoidal wall shear stress of 10 dynes/cm^2 at 1 Hz for 2 h at 37°C and 5% CO_2. MLO-Y4 cells in parallel plate flow chambers subjected to no flow served as controls.

Apoptosis Assay

MLO-Y4 cells were treated with supplemented α-MEM containing TNF-α (0, 1, 5, 10, 20 ng/ml) for 6 h to obtain a TNF-α concentration that would induce about 50% apoptosis. Cells were stained with APOPercentage dye according to manufacturer's protocol. A dye release agent was applied onto the osteocytes, and the absorbance of the accumulated APOPercentage dye was measured with a spectrophotometer at 550 nm. To verify the results, osteocytes treated with 0 or 10 ng/ml TNF-α were stained with terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL; R&D Systems) according to manufacturer's protocol (see Supplementary Materials). Cells with intense blue staining within the nucleus demonstrating fragmentation and condensation of nuclear material were identified as apoptotic. Cells were counterstained with Nuclear Fast Red that stained nonapoptotic cells pink. Three view points/slide were imaged, manually counted, and averaged to quantify the proportion of apoptotic cells.

To study the effect of fluid flow on osteocyte apoptosis, MLO-Y4 cells were exposed to flow or no flow, and were subsequently cultured in α-MEM supplemented media under apoptotic (10 ng/ml TNF-α; see Supplementary Material) or normal (no TNF-α) culture conditions for 6 h at 37°C and 5% CO_2 under static conditions. Cells were then stained with APOPercentage dye to assess apoptosis. Two view fields/slide were imaged, counted, and averaged to quantify the number of apoptotic cells.

Conditioned Media Collection

MLO-Y4 cells were cultured under apoptotic or normal conditions for 6 h. After TNF-α treatment, osteocytes were rinsed thoroughly with PBS to remove excess TNF-α and replaced with fresh supplemented α-MEM media. Media was incubated for 2 h at 37°C to create apoptotic and nonapoptotic osteocyte conditioned media, respectively. All conditioned media was stored at −20 °C until further use.

Proliferation Assay

Endothelial cells were seeded at 5,000 cells/cm^2 and treated with apoptotic or nonapoptotic osteocyte conditioned media for 72 h to observe proliferative effects. DNA in cell lysates was measured by the Cyquant Assay kit (Invitrogen). A cell number standard curve was used to convert sample fluorescence values into cell numbers.

Migration Assay

CellTracker Green-labeled endothelial cells (see Supplementary Material) were plated into culture inserts (8 μm pore size, Millipore, Billerica, MA) that were submerged in wells containing apoptotic or nonapoptotic osteocyte conditioned media. After culturing the endothelial cells for 8 h at 37°C and 5% CO_2, nonmigrated cells were removed from the upper chamber. Migrated cells were imaged with a fluorescence microscope and counted.

Tubule Formation

CellTracker Green-labeled endothelial cells were plated on 5 μg/ml Matrigel (BD Biosciences; see Supplementary Material) at 200,000 cells/well in a 24-well plate and cultured with apoptotic and nonapoptotic osteocyte conditioned media for 6 h. Resulting tubule networks were imaged under a fluorescent microscope. Tubule length and number of branches were quantified with AngioQuant software[15] for 10 view fields/sample.

Osteocyte Conditioned Media Characterization

A mouse angiogenic cytokine sandwich ELISA (Signosis, Inc., Sunnyvale, CA) was used to semi-quantitively profile cytokine expression in apoptotic and nonapoptotic osteocyte conditioned media, collected as above. Absorbance of the detection agent was measured with a spectrophotometer at 450 nm, and normalized to total cell lysate protein concentrations (see Supplementary Material). α-MEM supplemented media was used as a negative control to measure baseline cytokine levels in the media. Standard curves for each cytokine were not provided within the assay, and thus resulting expression levels were considered semi-quantitative.

The concentrations of VEGF in apoptotic and nonapoptotic osteocyte conditioned media were measured with a mouse VEGF ELISA (Quantikine, R&D Systems). A mouse VEGF standard ranging from 0 to 500 pg/ml was used. Serum-supplemented α-MEM media was used as a baseline VEGF measurement in the culture media. VEGF concentrations were normalized to total cell lysate protein concentration.

VEGF Supplementation and Blocking

A tubule formation assay was performed to determine whether the differential VEGF concentrations measured in apoptotic and nonapoptotic osteocyte conditioned media could elicit a significant difference in angiogenesis. Endothelial cells were treated with supplemented α-MEM with 127 pg/ml or 112 pg/ml of mouse recombinant VEGF 165 (Peprotech, Rocky Hill, NJ) to simulate the VEGF concentrations in apoptotic and nonapoptotic osteocyte conditioned media, respectively, as measured by ELISA. To verify the VEGF effect on tubule network formation on a higher resolution, a VEGF dose–response curve was performed by treating endothelial cells with 0–130 pg/ml VEGF and quantifying the subsequent tubule networks formed.

To determine whether VEGF-mediated osteocytic apoptosis-induced angiogenesis, endothelial cells were treated with or without 1 μg/ml of VEGF blocking antibody (AF493-NA, R&D Systems) in apoptotic or nonapoptotic osteocyte conditioned media, and tubule network formation was quantified. Endothelial cells treated with only apoptotic or nonapoptotic osteocyte conditioned media served as controls.

Data Analysis

All experiments were repeated at least in duplicate, with 3–6 replicates per condition. Student's *t*-tests and two-way ANOVAs were used to test significance. The Tukey test was used as a post-hoc test.

RESULTS

TNF-α-Induced Osteocyte Apoptosis in a Dose-Dependent Manner

With increasing concentrations of TNF-alpha treatment, a higher proportion of osteocytes underwent apoptosis. Treatment with 10 ng/ml of TNF-α for 6 h induced significantly more apoptosis compared to negative control (0 ng/ml TNF-α; *a:* $p < 0.01$), but significantly less apoptosis compared to the positive control (5 mM H_2O_2; *b:* $p = 0.01$, Fig. 1A). Osteocytes treated with 10 ng/ml TNF-α yielded $50 \pm 0.5\%$ osteocyte apoptosis detected by TUNEL, whereas no TNF-α treatment resulted in $6 \pm 5\%$ apoptosis ($n = 3$ per condition; *a:* $p < 0.001$, Fig. 1B).

Osteocyte Apoptosis Regulation by Flow

A higher percentage of apoptotic osteocytes was observed under no flow conditions compared to flow ($p < 0.05$, Fig. 2). When subjected to TNF-α treatment after flow or no flow, a greater percentage of apoptotic cells was observed in the no flow case ($p < 0.05$, Fig. 2). TNF-α treatment also caused more apoptosis in both flow ($p < 0.001$) and no flow ($p < 0.08$) conditions compared to osteocytes that were not treated with TNF-α. While both the no flow condition and TNF-α treatment alone promoted osteocyte apoptosis, we chose to chemically induce apoptosis with TNF-α treatment in subsequent experiments to focus on the role of apoptosis in regulating angiogenesis.

Osteocyte Apoptosis Promotes Angiogenesis In Vitro

Apoptotic osteocyte conditioned media treatment caused more endothelial cell proliferation ($p < 0.05$, Fig. 3A), migration across Transwell filters ($p < 0.05$, Fig. 3B), and formation of tubule networks on Matrigel that contained longer ($p < 0.01$) and more ($p < 0.001$) branches (Fig. 3C) compared to non-apoptotic osteocyte conditioned media treatment.

Differential Presence of Angiogenic Factors in Apoptotic Osteocyte Conditioned Media

The angiogenic cytokine screen detected greater amounts of VEGF, IL-6, and IGF in apoptotic osteocyte conditioned media, but no measurable difference in TNF-α, FGF, IFN, EGF, and Leptin (Fig. 4A). As VEGF is a potent angiogenic factor, a quantitative VEGF sandwich ELISA was performed to further quantify the VEGF concentration in the conditioned media. Apoptotic osteocyte conditioned media contained more VEGF than nonapoptotic osteocyte conditioned media ($p < 0.05$, Fig. 4B). The average VEGF concentrations in apoptotic and nonapoptotic osteocyte conditioned media were 127 and 112 pg/ml, respectively.

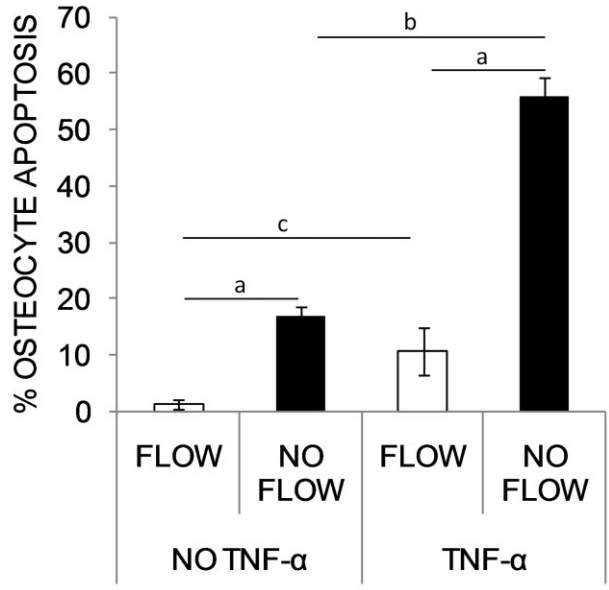

Figure 2. No flow conditions (0 ng/ml TNF-α) caused more apoptotic osteocytes than flow (*a:* $p < 0.05$). Treatment with 10 ng/ml TNF-α after flow or no flow caused more apoptosis in the no flow case (*a:* $p < 0.001$). TNF-α treatment caused significantly more apoptosis in both flow (*b:* $p < 0.001$) and no flow (*c*: $p < 0.08$) cases compared to osteocytes that were not treated with TNF-α ($n = 3$).

VEGF Concentrations in Apoptotic Osteocyte Conditioned Media Yielded More Complex Tubule Network Formation

Endothelial cells treated with complete media supplemented with 127 pg/ml VEGF formed networks with longer ($p < 0.05$) and more complex ($p < 0.02$) tubules compared to treatment of complete media supplemented with 112 pg/ml VEGF (Fig. 5A). Furthermore, endothelial cells treated with VEGF concentrations >115 pg/ml formed tubule networks with more junctions and longer tubules in a dose-dependent manner, while VEGF concentrations <115 pg/ml did not yield a difference in tubule network formation (Fig. 5B). These data confirmed that the differences in tubule network formation between apoptotic and nonapoptotic osteocyte conditioned media could be recapitulated by VEGF.

VEGF Blocking in Apoptotic Osteocyte Conditioned Media Abolishes Tubule Formation

Blocking VEGF abolished the pro-angiogenic effect of apoptotic osteocyte conditioned media, as the length ($p < 0.001$) and branching ($p < 0.001$) of endothelial tubule networks were

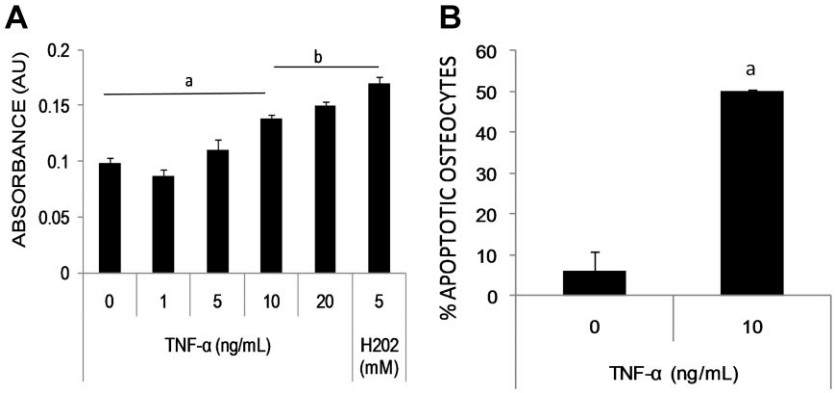

Figure 1. (A) APOPercentage labelling of apoptotic osteocytes. Treatment with 10 ng/ml of TNF-α for 6 h induced significantly more osteocyte apoptosis compared to negative control (0 ng/ml TNF-α; *a:* $p < 0.01$) but significantly less osteocyte apoptosis compared to the positive control (5 mM H_2O_2; *b:* $p = 0.01$). (B) TUNEL staining of apoptotic osteocytes. 10 ng/ml TNF-α treatment on osteocytes induced $50 \pm 0.5\%$ osteocyte apoptosis, whereas no TNF-α treatment resulted in $6 \pm 5\%$ osteocyte apoptosis ($n = 3$ per condition; *a:* $p < 0.001$).

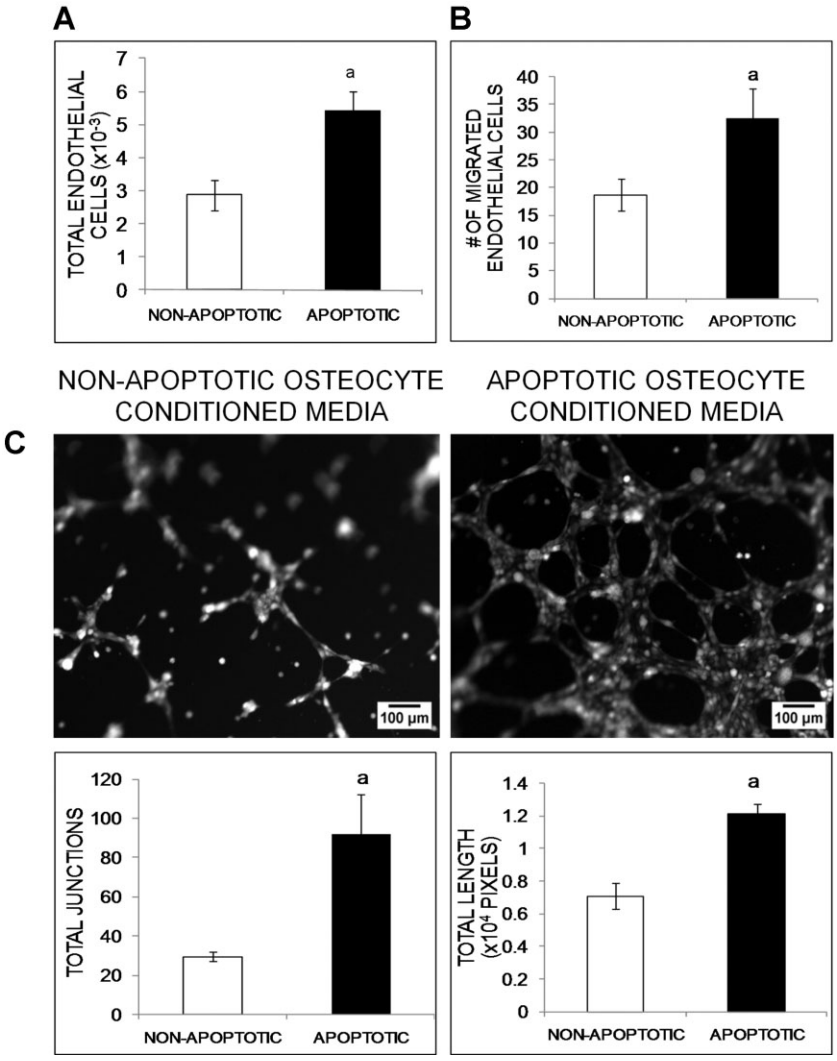

Figure 3. Apoptotic osteocyte conditioned media treatment caused more endothelial cell (A) proliferation ($n = 6$, $p < 0.05$), (B) migration ($n = 6$, $p < 0.05$), and (C) formation of complex tubule networks ($n = 3$) with longer ($p < 0.01$) and more ($p < 0.001$) branches than nonapoptotic osteocyte conditioned media.

reduced to levels similar to those treated with nonapoptotic osteocyte conditioned media alone ($p > 0.7$; Fig. 6). Blocking VEGF in nonapoptotic osteocyte conditioned media did not cause a reduction in the length ($p = 0.97$) or number of junctions ($p = 0.98$) in tubule networks formed by treatment with nonapoptotic osteocyte conditioned media alone (Fig. 6).

DISCUSSION

We investigated the effect of osteocyte apoptosis, and its regulation by flow, on angiogenesis as a potential mechanism for osteoclast precursor recruitment at the initial onset of bone resorption. We found that osteocyte apoptosis is flow-regulated and that soluble factors within apoptotic osteocyte conditioned media regulate endothelial cell proliferation, migration, and tubule formation. We found that apoptotic osteocyte conditioned media contains more VEGF, suggesting that osteocyte apoptosis-induced angiogenesis is regulated by paracrine VEGF-signaling. To verify this hypothesis, VEGF supplementation and blocking assays were performed. We could recapitulate angiogenic differences in tubule network formation with VEGF concentrations measured in apoptotic and nonapoptotic osteocyte conditioned media, respectively. In addition, neutralization of VEGF with an antibody was able to abolish angiogenic effects caused by apoptotic osteocyte conditioned media. Thus, our observations support the hypothesis that apoptotic osteocyte-induced angiogenesis is VEGF mediated.

Oscillatory fluid flow attenuated osteocyte apoptosis compared to no flow conditions, consistent with observations of Bakker et al.,[10] who showed that 1 h of pulsatile fluid flow inhibited chick calvaria-derived osteocyte apoptosis, while disuse promoted apoptosis. Our data also correspond with in vivo observations by Tami et al.,[16] who found apoptotic osteocytes near areas of disrupted interstitial fluid flow at microdamaged sites of fatigue-loaded bone. Further, to test whether mechanical loading can affect chemically induced osteocyte apoptosis, we investigated the effect of TNF-α treatment on osteocytes after the application of flow or no flow. TNF-α is an inflammatory cytokine found in elevated levels in osteoporotic patients[17] that is capable of inducing osteoblast and osteocyte apoptosis.[18,19] Osteo-

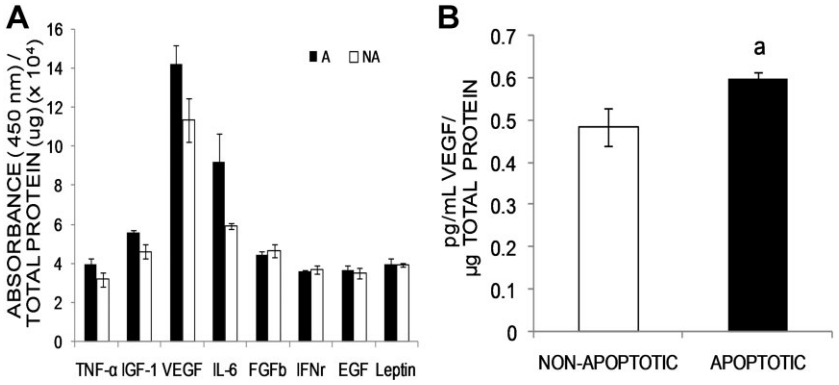

Figure 4. (A) VEGF, IL-6, and IGF were elevated in apoptotic osteocyte conditioned media ($n = 3$). (B) Apoptotic osteocytes secreted more VEGF than nonapoptotic osteocytes ($n = 6$, $p < 0.05$).

cytes subjected to no flow conditions and chemically induced to undergo apoptosis with TNF-α treatment yielded a level of apoptosis similar to that found in fatigue loaded bone[20] (Fig. 1). Under flow conditions, TNF-α-induced apoptosis was significantly attenuated (Fig. 2), consistent with findings by Tan et al.[19] Since, we used oscillatory flow, protection against apoptosis is likely a direct effect of fluid flow on the cells and not due to removal of soluble factors. The mechanism by which fluid flow or other mechanical loads prevent apoptosis is not

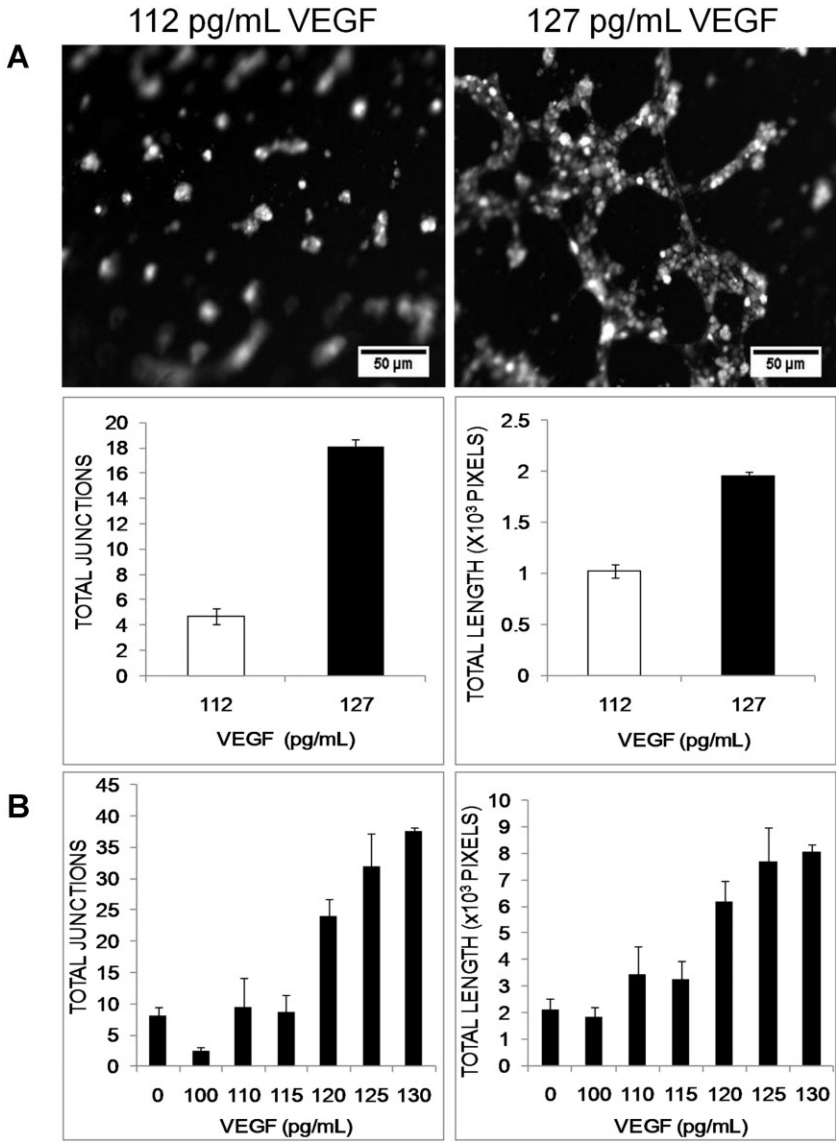

Figure 5. (A) Endothelial cells treated with 127 pg/ml VEGF formed tubule networks ($n = 3$) with longer ($p < 0.05$) and more ($p < 0.02$) branches than those treated with 112 pg/ml VEGF. (B) Endothelial cells treated VEGF concentrations >115 pg/ml formed tubule networks that contained more total junctions and longer tubules in a dose-dependent manner, while concentrations <115 pg/ml yielded no significant differences in total junctions or tubule lengths.

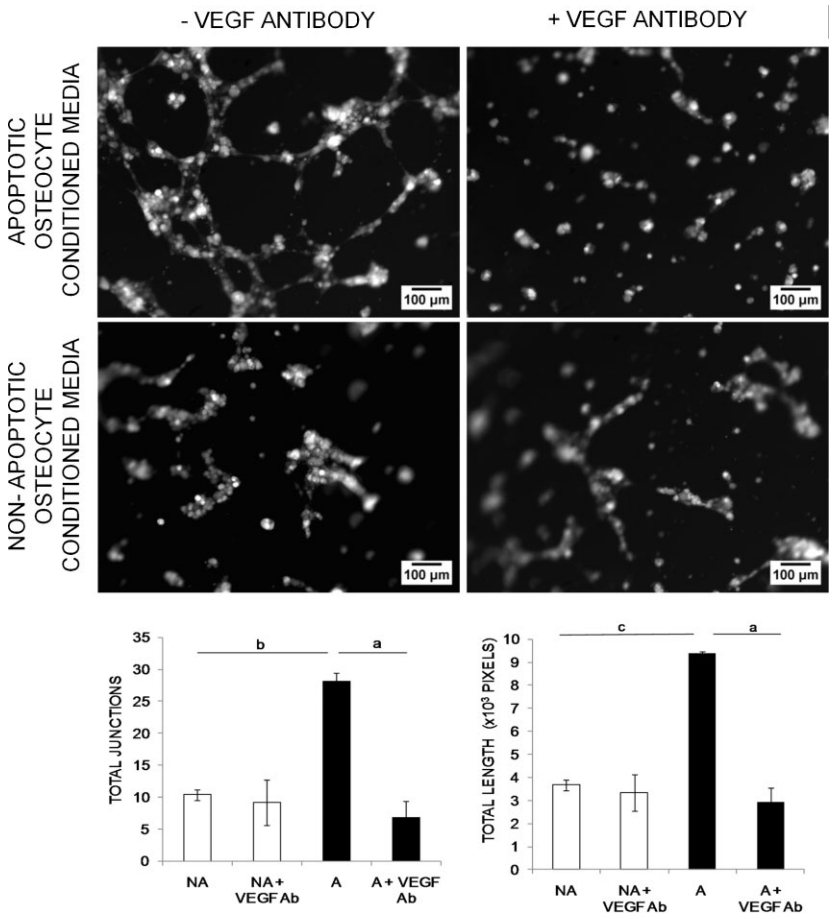

Figure 6. Blocking VEGF abolished the pro-angiogenic effect of apoptotic osteocyte conditioned media as evidenced by reduced tubule length and branching (a, $p < 0.001$). Blocking VEGF caused no differences in tubule length ($p = 0.97$) and total junctions ($p = 0.98$) in tubule networks formed from nonapoptotic osteocyte conditioned media treatment. Apoptotic osteocyte conditioned media treatment alone elicited a difference in branching (b, $p < 0.001$) and tubule length (c, $p < 0.004$) compared to nonapoptotic osteocyte conditioned media treatment ($n = 3$, for all conditions).

fully determined, but may involve mechanically induced nitric oxide production[21] or activation of the ERK-signaling pathway.[22]

The D4T endothelial cell line used in this study is an immortalized cell-line generated from day 4 embryoid body cells by infecting retroviruses expressing the polyoma middle T gene.[23] D4Ts exhibit normal cobblestone endothelial cell morphology when confluent, express common endothelial proteins such as CD31, and perform endothelial functions such as Dil-Ac-LDL uptake.[23] D4Ts have been used by embryonic stem cell scientists to study the precursors of hematopoietic and endothelial cell lineages[2324] and were recently used to vascularize cardiac tissue constructs.[25] With this cell line, we found that conditioned media collected from TNF-α-induced apoptotic osteocytes promoted endothelial cell proliferation, migration, and tubule network formation (Fig. 3), which are three requisite events of angiogenesis.[26]

Our findings suggest that apoptotic osteocyte conditioned media contains cytokines that induce angiogenesis to potentially assist in the delivery of osteoclast precursors directly to the remodeling site. By screening the conditioned media for angiogenic cytokines (Fig. 4A), we found elevated levels of VEGF, IL-6, and IGF-1 in TNF-α-induced apoptotic osteocyte conditioned media but no measurable difference in TNF-α expression. This suggests that the angiogenic response caused by TNF-α-induced apoptotic osteocyte conditioned media is not due to endogenous or exogenous TNF-α. Furthermore, conditioned media collected from H_2O_2-induced apoptotic osteocytes (~50% of the population; Supplementary Fig. 2) yielded a similar angiogenic response to conditioned media collected from apoptotic osteocytes induced with TNF-α (Supplementary Figs. 3 and 4). TNF-α regulates a variety of functions in many cell types[27] including the ability to dose-dependently increase VEGF mRNA expression in keratinocytes.[28] However, we determined that the conditioned media was angiogenic regardless of the method of apoptosis induction, suggesting that apoptosis of a portion of the population (here 50%), and not other TNF-α stimulated mechanisms, is responsible for the angiogenic response. Notably, osteocytes subjected to no flow without TNF-α yielded ~20% apoptosis (Fig. 2), but this did not elicit a significant angiogenic response (data not shown). This suggests that a threshold level of osteocyte apoptosis is required to induce a pro-angiogenic response.

The cell population responsible for VEGF production remains to be identified. Although healthy osteocytes express VEGF in situ,[29] apoptotic osteocytes themselves may express VEGF or they may signal neighboring

healthy osteocytes to secrete pro-angiogenic and pro-resorptive cytokines to induce angiogenesis and osteoclastogenesis. Verborgt et al.[3] reported a greater number of apoptotic osteocytes immediately adjacent to microcracks in fatigue-loaded bone compared to sites distant from microcracks. Similarly, Herman et al.[29] reported an exponential decrease in osteocytic VEGF-A production with distance from fatigue-induced microcracks. Since both studies reported 50% apoptosis near the microdamage site, Herman et al.[29] hypothesized that non-apoptotic osteocytes exposed to factors produced by apoptotic osteocytes are responsible for VEGF production. A separate in vitro study from our lab supports this hypothesis, although we also observed elevated VEGF production by osteocytes induced to undergo apoptosis relative to untreated cells.[30] Thus, both apoptotic and nonapoptotic subpopulations in our TNF-α and H_2O_2-treated cultures may contribute to the pro-angiogenic response.

Previous studies showed that VEGF is a known potent angiogenic cytokine and also promotes osteoclast recruitment and osteoclastogenesis.[31,32] Here, we confirmed that angiogenesis induced by apoptotic osteocyte conditioned media was mediated by VEGF (Figs. 5 and 6). Particularly striking was that a small increase in VEGF ($\sim$15 pg/ml) in apoptotic osteocyte conditioned media elicited a significant angiogenic response (Fig. 5A). By measuring VEGF dose–response at higher resolution, we found that concentrations $<$115 pg/ml yielded no significant changes in total number of junctions and tubule lengths in tubule networks, while concentrations $>$115 pg/ml yielded a dose-dependent response in total junctions and tubule lengths. This finding supports our initial discovery, as average concentrations from nonapoptotic or apoptotic osteocyte conditioned media were within the ranges of little or significant tubule network formation, respectively. This striking observation is similar to an "all-or-none switch-like behavior" that is observed in many signaling pathways and cell behaviors.[33,34]

We have shown that angiogenesis caused by apoptotic osteocyte conditioned media was VEGF-mediated, but our cytokine screen also showed differential expressions of IL-6 and IGF-1. Along with their roles in inducing angiogenesis during tumor progression[35] and brain development,[36] these cytokines may play a role in bone remodeling. Indeed, both cytokines have been implicated in osteoclast precursor recruitment, because they can activate the endothelium to assist in monocyte adhesion and transmigration,[3738] and subsequent induction of osteoclastogenesis.[3940] Thus, their elevated levels in our cytokine screen warrant further investigation.

In summary, our study showed that osteocyte apoptosis, which is regulated by flow, induces angiogenesis through VEGF paracrine signaling. Further studies aimed at determining the connections between mechanically regulated osteocyte apoptosis and osteoclast precursor recruitment may lead to better pharmacological and exercise-based treatments to control osteoclast activity in diseases such as osteoporosis and osteopetrosis.

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