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Review

Mechanical Regulation of Apoptosis in the Cardiovascular System

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Abstract—Apoptosis is a highly conserved physiological process of programmed cell death which is critical for proper organism development, tissue maintenance, and overall organism homeostasis. Proper regulation of cell removal is crucial, as both excessive and reduced apoptotic rates can lead to the onset of a variety of diseases. Apoptosis can be induced in cells in response to biochemical, electrical, and mechanical stimuli. Here, we review literature on specific mechanical stimuli that regulate apoptosis and the current understanding of how mechanotransduction plays a role in apoptotic signaling. We focus on how insufficient or excessive mechanical forces may induce apoptosis in the cardiovascular system and thus contribute to cardiovascular disease. Although studies have demonstrated that a broad range of mechanical stimuli initiate and/or potentiate apoptosis, they are predominantly correlative, and no mechanisms have been established. In this review, we attempt to establish a unifying mechanism for how various mechanical stimuli initiate a single cellular response, i.e. apoptosis. We hypothesize that the cytoskeleton plays a central role in this process as it does in determining myriad cell behaviors in response to mechanical inputs. We also describe potential approaches of using mechanomedicines to treat various diseases by altering apoptotic rates in specific cells. The goal of this review is to summarize the current state of the mechanobiology field and suggest potential avenues where future research can explore.

Keywords—Apoptosis, Cardiovascular disease, Valvular interstitial cells, Vascular smooth muscle cells, Mechanotransduction, Mechanobiology, Mechanomedicine.

INTRODUCTION

Apoptosis, also known as programmed cell death, involves a complex signaling cascade whereby extracellular or intracellular signals result in the orderly termination of cells. Although apoptosis results in the demise of cells, it is critical to the continuing health of the organism as a whole, especially during embryogenesis, growth, and tissue maintenance. Common examples of homeostatic apoptosis include digit individualization of the hands/feet, loss of tails in tadpoles, and cellular reorganization in dorsal closure in *Drosophila* (Fig. 1).[131] On the other hand, when apoptosis is incorrectly regulated, unchecked cell growth can lead to fibrosis and tumor formation, while excessive apoptosis can lead to degenerative diseases, such as degenerative disc disease and muscle atrophy (Fig. 2).[49,84] In fact, one of the hallmarks of cancer is the ability of malignant cells to evade apoptosis.[47]

There are many environmental factors that can initiate apoptosis, such as pH, oxygen concentration, radiation, infection, cytokines and inflammatory signaling molecules.[64,75,118] Increasingly, the mechanical environment is being recognized as a key regulator of apoptosis as well.[27,41] Externally applied mechanical stimuli (stretch and forces) and external physical factors (ECM stiffness, geometric constraints, topography) which regulate internally generated cell forces are all referred to as mechanical stimuli within this review. How mechanical stimulation is interpreted by the cell and transduced into an apoptotic response is an active area of research in cell mechanobiology. Currently, there are no well-defined mechanisms of action for how mechanical stimuli regulate cell health and initiate apoptosis. Teasing apart specific conditions which initiate apoptosis has been challenging, as factors inducing cell death are dependent upon the specific loading conditions and the extracellular mechanical environment is constantly evolving. Most studies correlate mechanical stimuli with observed cell responses. For example, elevated mechanical loading has been shown to initiate apoptosis by causing excessive DNA damage, while trauma from mechanical forces induces

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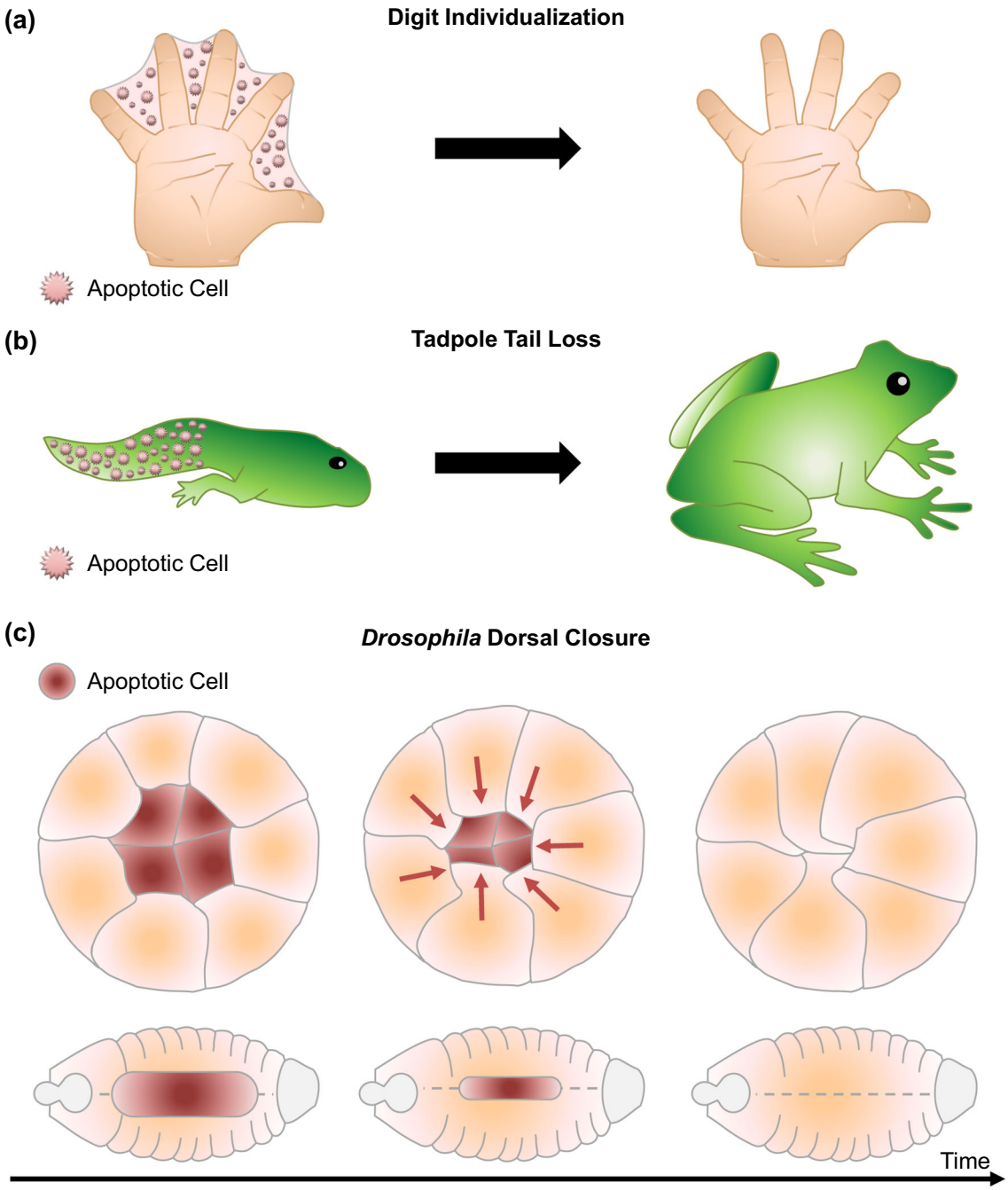

FIGURE 1. Apoptosis occurs naturally in organisms during various phases of their life, such as morphogenesis and embryogenesis. (a) Morphogenetic apoptosis helps separate digits in the hand and feet. (b) Apoptosis in tadpole tails helps transition their growth into frogs, causing tadpoles to lose their tails. (c) During the embryonic stage, *Drosophila* undergo a process called dorsal closure, where an elliptical gap in the dorsal region converges and undergoes compression and apoptosis.

apoptosis by altering mitochondria permeability.[19,89] Apoptosis also occurs following wound healing and results in the removal of excess myofibroblasts; these cells are shielded from stresses by the mature collagen matrix that they produce and remodel around themselves.[136] No real mechanisms have been offered linking mechanics to apoptosis; this is the direction that the field needs to improve next.

In this review, we analyze mechanically regulated apoptosis with a focus on the cardiovascular system. We give a general overview of apoptosis for the general biomedical and mechanobiology audience. In this regard, we provide a brief introduction to aberrant

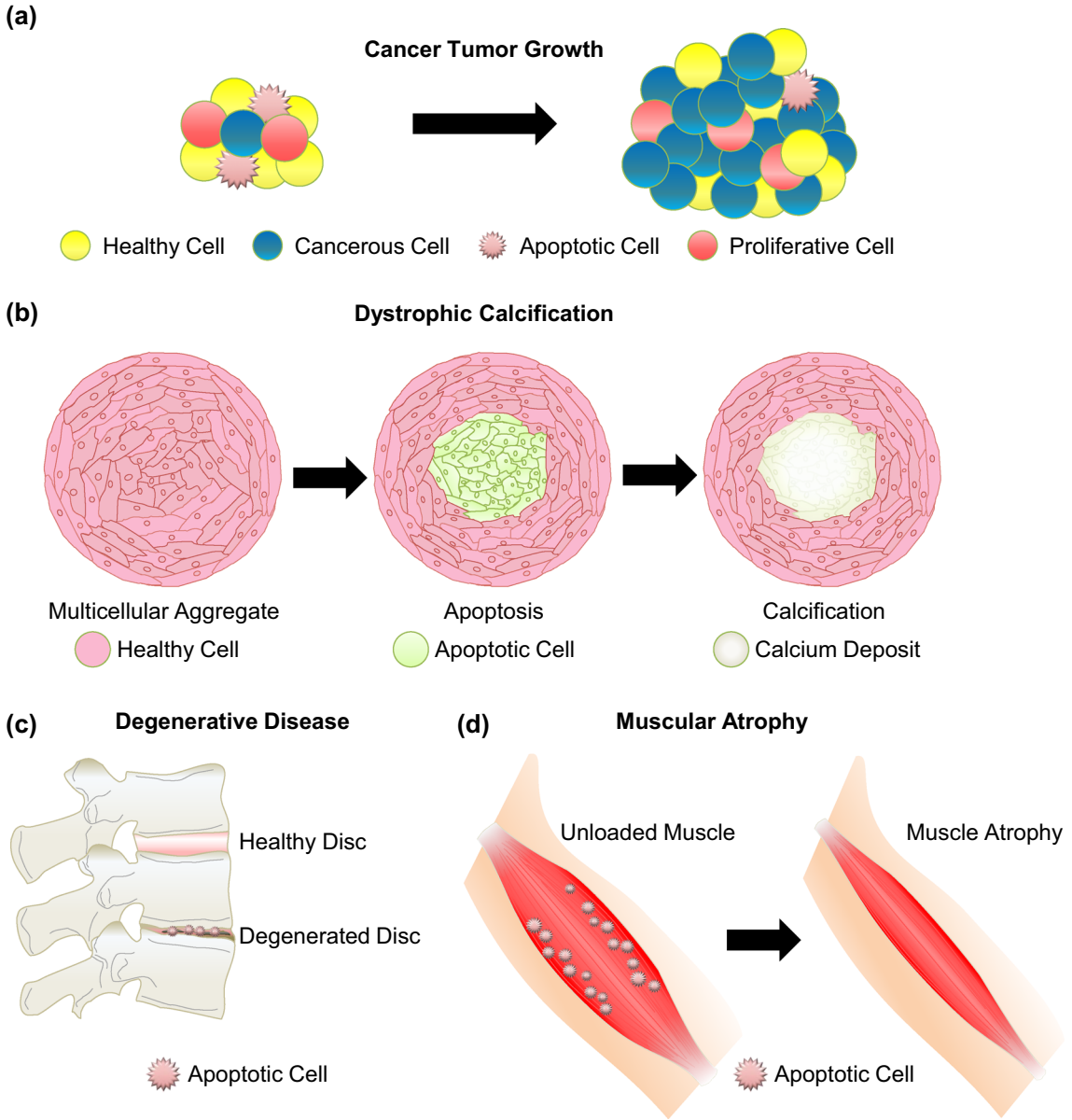

FIGURE 2. Unregulated apoptosis (insufficient or excessive) can lead to various diseases. (a) Healthy tissues have a balance between apoptotic and proliferative cells; in contrast, the unregulated growth of cancerous cells which evade apoptosis lead to tumor formation. (b) In dystrophic calcification, a symptom of heart valve disease, cells aggregate triggering apoptosis of the central compressed cells which promotes calcium deposition and disease progression. Uncontrolled apoptosis can also lead to degenerative diseases such as degenerated discs in the spine (c) and atrophy of the muscles (d).

apoptosis present in different types of cardiovascular diseases and describe the canonical signaling pathways of apoptosis. We then review the literature demonstrating mechanical-induced apoptosis and discuss potential mechanosensing pathways. Where and how mechanics acts on cells is not yet known. These are the questions research needs to explore in the future. We attempt to specify a potential unifying mechanism for how mechanical stimuli initiate apoptosis. We hypothesize that regulation of cytoskeletal stability may link mechanics to apoptosis. Finally, we conclude by examining the potential of mechanomedicines for treatment by regulation of apoptosis. The goal of this review is to summarize the current state of understanding of the mechanobiology of apoptosis for the

general reader and encourage promising directions to explore for future research.

THE ROLE OF APOPTOSIS IN CARDIOVASCULAR DISEASES

Apoptosis is required for the orderly removal of cells within an organism. In contrast, necrosis is premature cell death causes by external disease, injury, or lack of blood supply. As necrotic cells die, an inflammatory response can be triggered causing collateral damage to surrounding cells. Apoptosis allows the removal of cells without initiating undesirable immune responses. When apoptosis is unregulated, cell death can contribute to disease initiation and progression. Apoptosis is believed to contribute to various cardiovascular diseases such as heart failure, atherosclerosis, aneurysm formation, calcific aortic valve disease, and pulmonary arterial hypertension.[16,62,93,100,119] Combined, these diseases represent a significant source of morbidity and mortality in the United States, highlighting the immense burden aberrant apoptosis places on society.

Heart Failure

Chronic heart failure is marked by progressive loss of cardiomyocytes over time.[16,68] Numerous studies suggest that apoptosis of cardiomyocytes plays a role in cardiomyopathy and heart failure,[16,68] and apoptotic cardiomyocyte death has been shown to accompany irreversible congestive heart failure.[102] Further, examination of diseased explanted hearts from cardiac transplantation patients with idiopathic dilated cardiomyopathy and ischemic cardiomyopathy revealed high levels of apoptosis indicating a link between cardiomyocyte apoptosis and end-stage heart disease (Fig. 3).[96] Conversely, inhibiting apoptosis of cardiomyocytes has been shown to reduce the development of cardiac dilation and contractile dysfunction, both of which are hallmarks of heart failure.[148]

Atherosclerosis

Coronary heart disease is the most common form of cardiovascular disease, affecting 1 in 15 Americans each year.[140] A hallmark of coronary heart disease is the progressive narrowing and stiffening of the coronary arteries, also known as atherosclerosis. Atherosclerosis is characterized by the loss of integrity of the intimal (inner) surface of arteries and deposition of plaques of fatty material and cells. Although the early disease mechanisms are still an active area of research, it is known that the contiguous vascular endothelial cell (VEC) layer which lines the arterial wall is disrupted. The endothelial layer continuously proliferates to renew the intimal layer of blood vessels. As a result of overcrowding or injury, VECs undergo apoptosis and are then extruded from the endothelium to make room for new cells.[100] External stresses, such as mechanical stresses from overcrowding, can stimulate the sphingosine-1-phosphate pathway in endothelial cells.[92] This pathway activates contraction of actin and myosin II fibers of neighboring VECs and physically forces apoptotic cells from the endothelial layer while preventing any gap formation. This permits the elimination of aberrant or unfit cells from the endothelial layer while maintaining membrane integrity. On the other hand, when endothelial cells apoptose and are not properly extruded, their remnants may contribute to atherosclerosis. Apoptotic cells become more pro-coagulant and pro-adhesive for platelets, possibly through activated β1 integrin sig-

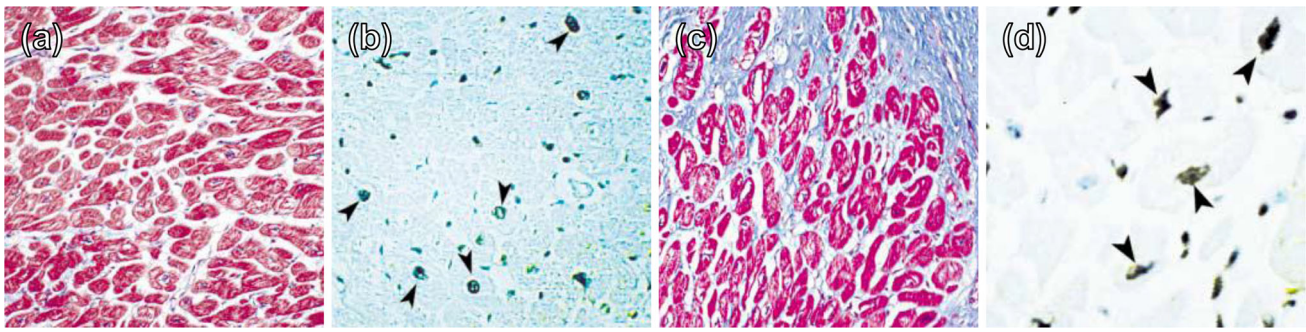

FIGURE 3. Apoptosis is present in various types of heart failure. (a) In end-stage idiopathic dilated cardiomyopathy, myocardial sectioning shows normal myocytes and no fibrosis. (b) Apoptotic myocytes (arrowheads) are generally observed in aggregated cells (not isolated) and varies regionally (TUNEL+ cells stained black) (× 250 magnification). (c) In ischemic cardiomyopathy, myocardial section shows mild myocardial hypertrophy and extensive interstitial fibrosis. (d) Apoptosis is found in myocytes (arrowheads) and can vary regionally in sections (TUNEL+ cells stained black) (× 350 magnification). These sections are evidence of the link between high levels of cardiomyocyte apoptosis and end-stage heart disease. (a–d) Images are adapted from Narula *et al.*[96]

naling, which can promote plaque formation.[155] Additionally, apoptotic cells can cause plaque instability leading to rupture.[155]

Another known mechanism for atherosclerosis progression occurs when smooth muscle cells, which normally reside in the medial layer of the arterial wall, migrate and proliferate into the intimal plaques. High rates of apoptosis in smooth muscle cells remaining in the tunica media accelerate the disease state, promoting medial layer degeneration.[21] Additionally, high rates of smooth muscle cell apoptosis within plaques decrease plaque stability and increase the risk of rupture.[20] Plaque rupture can be life threatening, causing complications including heart attack and stroke.

Aneurysm Formation

Aortic aneurysms contribute to over 10,000 deaths each year in the United States.[140] Aneurysms are localized bulges in an artery initiated from a weakened arterial wall. Examination of human pathological tissue specimens show higher rates of apoptosis and decreased vascular smooth muscle cell density in the medial layer of aneurysmal tissues compared to healthy control tissues.[119] Additionally, the rho kinase inhibitor, Fasudil, was shown to prevent apoptosis and formation of aneurysms when administered to ApoE-deficient mice.[145] These findings implicate excessive apoptosis as an aneurysm initiating mechanism.

Calcific Aortic Valvular Disease

Calcific aortic valvular disease (CAVD) is the most common valvular pathology, and the third most common type of cardiovascular disease.[29,35] CAVD arises from mineral deposits which form on the leaflets. A study of 350 explanted valves from surgeries found over 83% of the valves demonstrated evidence of dystrophic calcification.[93] Examination of diseased explanted valves show the fibrous protein structure highly disarrayed with loss of the tri-layer tissue architecture.[78,117] Valvular interstitial cell aggregation coinciding with large increases in cell density is linked to increased instances of apoptosis and subsequent dystrophic calcification (Fig. 4).[18,60,133] Studies utilizing *in vitro* models of valve calcification demonstrate that preventing apoptosis with pan-caspase inhibitor Z-VAD-FMK prevents calcifications from forming, suggesting that apoptosis is a critical step in the disease process.[18,60]

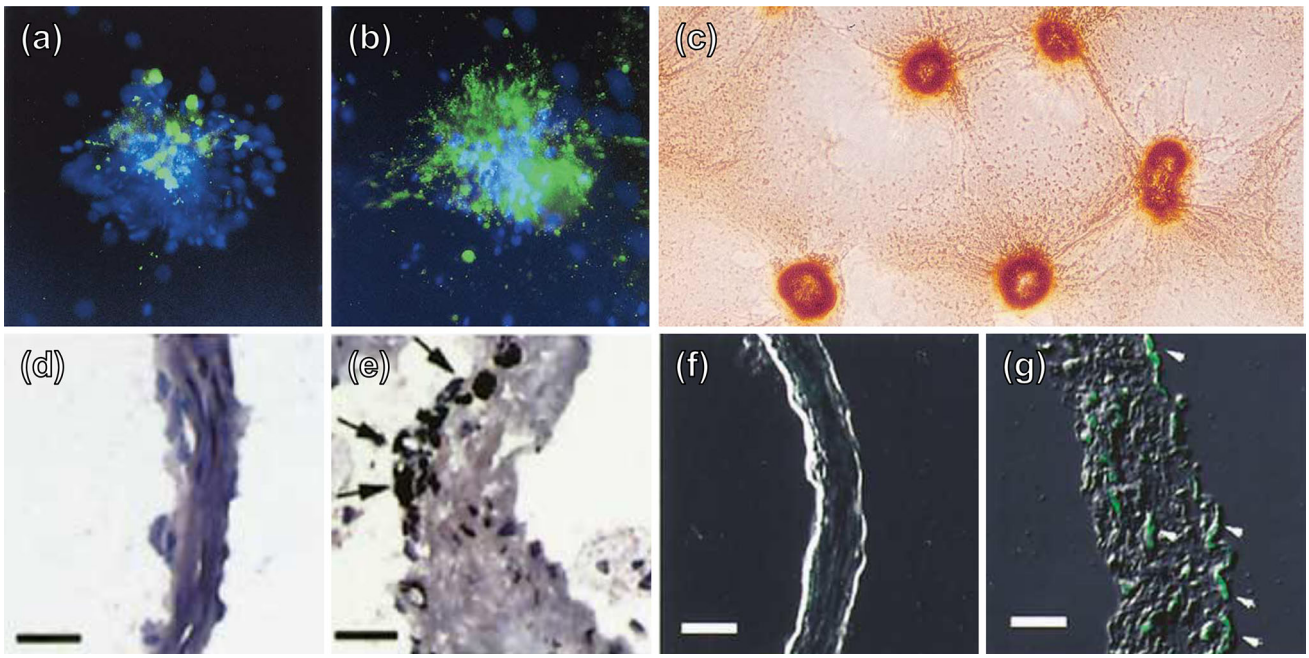

FIGURE 4. In *in vitro* models of CAVD, TGF-β1 causes cell aggregation to occur in monolayers of valvular interstitial cells. Cells in the center of the aggregates begin to apoptose after 3 days (a) and show high rates of apoptosis after 7 days (b). Cells are stained for nuclei (blue, DAPI) and apoptosis (green, Annexin V) (× 200 magnification). (c) Cell aggregates stain positive for calcification after 14 days (red, Alizarin Red S) (× 100 magnification). In *in vivo* models, hearts from wild-type mice (d) are negative for calcification, while ApoE deficient mice (e) stain positive for ectopic calcification (von Kossa). Arrows indicate positive area. Apoptotic death is absent in wild-type mice (f) but present in ApoE deficient mice (g). Arrows indicate apoptotic cells, which are TUNEL stained (green). Scale bars = 20 μm. (a–c) Images are adapted from Jian *et al.*[60] (d–g) Images are adapted from Tanaka, *et al.*[133].

Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a rare disease with very poor prognosis.[107] PAH results from adaptive pulmonary remodeling, vasoconstriction, and thrombosis.[46] Interestingly, unlike heart failure, CAVD, aneurysm formation, and atherosclerosis where abnormal apoptosis increases the severity of the disease, increasing apoptosis in pulmonary arterial smooth muscle (PASM) cells has been proposed as a possible treatment to reverse the hyper-proliferative state of PASMs.[53]

CANONICAL AND NON-CANONICAL APOPTOTIC PATHWAYS

Currently, there are two defined canonical pathways recognized in the apoptotic process: the intrinsic pathway and the extrinsic pathway (Fig. 5). There is growing evidence of other non-canonical pathways that initiate apoptosis in cells, such as ones that are mechanosensitive. In these pathways, mechanical forces can induce and modulate the extrinsic and intrinsic pathways.

The extrinsic pathway is activated by external stimuli that bind to various cell transmembrane "death" receptors. Two common death receptors are TNF-α and FAS, which are part of the tumor necrosis factor (TNF) family.[83] Upon activation of the receptors, multiple intracellular proteins interact with one another in order to form a death-inducing signaling complex (DISC). These complexes then recruit and activate downstream initiator caspases, such as caspases 8 and 10. These initiator caspases can then activate parts of the intrinsic pathway by inducing mitochondrial stress, or can activate downstream effector caspases, such as caspase-3 and 7, which directly initiate degradation of cellular components and facilitate cell death. When proceeding through the stages of apoptosis, caspases interact with numerous downstream proteins as a form of intracellular chemical signaling. Although distinct from one another, there is evidence that the intrinsic and extrinsic pathways are connected and that signaling molecules in one pathway can stimulate the other.[56]

The intrinsic pathway, also known as the mitochondrial pathway, is activated through signals generated inside the cell. This pathway can be triggered by various positive or negative stimuli. Positive stimuli include DNA damage,[98] oxidative stress,[64] heat shock,[123] radiation,[147] viral infection,[48] hypoxia,[121] as well as a positive induction from cytokines.[4] Negative stimuli can include the absence of specific hormones, such as growth factors[3] and cytokines,[85] which results in a failure to repress apoptosis. The Bcl-2 protein family plays a major role in regulating the intrinsic pathway, as it serves dual functions in apoptosis with some protein members serving as anti-apoptotic stimuli and others being pro-apoptotic.[24,137] Mechanical stimuli have been found to cause changes in Bcl-2 anti-apoptotic and pro-apoptotic protein levels and thus interact with the intrinsic pathway.[72,129] Bcl-2 and Bcl-xL proteins act as anti-apoptotic cues, prompting cell survival and inhibiting apoptosis, while Bax, Bad/Bid, and Bik proteins serve as pro-apoptotic cues, which advance apoptosis by promoting permeabilization of the mitochondrial membrane.[24,137] Cytochrome c, the main chemical mediator of downstream apoptosis signaling, is released from the permeabilized mitochondrial intermembrane compartment into the cytosol.[137] Cytochrome c then binds with apoptotic protease activating factor 1 (APAF1) to create apoptosomes. Apoptosomes activate downstream caspases leading to the final stages of apoptosis, such as membrane blebbing and the formation of apoptotic bodies.[150]

In addition to the traditional extrinsic and intrinsic pathways, there exist many secondary signaling apoptotic pathways, some of which are mechanically linked. Mechanical stretch has been found to activate both the extrinsic and intrinsic pathways but the mechanisms remain unknown.[81] Mechanically induced apoptosis may be a result of cytoskeletal destabilization, which can cause deformities in mitochondrial and nuclear structure.[1,50] These organelles are directly connected to the cytoskeleton, where external mechanical forces can transfer directly from the cell boundary to internal organelles.[69,79] Cytoskeletal disruption can cause changes in gene expression, subsequently altering cell behavior and possibly inducing apoptosis.[59,132] Additionally, mechanical stresses have been found to activate various caspases and death receptors in the apoptotic pathway.[120,152] The mechanisms behind transducing external mechanical signals into an apoptotic response remain unknown but are possibly accomplished via cytoskeletal reorganization.

The state of the cytoskeleton can also regulate mitochondrial permeabilization. Reduced actin dynamics increase instances of apoptosis in yeast with actin point mutations.[42] F-actin with decreased turnover forms clumps and results in increased accumulation of reactive oxygen species (ROS) as well as prolonged opening of voltage dependent anion channels (VDAC). Open VDACs result in a loss of mitochondrial membrane potential and increased apoptosis. To this effect, the enzyme gelsolin helps promote F-actin turnover. Gelsolin overexpression results in the closure of VDACs resulting in a reduc-

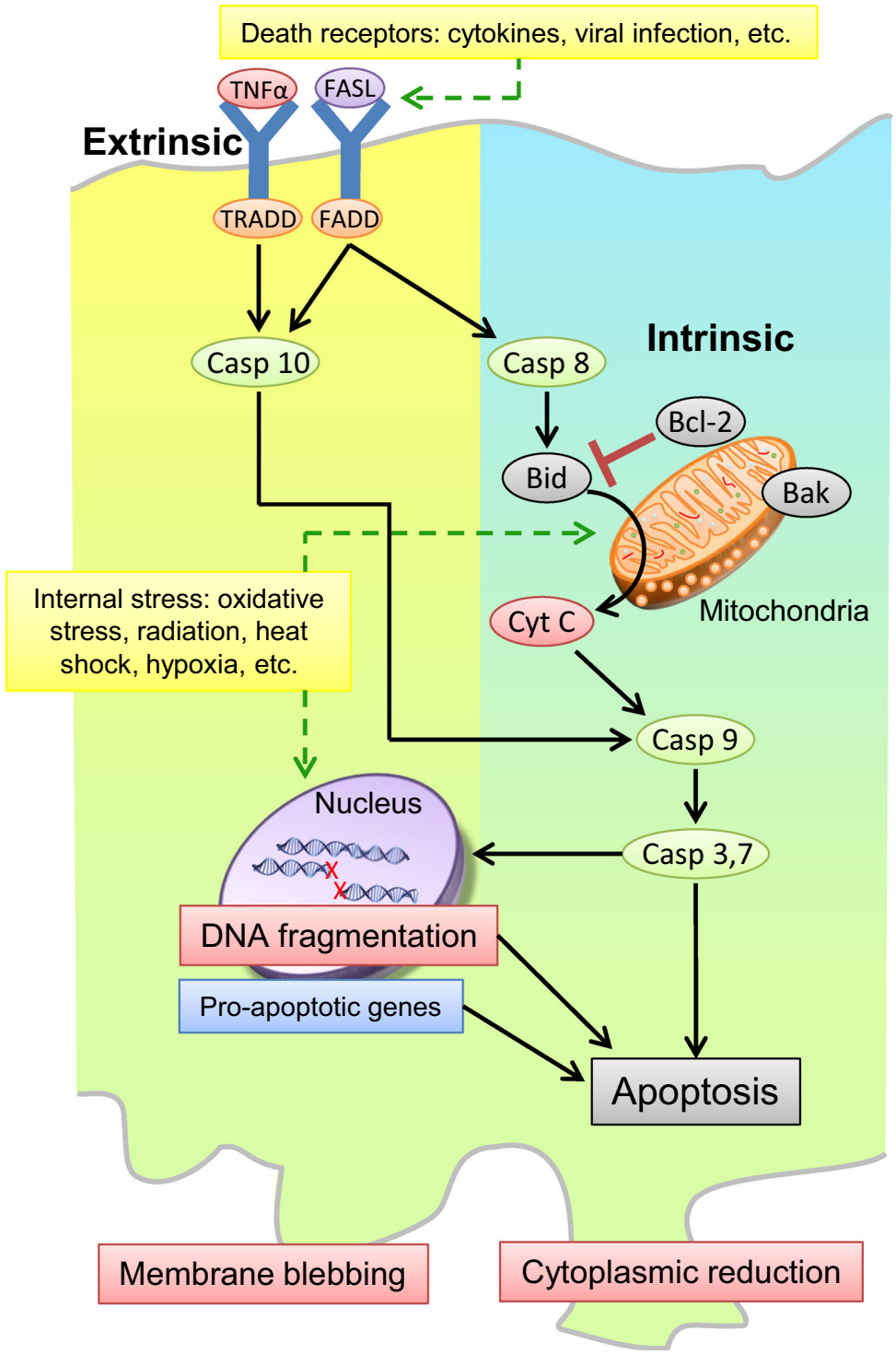

FIGURE 5. Overview of apoptotic pathways. Apoptosis can be triggered by the intrinsic pathway (mitochondria mediated) or extrinsic pathway (receptor mediated). External factors, such as cytokines and infection, can initiate the extrinsic pathway, while internal factors, such as oxidative stress and radiation, can initiate the intrinsic pathway. The two pathways can interact with one another through mechanical and chemical signaling, and progress with activated caspases.

tion of cytochrome c release, maintenance of membrane potential, and reduction in apoptosis.[74]

Actin-associated molecules can initiate apoptosis when they dissociate from cytoskeletal fibers. Bmf, a pro-apoptotic protein, is normally sequestered within actin-associated myosin motors and inactive.[114] CD95/FAS, another pro-apoptotic protein, associates with actin at the cellular membrane via the protein ezrin.[105]

Additionally, Akt (protein kinase B, PKB) is activated through integrin signaling and the phosphatidylinositol 3-kinase (PI3K) pathway.[34] Upon actin depolymerization by cell detachment or cytochalasin D treatment, Bmf is released from myosin, initiating mitochondrial-dependent apoptosis, ezrin-FAS association is reduced, initiating FAS-dependent apoptosis, and Akt activity is reduced, which increases instances of apoptosis (Fig. 7).

STAGES OF APOPTOSIS AND STAGE-SPECIFIC MARKERS

Studying the timing of apoptosis is important to understand how mechanics plays a role in the different stages of apoptosis. Apoptosis is a process that progresses over time, with specific characteristics within each stage. Determining the stages in which mechanics plays a role may lead to new targets in the apoptotic pathway aimed at increasing or reversing apoptosis. There are five common apoptosis detection assays: morphological changes, membrane alteration, caspase detection, DNA fragmentation, and mitochondrial membrane potential.[6,37,40,51,113] There are pros and cons to each assay as each one is used to measure a different characteristic of apoptosis. Some proteins, such as caspases, are temporary and need to be measured at the correct times, while others are permanent, such as DNA fragmentation.

Early Stage Apoptosis

In early-stage apoptosis, signaling cascades begin to activate after death-receptors or internal triggers are activated. The cascades include the activation of caspases, which are transient proteins.[73,91] Caspases can be detected by numerous means including western blots, immunoprecipitation, and immunostaining. Caspase-3 and 7 are two of the most commonly activated caspases during apoptosis and are therefore a common target for researchers.[113] Staining for caspase can be done on live cells, fixed cells, or cell pellets. Additionally, mitochondria begin to permeabilize in early apoptosis leading to changes in the mitochondrial membrane potential due to ion leakage. This depolarization can be detected through the use of fluorescent dyes, which bind to ions that are translocated across the mitochondrial membrane, coupled with fluorescent microscopy.[109]

Mid Stage Apoptosis

During mid-stage apoptosis, caspase signaling cascades continue to be active, and the mitochondria continue to permeabilize, causing more ion leakage. Also, during mid-stage apoptosis, membrane alteration in apoptotic cells occurs which is characterized by the externalization of phosphatidylserine, a membrane residue. At first, phosphatidylserine translocation may be reversible, but as apoptosis progresses, this process becomes permanent. These residues can easily be visualized by staining cells with Annexin V.[32] This allows simple detection of single apoptotic cells; however, this marker is also shared with cells that are undergoing necrosis. Therefore, a control is necessary to distinguish between these two types of cell death, such as co-staining with propidium iodide. Morphological changes such as cytoplasmic reduction may also occur during the mid-stage of apoptosis. Most of these changes are visually distinguishable via light microscopy.

Late Stage Apoptosis

In late-stage apoptosis, permanent transformations occur in apoptotic cells. Additional morphological changes occur including nuclear fragmentation/condensation, disorganization of cytoplasmic organelles, formation of apoptotic bodies, and blebbing of the cell membrane.[37,66] Further, DNA fragmentation occurs within the nucleus.[25,26] A terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, which labels the ends of DNA breaks, can be used to observe fragmented DNA in apoptotic cells.[26,95]

Apoptosis has previously been considered a unidirectional process; once initiated, the process ends in cell death. However, it is possible to reverse apoptosis if cells have not progressed too far down the apoptotic cascade (Fig. 6). It has been shown in numerous studies that removing an apoptotic inducer can allow cells to revert back to a healthy state, sometimes as late as mid-stage apoptosis.[38,134,142] For example, murine hepatocytes show increased levels of caspase-3 and caspase-8 when cells are induced with glycochenodeoxycholate (GCDC), yet they return to control levels after the removal of GCDC.[142] After returning to higher culture temperatures, low-temperature-stressed p53 MOD cells have decreased levels of Annexin V, which is indicative of phosphatidylserine internalization, and undergo DNA repair (low temperature is apoptotic inducer in these cells).[38] HeLa cells revert to their typical morphology and have decreased rates of apoptosis when inducers like jasplakinolide, ethanol, or staurosporine are removed if early in the apoptotic process.[134] However, if nuclear fragmentation occurs, cells cannot be rescued even after the removal of the apoptotic inducer. Finally, in various cancer lines, such as human A375 (skin), HepG2 (liver), MCF7 (breast), and PC3 (prostate) cancer cells, cells treated with jas-

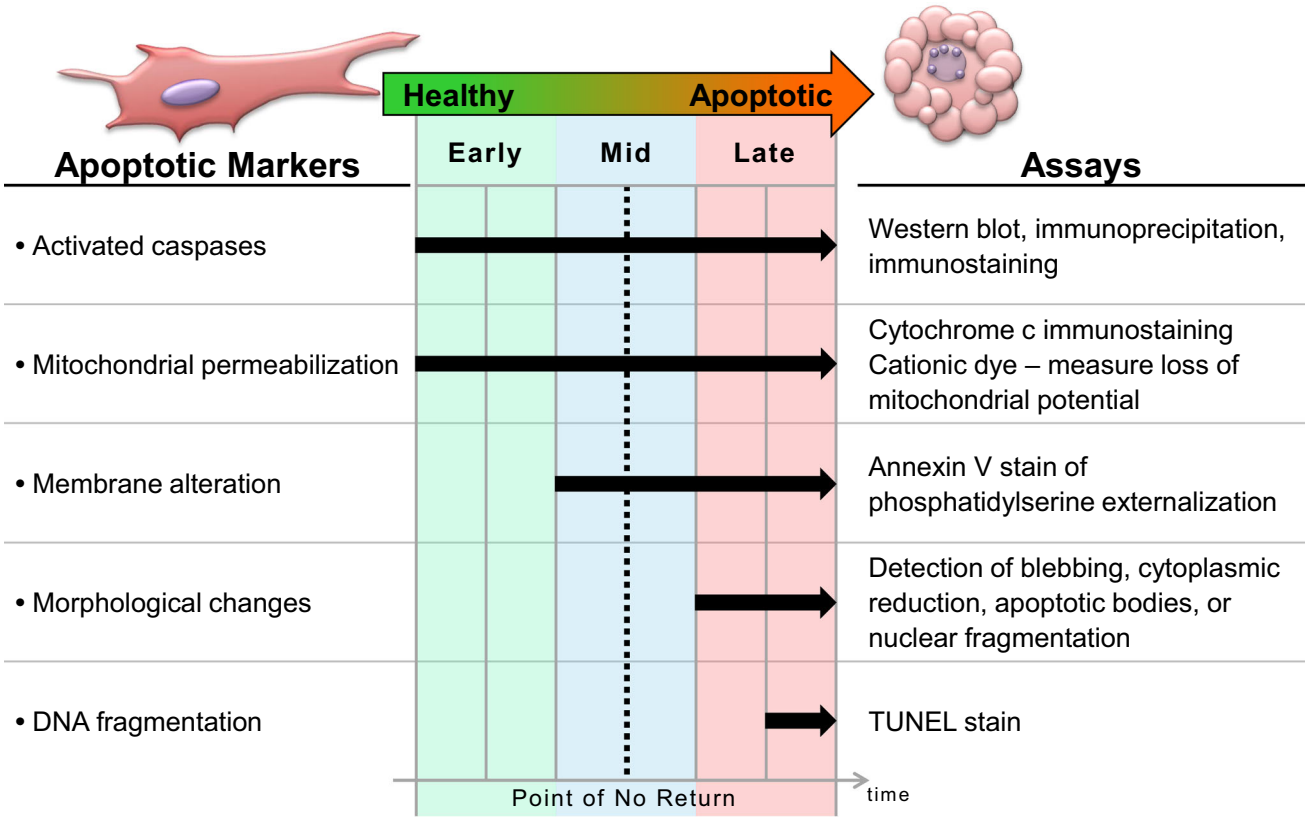

FIGURE 6. Apoptosis occurs in three different stages: early, mid, and late. Different stage-specific markers (left list) are activated/initiated at specific times within the apoptotic process and can be measured with associated assays (right list). Apoptosis is a reversible process up until the dotted line, which indicates the point of no return, where a cell reaching this point will always complete apoptosis.

plakinolide (a reagent that disrupts actin filaments and induces polymerization of monomeric actin into amorphous masses) exhibit early apoptotic markers, yet the cells revert back to their apoptosis-resistant homeostatic state if the chemical inducer is washed before the later stages of apoptosis are reached. These findings indicate that certain stages of the apoptotic pathway can be reversible while others are permanent (Fig. 6).

MECHANICAL INDUCTION OF APOPTOSIS

Recently, there is increasing evidence that the mechanical environment plays a critical role in cardiovascular apoptosis.[27,41] It includes external forces applied to the cells and the material properties of the extracellular matrix (ECM) which resist cell-generated forces. The mechanical environment affects cells through mechanotransduction, the processes by which mechanical stimuli are sensed, transferred, and then converted into biochemical signals and subsequent gene expression that regulate cell fate (Fig. 7). There are various mechanical forces that are physiologically and pathologically present within the cardiovascular system. The chambers of the heart, heart valves, and blood vessels reside in diverse conditions which give rise to heterogeneous structures and properties of each component. These cell environments have varying levels of stiffness, are exposed to cyclical mechanical loading, and can experience fluid shear stress from flowing blood. To study the effects of these stimuli in controlled environments, researchers recapitulate substrate stiffness, stretch, and shear *in vitro*. Culture on compliant hydrogels allow for the tuning of the substrate stiffness, enabling the study of cells on a variety of elastic moduli, which can replicate a range of environments found within the body. A number of devices have been developed commercially or custom-designed within research labs that can apply stretch to cells, either uniaxially or biaxially. Cells cultured within compliant stretchable wells fit within device actuators, and when the device is run, the substrate and adhered cells undergo mechanical stretch. Various stretch waveforms are programmable, allowing for the study of different mechanical stretch cues and potential thresholds. Shear stress is applied by replicating fluid flow patterns in microfluidic devices, parallel flow

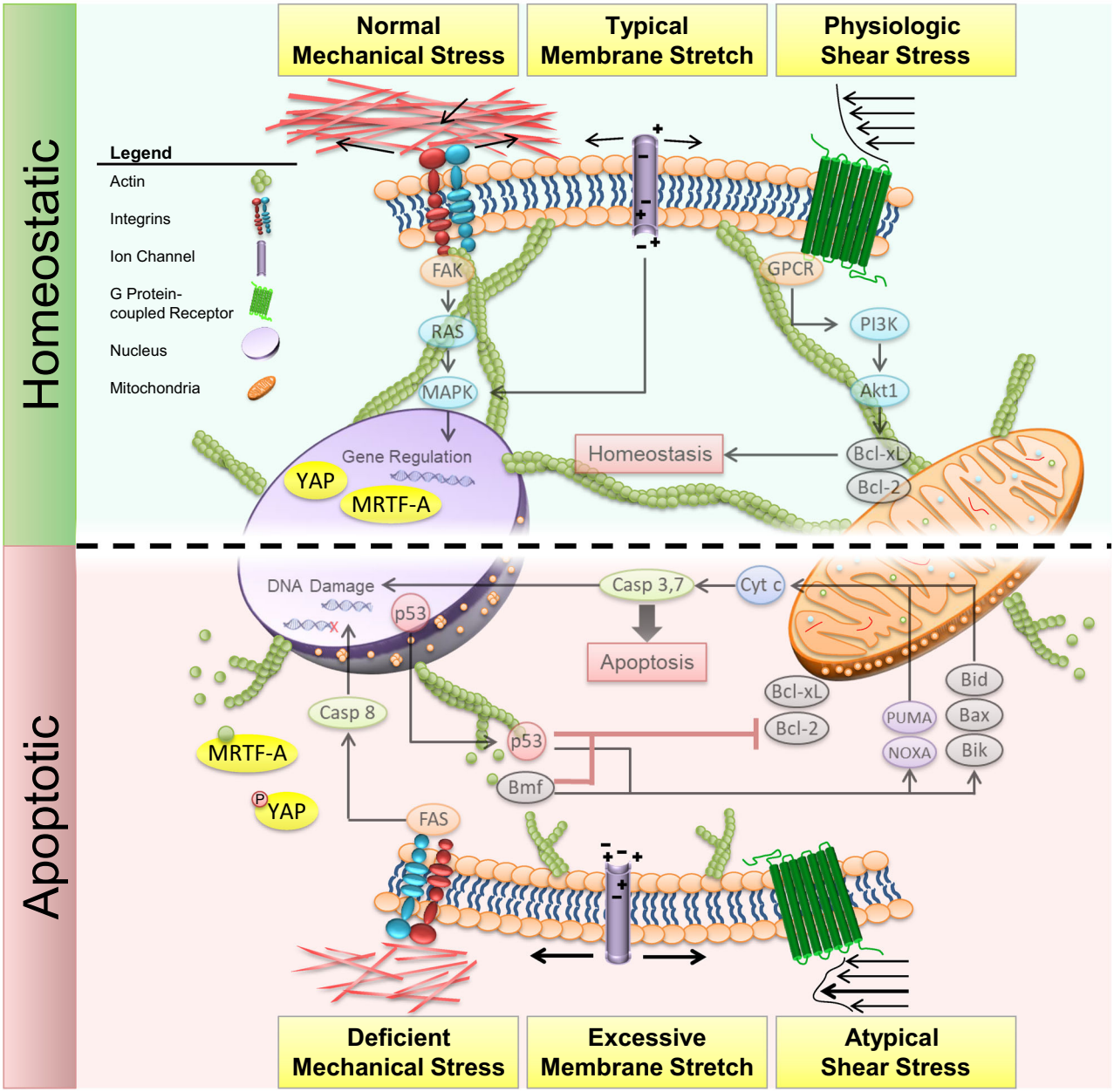

FIGURE 7. Mechanical stimuli that regulate apoptosis include mechanical stress from the ECM, stretching of the cellular membrane, and shear stress, which activate signaling pathways via integrins, ion channels, and G-protein coupled receptors (GPCR). *Top* mechanical stimuli at physiological levels activate pro-survival pathways, such as the PI3K and MAPK pathways, which maintain cells at a homeostatic state. The cytoskeletal network is highly stable and remains as a fiber network. Mechanosensitive proteins, regulated by the cytoskeleton, such as YAP and MRTF-A, are activated and localized to the nucleus. *Bottom* mechanical stimuli at sub- or supra-physiological levels activate pro-apoptotic pathways, such as the p53 tumor-suppressor and Bmf pathways, which inhibit anti-apoptotic molecules and activate pro-apoptotic molecules, subsequently advancing the apoptotic process. These mechanical stimuli can be excessive or deficient mechanical stress from a fragmented ECM, excessive stretch of transmembrane channels, or abnormal shear stress. The cytoskeletal network is destabilized and fragments. Mechanosensitive proteins, such as YAP and MRTF-A, are deactivated and localized to the cytosol.

plates, and cone-in-plate devices. Combinations and interactions between mechanical stimuli are also studied by integrating multiple of the aforementioned platforms together, e.g., stretching cells cultured on tunable modulus substrates.[17,23] These mechanical stimuli have different effects on cell survival individually and in combination (Table 1).

Mechanical Stretch

Increased rates of apoptosis have been observed in tissues exposed to supraphysiologic stretch, such as in individuals with hypertension and subsequent pathological vascular remodeling.[8,62,150] Different locations within the cardiovascular system undergo different magnitudes of mechanical stretch and therefore stress. It is well known that the pressures and loads experienced by arteries are much greater than those of veins. Cells within each environment are accustomed to those specific loading conditions and aberrant apoptosis is seen when this loading is changed. For example, in *in vivo* murine models, higher rates of apoptosis were seen in vein vascular smooth muscle cells (VSMC) grafted to arteries when compared to veins grafted to other veins, indicating that the increase in arterial pressure resulted in more apoptotic events. A potential molecular mechanism was later discovered that VSMCs subjected to this cyclic stretch activated the p53 tumor-suppression pathway.[89] The activation of p53 subsequently changed the ratios of pro- and anti-apoptotic markers (Bax; Bcl-2 and Bcl-xL), therefore stimulating the apoptosis process (Fig. 7). These marker levels changed in parallel with the observed apoptosis rates from the previous experiment. In contrast, it has been shown that cells naturally found in dynamically stretching environments can have increased rates of apoptosis with the cessation of mechanical stretch. Yoganathan *et al*. excised porcine aortic valves and found no differences in leaflet cell (endothelial cells, smooth muscle cells, and fibroblasts) death between fresh controls and dynamically cultured valves. However, when valves were inserted into static culture, apoptosis increased suggesting that reduction of cyclic stretch can induce apoptosis.[71]

In *in vitro* studies, the magnitude, rate, and type of stretch have been shown to have varying effects on apoptosis in isolated cells. High, pathological-level strains of 20–25% in cyclically stretched VSMCs and endothelial cells have been shown to increase apoptotic rates.[82,90,125] Increasing stretch amplitude (5–25%) has also been shown to increase apoptosis.[82,143] On the other hand, when apoptosis was induced in endothelial cells via TNFα, stretching cells at physiological levels of 6–10% had positive effects on cell survival.[82] VSMCs exhibit higher rates of apoptosis over longer time durations (6 h) at high strain (15%).[90] Additionally, the stretch waveform has been shown to affect apoptosis. Human carcinoma tongue cells, Tca8113, have increases in apoptotic occurrences when the waveform of stretch changes from a sine wave to a triangular wave to a square wave.[143]

Cell adhesion proteins and their respective signaling pathways play a large role in transducing mechanical signals inside a cell. Wernig *et al*. found that murine VSMCs cultured on collagen undergo higher rates of apoptosis when cyclically stretched compared to VSMCs cultured on elastin, laminin, or Pronectin.[149] This result suggests that β1-integrin signaling pathways are associated with apoptosis through mechanical mechanisms.

Fluid Shear Stress

Hemodynamic shear stress is an important mechanical stimulus that helps regulate function, structure, and gene expression in endothelial cells in blood vessels. Both increasing and decreasing shear stress *in vitro* via fluid flow has been shown to alter the prevalence of apoptosis in endothelial cells. Directional shear flow parallel to the long axis of human umbilical vein endothelial cell (HUVEC) was found to enhance stress fiber polymerization and reduce instances of apoptosis, while perpendicular flow did not counteract apoptotic events.[151] In an environment lacking mechanical stimuli, the absence of shear flow triggered apoptosis in vascular endothelial cells.[63] Reduced levels of shear stress appears to induce apoptosis of endothelial cells in human samples, which may lead to the onset of cardiovascular diseases such as atherosclerosis.[5,87]

Microgravity

Decreased gravitational forces, as experienced in a microgravity environment simulated with the use of a random positioning machine (RPM) on Earth or in space flight, can inhibit survival signaling pathways and have been shown in initiate apoptosis. Microgravity conditions cause mechanical unloading on cells and are known to cause cellular changes in shape, size, and migration related to modifications to the cytoskeleton.[54,57] In an RPM, ONCO-DG 1 cells (papillary thyroid cancer cells) underwent significant cytoskeletal depolymerization followed by apoptosis when under hypogravitational conditions.[57] Additionally, in porcine aortic vascular endothelial cells and BL6-10 cells, cells downregulate anti-apoptotic genes, such as Bcl-2 and Bnip3, express higher levels of pro-apoptotic genes, such as p53, Bax, FasL, Bok, caspases-3, 7, and 8, and various death-domain genes, and

TABLE 1. Mechanical stimuli cause cellular apoptosis or survival.

Stimulus	Parameter	Cell Line	Source	Result	References
Substrate stiffness	Low modulus (<1 kPa) vs high modulus (~ 5 kPa) (moduli estimated from acrylamide/bis-acrylamide concentrations)	Stiffness-dependent NIH 3T3 fibroblasts Stiffness-independent H-*ras*-transformed NIH 3T3 cells	murine (mouse)	↓ Proliferation (BrdU) ↑ Apoptosis (TUNEL) - Proliferation - Apoptosis	Wang[144]
	Low modulus (1 kPa) vs intermediate modulus (32 kPa) vs high modulus (63 kPa)	Annulus fibrosus cells	Murine (rat)	↓ Proliferation (hemocytometer) ↑ Apoptosis (Annexin V, caspase-3)	Zhang[156]
	Low modulus (0.4 kPa) vs intermediate modulus (5 kPa) vs high modulus (60 kPa)	Normal murine mammary gland epithelial cells (NMuMG)	Murine (mouse)	↑ Apoptosis (caspase-3)	Leight[77]
		Madin–Darby canine kidney epithelial cells (MDCK)	Canine	↑ Apoptosis (caspase-3)	
	Low modulus (0.15 kPa) vs high modulus (4.8 kPa)	Stiffness-dependent A549 (lung) MDA-MB-231 (breast) Stiffness-independent PC-3 (prostate) mPanc96 (pancreas)	Human	↓ Proliferation (CyQuant kit) ↑ Apoptosis (TUNEL) - Proliferation - Apoptosis	Tilghman[135]
	No stiffness—suspended in solution (anoikis)	Capillary endothelial cells	Human, bovine	↓ Proliferation (BrdU) ↑ Apoptosis (TUNEL)	Chen[15]
	No stiffness—suspended in solution (anoikis)	Capillary endothelial cells	Bovine	↑ Apoptosis (TUNEL, caspase-3)	Flusberg[34]
	No stiffness—suspended in solution (anoikis)	FSK-7 mammary epithelial cells	Murine (mouse)	↑ Apoptosis (caspase-3, cytochrome c release, Hoechst 33258)	Wang[146]
Substrate strain	7, 25% strain	Vascular smooth muscle cells (VSMC)	Porcine	↑ Apoptosis (LM-PCR) in 25% strain	Sotoudeh[125]
	5, 10, 15% strain Sine, triangle, square wave	Tca8113 - human tongue squamous carcinoma cells	Human	↑ Apoptosis (Annexin V) with increasing strain ↑ Apoptosis (Annexin V) in square > triangle > sine wave	Wang[143]
	5, 15, 25%	Vascular smooth muscle cells	Murine (rat, mice), human	↑ Apoptosis (annexin v, tunel) with higher strains in all cell lines Human > rat > mouse	Mayr[90]
	15% cyclic strain	Vascular smooth muscle cells	Murine (rat)	↑ Apoptosis (Annexin V, TUNEL) only on collagen I	Wernig[149]
	100 mmHg in organ culture system	Aortic heart valve	Porcine	↓ Proliferation (BrdU) in static condition ↑ Apoptosis (caspase-3) in static condition	Konduri[71]
Fluid flow shear stress	Fluid flow present 12 ± 4 dyn/cm^2	HUVEC	Human	↓ Apoptosis (Annexin V) Flowed parallel to long axis of patterned cells – Apoptosis (Annexin V) Flowed perp. to long axis of patterned cells	Wu[151]
	Fluid flow present 0.05–0.1 dyn/cm^2	HUVEC	Human	↓ Apoptosis (DAPI)	Kaiser[63]

TABLE 1. continued

Stimulus	Parameter	Cell Line	Source	Result	References
Surface patterning	Small cell area (78 μm^2 vs ~ 314 μm^2 vs unrestricted)	Capillary endothelial cells	Human, bovine	↓ Proliferation (BrdU) ↑ Apoptosis (TUNEL)	Chen[15]
	Small cell area (78 μm^2 vs unrestricted)	Capillary endothelial cells	Bovine	↑ Apoptosis (TUNEL, caspase-3)	Flusberg[34]
	Small cell area (314 μm^2, 615 μm^2, 1256 μm^2) Increased circularity (0.1, 0.2, 0.4, 1)	MC3T3-E1	Murine (mouse)	↑ Apoptosis (TUNEL) ↑ Apoptosis (TUNEL)	Fu[36]
	Small cell area (300 μm^2, 1024 μm^2, 2025 μm^2, 10,000 μm^2)	Human mesenchymal stem cells (MSC) Human lung microvascular endothelial cells (HMVEC)	Human human	↓ Proliferation (BrdU) ↑ Apoptosis (TUNEL)	Dupont[28]
	Multicellular aggregate (heterogeneous stress)	Valvular interstitial cells (VIC)	Porcine	↑ Apoptosis in central region (caspase-3)	Cirka[18]
	Multicellular aggregate (heterogeneous stress)	Valvular interstitial cells (VIC)	Porcine	↑ Apoptosis in central low stress region (caspase-3)	Goldblatt[39]
Surface composition	Fibronectin (FN) Matrigel Collagen I	Normal murine mammary gland epithelial cells (NmuMG)	Murine (mouse)	↑ Apoptosis (caspase-3) collagen I higher than Matrigel and FN	Leight[77]
	Collagen I Elastin Laminin Pronectin	Vascular smooth muscle cells	Murine (rat)	↑ Apoptosis (Annexin V, TUNEL) only on collagen I with stretch	Wernig[149]

"↑" indicates increase, "↓" indicates decrease, and "–" indicates no change. All cell lines listed under *substrate stiffness* are stiffness-dependent unless otherwise indicated.

undergo higher rates of apoptosis than those in earth-level gravity conditions.[94,157] Further, Vidyasekar *et al.* found microgravity causes increases in apoptotic rates in DLD-1 cells (colorectal cancer cells) and MOLT-4 cells (lymphoblast leukemic cells).[139] Apoptosis was coupled with inhibition of anti-apoptotic protein Bcl-2 and had up regulation of pro-apoptotic proteins, such as PARP, p-53, and BAX.

Regulation of Cell Spread Area

In high density monolayers, there are higher instances of apoptosis compared to sparse cultures.[115] High density monolayers naturally cause size restrictions of cell spread area. Geometric constraints also have significant repercussions on other cell behavior such as changes in cytoskeleton dynamics,[86] cell migration,[14] proliferation,[128] and differentiation.[67] Micro-contact printing of small protein "island" enables the creation of patterned substrates with varying size and shapes. Cells are non-adhesive to the areas outside of the protein geometries, allowing high control of both individual cell shape and multicellular assembly shape. In these single-cell cultures, restricting cell spread area in human capillary endothelial cells and MC3T3-E1 osteoblast-like cells induces apoptosis in a dose-dependent manner with rates of apoptosis increasing from 30 to 50% as the protein island size is decreased from as high as 1256 μm^2 (40 μm diameter circle) to as low as 78 μm^2 (10 μm diameter circle).[15,36] Chen *et al.* further demonstrated that the amount of integrin binding was not responsible for the altered rates of apoptosis. By constricting cells to small sizes, they are unable to generate high traction forces on their ECM. It is possible that it is the decrease in cell-generated stress, rather than the restriction in cell area, that initiates apoptosis,[18,28] as decreased cell forces and increased apoptosis are also observed on low modulus substrates compared to stiff substrates as described below.[67]

Control of Substrate Modulus

Similar to cell spread area, substrate modulus effects many cellular functions including cell migration, proliferation, and differentiation.[13,70,135] Cells need to generate mechanical tension through cell-ECM adhesions to maintain homeostatic stress conditions.[30,55] When cultured on low modulus substrates (often referred to as "soft" substrates), cells are unable to generate substantial traction forces and often appear rounded, unlike the spread morphology of cells cultured on high modulus ("stiff") substrates;[10,122] this has been correlated to higher incidences of apoptotic

events.[77,135,144,156] Wang *et al.* demonstrated that primary fibroblasts generate lower traction forces and have smaller spread area on soft substrates compared to stiff substrates; in contrast, transformed cells have no observable differences in generated traction forces or spread area between soft and stiff substrates.[144] Interestingly, when cells are confluent, mechanical interactions between multiple cells abrogate the effects of substrate modulus.[154] These cell monolayers show indistinguishable morphologies between soft and stiff substrates. Complete lack of cell-ECM ("zero modulus") attachment results in a specific type of apoptosis called anoikis. The detachment of cells or inhibition of focal adhesions signals anoikis and leads to subsequent programmed cell death.[104] It is unclear if it is the lack of physical feedback that cells receive from their environment, the lack of integrin binding, or other related signals that activate the apoptotic pathways in anoikis. There may be a common mechanism between cells in different low modulus conditions (e.g. cytoskeletal disassembly); however, studies that tease out the varying mechanical inputs need to be performed.

Area-Restricted Multicellular Systems

Mechanical stress fields arise from force transfer between cells and the ECM as well as cells with other cells. As previously stated in the *restricted cell spread area* section, apoptotic rates are higher for single cells cultured on small protein islands compared to large islands. In multicellular systems, constricting monolayers is a powerful means of creating heterogeneous monolayers with reproducible regions of differing cell stress, density, and spread areas which emerge from collective cell interactions. In multicellular aggregates, high cell densities and low spread areas localize in the central region contrary to the peripheral region, which has lower cell densities and high spread areas. We have previously shown that enhanced rates of apoptosis occur in these central regions with restricted valvular interstitial cell spreading and low traction forces (Figs. 8a, 8b).[18,39] Additionally, other studies have shown increased proliferation in the peripheral regions of aggregates (aggregates consisting of NIH 3T3 fibroblasts or bovine pulmonary artery endothelial cells) where cells are elongated and have high spread area.[80,97] We are currently examining possible mechanisms for apoptotic signaling that correlate with cell-stresses. Previous models that predict and calculate stresses in the cell aggregates assume homogeneous cell properties which produces high stresses in aggregate centers (opposite of where proliferation, apoptosis, and traction forces localize). Using more realistic heterogeneous parameters, our models predict low cell-layer stress in central regions, which correlates well with apoptosis. Additionally, we observe higher G/F-actin ratios in central regions which indicates cytoskeletal remodeling may play a role (Fig. 8c). Accurate estimation of cell-stresses in these aggregates will lead to a better understanding how specific mechanical factors drive cell fate (Fig. 8d). Further, being able to accurately determine where stresses are localized within cell monolayers will allow for more precise design of methods for mechanical control of cell fate.

Separating Correlated Mechanical Stimuli

The mechanical parameters initiating apoptosis remain convoluted in that multiple mechanical stimuli occur simultaneously. For instance, both culturing cells on soft substrates and restricting cell spread area by micro-contact printing inhibit the ability of cells to

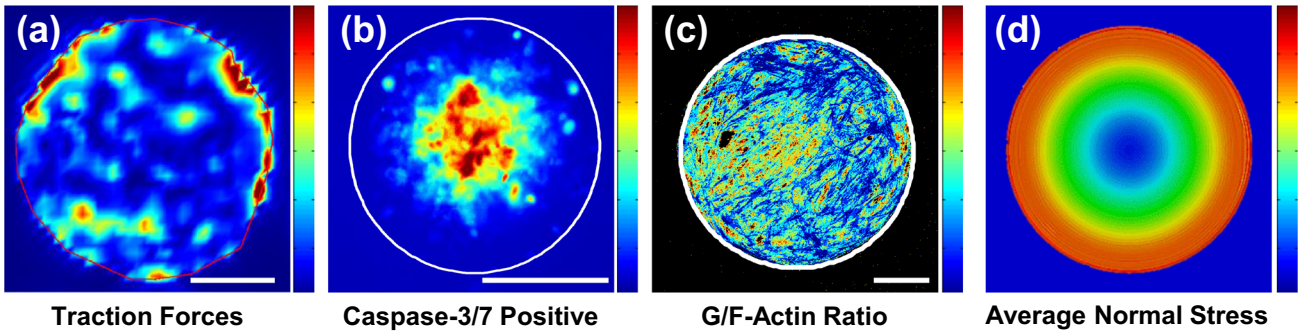

FIGURE 8. High cell-stress markers localize to aggregate periphery while low cell-stress markers localize to aggregate center. Heat map of traction forces (a) shows localization at aggregate periphery, while heat maps of active caspase-3/7 (b) and G/F-actin ratio (c) indicate low-stress localization in central region. (d) Heat map of average normal stress from thermal contraction model using heterogeneous mechanical properties predicts low cell-layer stress in aggregate center colocalizing with the apoptotic cells. Scale bars = 100 μm. Images are adapted from Cirka *et al.*[18], and Goldblatt *et al.*[39].

spread and generate traction forces and internal cell stress. Do low spread area, low traction forces, and/or low cell stress initiate apoptosis? Isolation experiments are needed to determine which mechanical signals and subsequent mechanotransduction pathways most directly regulate apoptosis. The pathways that tie these different mechanical signals together and induce similar cellular responses also need to be elucidated. It is likely that similar mechanisms connect all of these mechanical stimuli together in order to regulate apoptosis, such as their combinatorial roles on actin dynamics. For instance, if apoptosis is strongly regulated by actin dynamics, then inhibition of spread area and low traction force generation could induce apoptosis, as both independently inhibit actin dynamics. Further, a decrease in the ability of the actin cytoskeleton to remodel would have reciprocal effects on spread area and traction forces contributing to a positive feedback loop.

POTENTIAL MECHANICAL MECHANISMS REGULATING APOPTOSIS

Canonical Mechanotransduction Pathways

Very little is known regarding the mechanisms by which mechanical stimuli regulate apoptosis. Many of the signaling pathways already known to be critical for mechanotransduction interact with and modulate the canonical intrinsic and/or extrinsic apoptotic pathways. There is evidence that signaling molecules associated with mitogen-activated protein kinases (MAPK), protein kinase C (PKC), and phosphatidylinositol 3-kinase (PI3K) pathways play a role in cell survival (Fig. 9).

There are three main MAPK cascades that modulate cell fate: extracellular signal-related kinases (ERK), c-Jun N-terminal kinases (JNK), and p38-MAPKs.[141,150] The ERK pathway tends to activate survival signals, while JNK and p38-MAPK pathways have been shown to contribute to both cell survival and apoptotic signaling.[90,141]

PKC and PI3K pathways are also signaling cascades that play a role in cell survival. PKCs can activate survival proteins such as NF-κB and inhibit apoptotic proteins such as BAD. PI3K activates Akt1, which activates survival proteins like Bcl-xL, while inactivating pro-apoptotic proteins like BAD (Fig. 9). Mechanical stretch can also induce ion transport within transmembrane ion channels in cardiomyocytes.[110,153] Calcium as well as chloride ion influxes have been shown to activate caspases and subsequent apoptosis.[9]

Tension in the Cytoskeleton

Cells require an intracellular tension homeostasis for continued proper cell health.[11] The actomyosin cytoskeleton directly controls the tension state of cells, which plays a critical role in apoptotic pathways. Cytoskeletal reorganization can initiate apoptosis and also plays a role throughout the entire process.[42,43,76] Healthy cells under homeostatic conditions have low instances of apoptosis and high cytoskeletal stability (Fig. 10, region *B*). In supraphysiological conditions, such as when cells are excessively stretched *in vitro* or in fibrosis *in vivo*, cells experience atypically high inputs of external stresses which increases their intracellular stress levels (Fig. 10, region *C*). Here, cytoskeletal stability decreases and occurrences of apoptosis increase. Cells will attempt to revert to homeostatic levels (region *B*) through reorganization of the cytoskeleton (e.g., strain avoidance in cyclically stretched cells); however, they will apoptose if they cannot reduce the stress on them. In subphysiological conditions, such as when cells are seeded on soft substrates or restricted to small areas through microcontact printing, cells experience unusually low inputs of external stress which decreases their intracellular stress levels (Fig. 10, region *A*). Low internal cell tension will cause cytoskeletal stability to decrease and apoptosis to increase. In the central region of multicellular aggregates, we have found that low cell-stresses co-localize with high levels of G/F-actin ratio (low cytoskeletal stability) and high instances of apoptosis (Fig. 8c).[39] By introducing external stresses (such as mechanical stretch), cells can increase their intracellular tension to reach homeostatic levels (region *B*). A mechanomedicine could help cells in low stress (region *A*) or high stress (region *C*) re-achieve homeostasis.

Actin stress fibers can directly regulate apoptosis by pulling on organelles, such as the nucleus or mitochondria, or indirectly via actin-associated molecules or activation of mechanosensitive pathways regulated by cytoskeletal dynamics.[1,50,88] Studies have shown that stabilizing cytoskeletal dynamics can allow cells to overcome apoptosis (observed in Jurkat T, HeLa, and NIH3T3 cells).[41,74,101] Overexpression of gelsolin helps promote F-actin turnover and has been shown to reduce apoptotic rates. By depolymerizing F-actin, gelsolin stimulates VDAC closure in mitochondria thereby preventing cytochrome c release. Additionally, gelsolin causes the accumulation of short F-actin segments; once it dissociates from the F-actin caps, these segments are readily available for repolymerization into longer F-actin stress fibers again.

On the other hand, destabilizing the cytoskeleton through excessive polymerization or depolymerization has been shown to initiate the apoptotic pro-

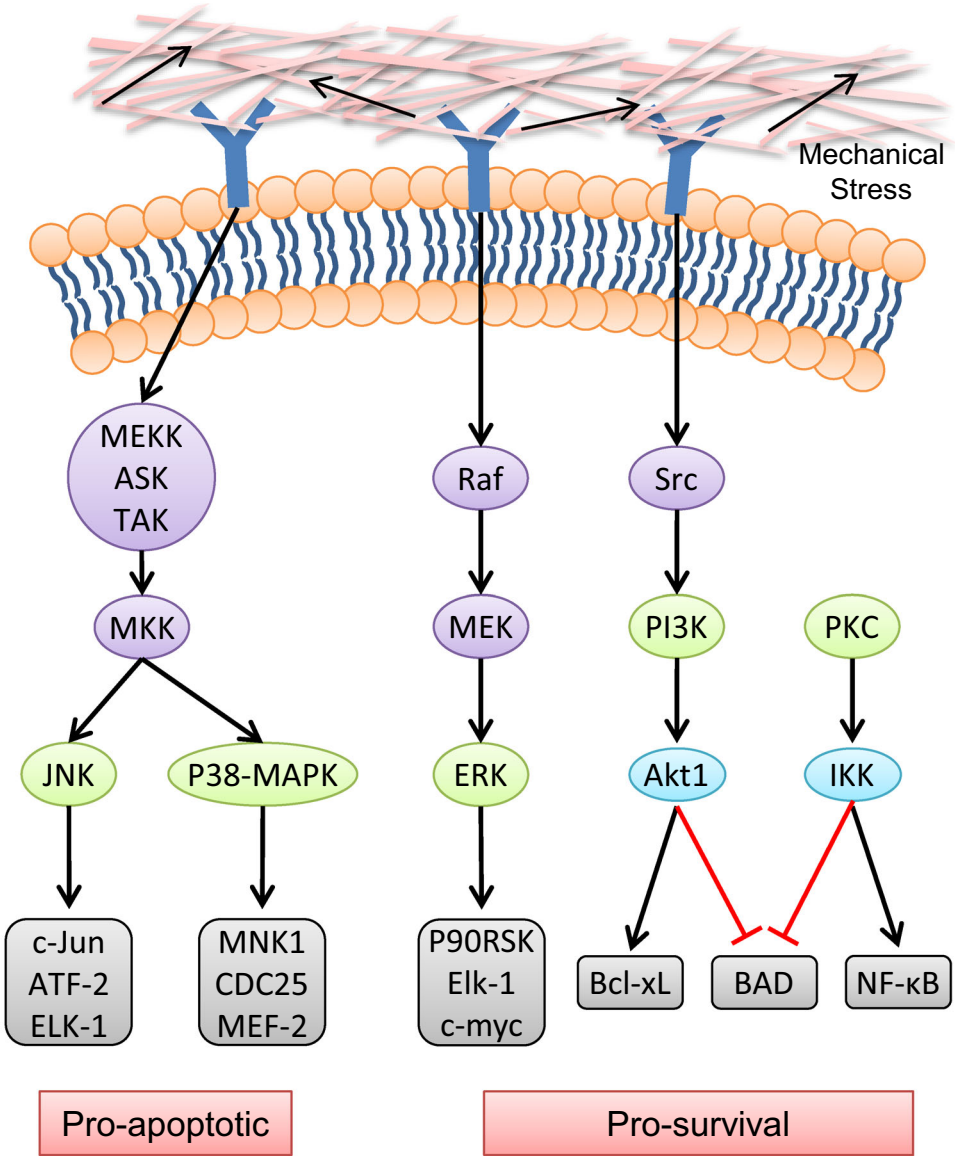

FIGURE 9. Both excessive and insufficient mechanical stress can activate apoptotic signaling pathways leading to cell death since both pro-apoptotic and pro-survival pathways are mechanically regulated. Mechanosensitive signaling pathways of apoptosis include the MAPK (JNK, p38-MAPK, ERK), PI3K, and PKC pathways. Certain branches of the JNK and p38-MAPK pathways can also promote cell survival (not shown).

cess.[27,43,76,99] Jasplakinolide causes robust actin stabilization and promotes further polymerization of F-actin. This polymerization can cause F-actin fibers to clump together into aggregates as well as distinct changes in cellular activity indicative of apoptosis. An increase in caspase-3 activation has been shown in jasplakinolide treated yeast and Jurkat T cells, in addition to DNA fragmentation in rat thymocytes, lymph node cells, and COS cells, potentially from DNAse-1 that is dissociated from G-actin monomers that are newly polymerized into F-actin.[43,99,108] Conversely, depolymerization of the cytoskeletal network via cytochalasin D also initiates apoptosis. It has been shown to induce caspase activation as well as cytochrome c release in fibrosarcoma L929 and 3C6 T cells.[106,130] Furthermore, organelles require mechanical feedback for proper function, and a decrease in cytoskeletal integrity may activate apoptotic pathways. The cytoskeleton directly connects to nuclear actin via linker proteins, such as nesprins and lamins. Both of these proteins are critical for nuclear mechanosensing and can cause alterations in gene expression due to transduced forces into the nucleus. When nesprins or lamins are inhibited, the nucleus cannot respond to mechanical signals, and as seen in neonatal mouse

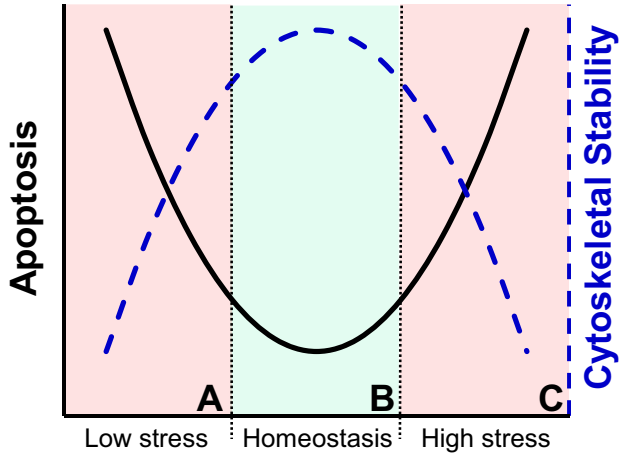

FIGURE 10. Schematic of the relationship between intracellular stress levels, apoptosis (black solid line), and cytoskeletal stability (blue dotted line). Healthy cells in physiological conditions are under standard stress levels and exist in region *B*. Cells experiencing atypically low (region *A*) or high (region *C*) inputs of external stress undergo higher instances of apoptosis with lower cytoskeletal stability. Figure is adapted from Chan *et al.*[11]

cardiomyocytes and HeLa cells, cells have been shown to undergo apoptosis.[7,126]

Cytoskeletal stability indirectly regulates various intracellular proteins such as transcriptional factors like yes-associated protein (YAP) and myocardin-related transcriptional factor-A (MRTF-A). YAP is primarily studied regarding its role in cell proliferation; however, it has also been shown to play a role in apoptosis. YAP can stimulate expression of prominent apoptosis inhibitors, such as BIRC3, BIRC5, and CYR61.[22] Further, YAP inhibits pro-apoptotic genes such as dendrin and Bcl-2 family proteins.[124] The mechanical environment has been shown to directly regulate YAP via the tensional state of the cytoskeleton.[2] Angiomotins bind to F-actin and are released upon F-actin depolymerization.[88] Free angiomotins can bind and sequester cytoplasmic YAP thus keeping them inactive. YAP activation may also occur independent of the cytoskeleton; a recent study showed that direct application of force onto the nucleus of embryonic fibroblasts (Talin 1 knockout mice) and MCF10As caused YAP activation and translocation into the nucleus.[31]

MRTF-A, a potent coactivator of serum response factor (SRF) which induces transcription of many proliferative and cytoskeletal genes, is another mechanosensitive protein regulated by the cytoskeleton that may play a role in apoptosis. Due to F-actin polymerization, G-actin concentrations decrease, allowing activation of MRTF-A.[103] Upstream from both YAP and MRTF-A are Rho and ROCK signaling. It was previously found that inhibition of ROCK can reduce the response to pressure induced cardiomyocyte apoptosis,[111] while constitutively active ROCK can enhance cardiomyocyte apoptosis.[12]

MECHANOMEDICINES AS A POTENTIAL THERAPEUTIC SOLUTION

Understanding the mechanical mechanisms that regulate cell health in response to mechanical stress may inspire new therapeutic strategies which target these mechanically sensitive pathways. Chan *et al.* first introduced the concept of mechanomedicines as a class of therapies which exploit mechanotransduction pathways and restore cells to their homeostatic stress state.[11] As both excessive apoptosis (e.g., atrophy and CAVD) and the absence of apoptosis (e.g., fibrosis and cancer) contribute to pathologies, mechanomedicines could treat disease by modulating rates of apoptosis as appropriate (Fig. 11). Not only do mechanomedicines include pharmacological agents and inhibitory antibodies which may trigger or prevent apoptosis by interacting with the intrinsic/extrinsic apoptotic signaling cascade, but also potential biomimetic injectables that can modify the extracellular matrix environment.[58] The mechanical environment created by the native ECM has been shown to regulate growth,[112] differentiation,[45] proliferation,[138] and sensitivity to cytokine-induced apoptosis.[33] Pathologies are often accompanied by noticeable changes in tissue properties (e.g., by physical palpitation in tumor discovery),[52] and it has been proposed that many diseases arise due to degrading tissue architecture. Restoring the correct mechanical properties through ECM modification could help restore homeostasis by either preventing apoptosis or sensitizing target cells to apoptotic mechanical cues.

To date, no pharmacologic treatment has been designed with the sole purpose of altering the mechanosensitive signaling pathways of cells, although there are medications that cause altered cytoskeletal tension as a side-effect. One such example is statins which are prescribed for treatment of high cholesterol or blood pressure. Statins inhibit HMG-CoA reductase which ultimately leads to the inhibition of isoprenylation of small GTPases such as Ras, Rho, Rab, and Rap.[116] As Rho signaling is involved in cytoskeletal dynamics, decreased Rho activity from statin use results in reduced cell contractility, as shown *in vitro* with decreased contractile protein expression in valvular interstitial cells.[44]

Pharmacological agents that affect the cytoskeleton are of interest for cancer therapies to promote apoptosis in diseased cells, as well as impede cell metastasis. A number of groups have reported altered cytoskeletal

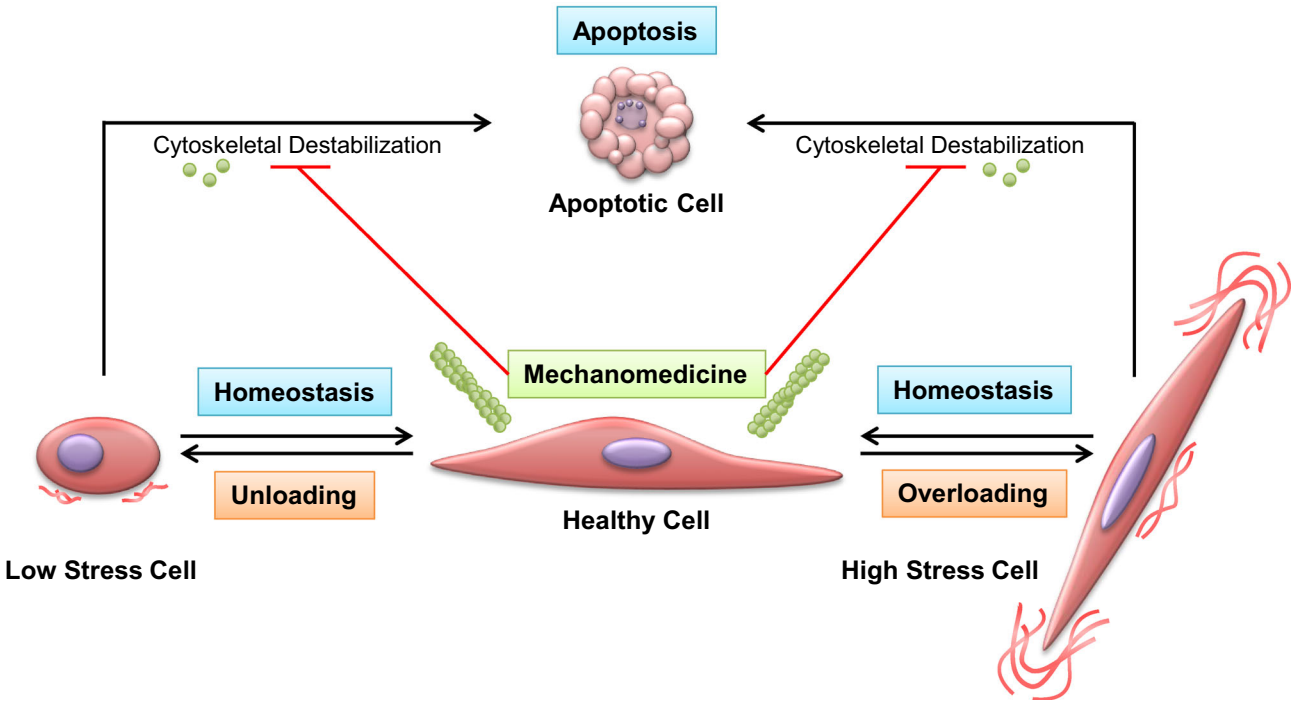

FIGURE 11. Schematic of the promise of mechanomedicines to bring the cell-stress state back to homeostatic levels. Overloading and underloading of cells can cause cytoskeletal destabilization which mechanomedicines can potentially target, allowing cells to reduce excessive apoptosis in degenerative diseases or uncontrolled growth (deficient apoptosis) in cancers.

dynamics within transformed cells, including altered G- to F-actin ratios and an inability to sense substrate stiffness in normal and malignant human lymphocytes and endometrial cells.[127] Additionally, a number of drugs which interact with microtubules or tubulin in order to prevent mitosis/proliferation, such as taxol and taxol-like compounds, molide and epothilone, alter microtubule dynamics and have been shown to promote apoptosis in transformed cells.[61,65] As the cytoskeleton is critical to numerous cell processes such as migration and proliferation, which can be exploited in cancer during metastasis and tumorigenesis, the cytoskeleton has become a promising target for pharmacological intervention. A number of compounds have been identified which interfere with actin polymerization and dynamics through a variety of mechanisms. These compounds include cytochalasins, jasplakinolide, misakinolide and latrunculins.[103] Although these compounds are heavily used *in vitro* to gain an understanding of the relationship between actin dynamics and apoptosis, similar acting pharmaceuticals have yet to be approved for clinical use. There is promise in cytoskeletal tension modulation as a therapy for cancer; however, this approach has yet to be taken for development of mechanomedicines for cardiovascular diseases.

CONCLUSIONS

Apoptosis is a highly regulated cellular event which plays a critical role in organism development and tissue maintenance. Dysregulation of apoptosis is implicated in a number of disease processes. Despite numerous correlations between physical stimuli and apoptosis, there remains a significant gap in our understanding in how particular mechanical inputs trigger apoptosis through mechanotransduction signaling pathways. Continued work is needed in identifying the mechanotransductive events which result in apoptosis via both extracellular and intracellular cues. Uncovering these mechanical mechanisms that regulate cell health is the first step in creating ways to manipulate mechanically sensitive pathways. We believe one such mechanism is the regulation of cytoskeletal stability and how it directly modulates cell health in response to mechanical stimuli. The reversibility of apoptosis highlights the potential of developing promising mechanomedicines that alter cellular mechanics to reverse disease progression. By controlling rates of apoptosis, mechanomedicines could treat diseases ranging from cancer (increasing apoptosis) to degenerative diseases (decreasing apoptosis). Discovering how specific mechanotransduction pathways regulate apoptosis is critical for identifying therapeutic targets that may be

treated with mechanomedicines and mechanotherapies.

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REFERENCES

[1]Anesti, V., and L. Scorrano. The relationship between mitochondrial shape and function and the cytoskeleton. *Biochim. Biophys. Acta Bioenerg.* 1757:692–699, 2006.

[2]Aragona, M., T. Panciera, A. Manfrin, S. Giulitti, F. Michielin, N. Elvassore, S. Dupont, and S. Piccolo. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* 154:1047–1059, 2013.

[3]Araki, S., Y. Shimada, K. Kaji, and H. Hayashi. Apoptosis of vascular endothelial cells by fibroblast growth factor deprivation. *Biochem. Biophys. Res. Commun.* 168:1194–1200, 1990.

[4]Arstall, M. A., D. B. Sawyer, R. Fukazawa, and R. A. Kelly. Cytokine-mediated apoptosis in cardiac myocytes. *Circ. Res.* 85:829–840, 1999.

[5]Asakura, T., and T. Karino. Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *Circ. Res.* 66:1045–1066, 1990.

[6]Bailey, R. W., T. Nguyen, L. Robertson, E. Gibbons, J. Nelson, R. E. Christensen, J. P. Bell, A. M. Judd, and J. D. Bell. Sequence of physical changes to the cell membrane during glucocorticoid-induced apoptosis in S49 lymphoma cells. *Biophys. J.* 96:2709–2718, 2009.

[7]Banerjee, I., J. Zhang, T. Moore-Morris, E. Pfeiffer, K. S. Buchholz, A. Liu, K. Ouyang, M. J. Stroud, L. Gerace, S. M. Evans, A. McCulloch, and J. Chen. Targeted ablation of nesprin 1 and nesprin 2 from murine myocardium results in cardiomyopathy, altered nuclear morphology and inhibition of the biomechanical gene response. *PLoS Genet.* 10:e1004114, 2014.

[8]Bing, O. H. L. Hypothesis: apoptosis may be a mechanism for the transition to heart failure with chronic Pressure overload. *J. Mol. Cell. Cardiol.* 26:943–948, 1994.

[9]Bortner, C. D., and J. A. Cidlowski. Ion channels and apoptosis in cancer. *Philos. Trans. R. Soc. B* 369:1–9, 2014.

[10]Califano, J. P., and C. A. Reinhart-King. Substrate stiffness and cell area predict cellular traction stresses in single cells and cells in contact. *Cell. Mol. Bioeng.* 3:68–75, 2010.

[11]Chan, D. D., W. S. Van Dyke, M. Bahls, S. D. Connell, P. Critser, J. E. Kelleher, M. A. Kramer, S. M. Pearce, S. Sharma, and C. P. Neu. Mechanostasis in apoptosis and medicine. *Prog. Biophys. Mol. Biol.* 106:517–524, 2011.

[12]Chang, J., M. Xie, V. R. Shah, M. D. Schneider, M. L. Entman, L. Wei, and R. J. Schwartz. Activation of Rho-associated coiled-coil protein kinase 1 (ROCK-1) by caspase-3 cleavage plays an essential role in cardiac myocyte apoptosis. *Proc. Natl. Acad. Sci. USA* 103:14495–14500, 2006.

[13]Chaudhuri, O., L. Gu, D. Klumpers, M. Darnell, S. A. Bencherif, J. C. Weaver, N. Huebsch, H.-P. Lee, E. Lippens, G. N. Duda, and D. J. Mooney. Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat. Mater.* 15:326–334, 2015.

[14]Chen, B., G. Kumar, C. C. Co, and C.-C. Ho. Geometric control of cell migration. *Sci. Rep.* 3:2827, 2013.

[15]Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber. Geometric control of cell life and death. *Science* 276:1425–1428, 1997.

[16]Chiong, M., Z. V. Wang, Z. Pedrozo, D. J. Cao, R. Troncoso, M. Ibacache, A. Criollo, A. Nemchenko, J. Hill, and S. Lavandero. Cardiomyocyte death: mechanisms and translational implications. *Cell Death Dis.* 2:e244, 2011.

[17]Cirka, H., M. Monterosso, N. Diamantides, J. Favreau, Q. Wen, and K. Billiar. Active traction force response to long-term cyclic stretch is dependent on cell pre-stress. *Biophys. J.* 110:1845–1857, 2016.

[18]Cirka, H. A., J. Uribe, V. Liang, F. J. Schoen, and K. L. Billiar. Reproducible *in vitro* model for dystrophic calcification of cardiac valvular interstitial cells: insights into the mechanisms of calcific aortic valvular disease. *Lab Chip* 17:814–829, 2017.

[19]Clark, R. S. B., P. M. Kochanek, M. Chen, S. C. Watkins, D. W. Marion, J. Chen, R. L. Hamilton, J. E. Loeffert, and S. H. Graham. Increases in Bcl-2 and cleavage of caspase-1 and caspase-3 in human brain after head injury. *FASEB J.* 13:813–821, 1999.

[20]Clarke, M. C. H., N. Figg, J. J. Maguire, A. P. Davenport, M. Goddard, T. D. Littlewood, and M. R. Bennett. Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. *Nat. Med.* 12:1075–1080, 2006.

[21]Clarke, M. C. H., T. D. Littlewood, N. Figg, J. J. Maguire, A. P. Davenport, M. Goddard, and M. R. Bennett. Chronic apoptosis of vascular smooth muscle cells accelerates atherosclerosis and promotes calcification and medial degeneration. *Circ. Res.* 102:1529–1538, 2008.

[22]Codelia, V. A., G. Sun, and K. D. Irvine. Regulation of YAP by mechanical strain through Jnk and Hippo signaling. *Curr. Biol.* 24:2012–2017, 2017.

[23]Cui, Y., F. M. Hameed, B. Yang, K. Lee, C. Q. Pan, S. Park, and M. Sheetz. Cyclic stretching of soft substrates induces spreading and growth. *Nat. Commun.* 6:6333–6340, 2015.

[24]Czabotar, P. E., G. Lessene, A. Strasser, and J. M. Adams. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat. Rev. Mol. Cell Biol.* 15:49–63, 2014.

[25]Darzynkiewicz, Z., D. Galkowski, and H. Zhao. Analysis of apoptosis by cytometry using TUNEL assay. *Methods* 44:250–254, 2008.

[26]Darzynkiewicz, Z., and H. Zhao. Detection of DNA strand breaks in apoptotic cells by flow- and image-cytometry. *Methods Mol. Biol.* 682:91–101, 2011.

[27]Desouza, M., P. W. Gunning, and J. R. Stehn. The actin cytoskeleton as a sensor and mediator of apoptosis. *Bioarchitecture* 2:75–87, 2012.

[28]Dupont, S., L. Morsut, M. Aragona, E. Enzo, S. Giulitti, M. Cordenonsi, F. Zanconato, J. Le Digabel, M. Forcato, S. Bicciato, N. Elvassore, and S. Piccolo. Role of YAP/TAZ in mechanotransduction. *Nature* 474:179–183, 2011.

[29]Dweck, M. R., N. A. Boon, and D. E. Newby. Calcific aortic stenosis: a disease of the valve and the myocardium. *J. Am. Coll. Cardiol.* 60:1854–1863, 2012.

[30]Egerbacher, M., S. P. Arnoczky, O. Caballero, M. Lavagnino, and K. L. Gardner. Loss of homeostatic tension induces apoptosis in tendon cells: an *in vitro* study. *Clin. Orthop. Relat. Res.* 466:1562–1568, 2008.

[31]Elosegui-Artola, A., I. Andreu, A. E. M. Beedle, A. Lezamiz, M. Uroz, A. J. Kosmalska, R. Oria, J. Z. Kechagia, P. Rico-Lastres, A.-L. L. Le Roux, C. M. Shanahan, X. Trepat, D. Navajas, S. Garcia-Manyes, and P. Roca-Cusachs. Force triggers YAP nuclear entry by regulating transport across nuclear pores. *Cell* 171:1397–1410.e14, 2017.

[32]Fadeel, B. Plasma membrane alterations during apoptosis: role in corpse clearance. *Antioxid. Redox Signal.* 6:269–275, 2004.

[33]Farrelly, N., Y.-J. Lee, J. Oliver, C. Dive, and C. H. Streuli. Extracellular matrix regulates apoptosis in mammary epithelium through a control on insulin signaling. *Cell* 144:1337–1347, 1999.

[34]Flusberg, D. A., Y. Numaguchi, and D. E. Ingber. Cooperative control of Akt phosphorylation, bcl-2 expression, and apoptosis by cytoskeletal microfilaments and microtubules in capillary endothelial cells. *Mol. Biol. Cell* 12:3087–3094, 2001.

[35]Freeman, R. V., and C. M. Otto. Spectrum of calcific aortic valve disease: pathogenesis, disease progression, and treatment strategies. *Circulation* 111:3316–3326, 2005.

[36]Fu, R., Q. Liu, G. Song, A. Baik, M. Hu, S. Sun, X. E. Guo, M. Long, and B. Huo. Spreading area and shape regulate apoptosis and differentiation of osteoblasts. *Biomed. Mater.* 8:055005, 2013.

[37]Galluzzi, L., M. C. Maiuri, I. Vitale, H. Zischka, M. Castedo, L. Zitvogel, and G. Kroemer. Cell death modalities: classification and pathophysiological implications. *Cell Death Differ.* 14:1237–1243, 2007.

[38]Geske, F. J., R. Lieberman, R. Strange, and L. E. Gerschenson. Early stages of p53-induced apoptosis are reversible. *Cell Death Differ.* 8:182–191, 2001.

[39]Goldblatt, Z. E., H. Ashouri Choshali, H. A. Cirka, V. Liang, Q. Wen, D. McCollum, N. Rahbar, and K. L. Billiar. Heterogeneity profoundly alters emergent stress fields in constrained multicellular systems. *Biophys. J.* 118:15–25, 2020.

[40]Gottlieb, E., S. Armour, M. Harris, and C. Thompson. Mitochondrial membrane potential regulates matrix configuration and cytochrome c release during apoptosis. *Cell Death Differ.* 10:709–717, 2003.

[41]Gourlay, C. W., and K. R. Ayscough. The actin cytoskeleton: a key regulator of apoptosis and ageing? *Nat. Rev. Mol. Cell Biol.* 6:583–589, 2005.

[42]Gourlay, C. W., and K. R. Ayscough. A role for actin in aging and apoptosis. *Biochem. Soc. Trans.* 33:1260–1264, 2005.

[43]Gourlay, C. W., L. N. Carpp, P. Timpson, S. J. Winder, and K. R. Ayscough. A role for the actin cytoskeleton in cell death and aging in yeast. *J. Cell Biol.* 164:803–809, 2004.

[44]Gu, X., and K. S. Masters. Role of the Rho pathway in regulating valvular interstitial cell phenotype and nodule formation. *Am. J. Physiol. Circ. Physiol.* 300:H448–H458, 2011.

[45]Guilak, F., D. M. Cohen, B. T. Estes, J. M. Gimble, W. Liedtke, and C. S. Chen. Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell* 5:17–26, 2009.

[46]Gurbanov, E., and X. Shiliang. The key role of apoptosis in the pathogenesis and treatment of pulmonary hypertension. *Eur. J. Cardio-thoracic Surg.* 30:499–507, 2006.

[47]Hanahan, D., and R. A. Weinberg. Hallmarks of cancer: the next generation. *Cell* 144:646–674, 2011.

[48]Hardwick, J. M. Apoptosis in viral pathogenesis. *Cell Death Differ.* 8:109–110, 2001.

[49]Hinz, B., and D. Lagares. Evasion of apoptosis by myofibroblasts: a hallmark of fibrotic diseases. *Nat. Rev. Rheumatol.* 16:11–31, 2020.

[50]Houben, F., F. C. S. Ramaekers, L. H. E. H. Snoeckx, and J. L. V. Broers. Role of nuclear lamina-cytoskeleton interactions in the maintenance of cellular strength. *Biochim. Biophys. Acta Mol. Cell Res.* 1773:675–686, 2007.

[51]Huang, X., H. D. Halicka, F. Traganos, T. Tanaka, A. Kurose, and Z. Darzynkiewicz. Cytometric assessment of DNA damage in relation to cell cycle phase and apoptosis. *Cell Prolif.* 38:223–243, 2005.

[52]Huang, S., and D. E. Ingber. Cell tension, matrix mechanics, and cancer development. *Cancer Cell* 8:175–176, 2005.

[53]Huang, J., Y. Liu, P. Sun, X. Lv, K. Bo, and X. Fan. Novel strategy for treatment of pulmonary arterial hypertension: enhancement of apoptosis. *Lung* 188:179–189, 2010.

[54]Hughes-Fulford, M., R. Tjandrawinata, J. Fitzgerald, K. Gasuad, and V. Gilbertson. Effects of microgravity on osteoblast growth. *Gravit. Space Biol. Bull.* 11:51–60, 1998.

[55]Humphrey, J. D., E. R. Dufresne, and M. A. Schwartz. Mechanotransduction and extracellular matrix homeostasis. *Nat. Rev. Mol. Cell Biol.* 15:802–812, 2014.

[56]Igney, F. H., and P. H. Krammer. Death and anti-death: tumour resistance to apoptosis. *Nat. Rev. Cancer* 2:277–288, 2002.

[57]Infanger, M., P. Kossmehl, M. Shakibaei, J. Bauer, S. Kossmehl-Zorn, A. Cogoli, F. Curcio, A. Oksche, M. Wehland, R. Kreutz, M. Paul, and D. Grimm. Simulated weightlessness changes the cytoskeleton and extracellular matrix proteins in papillary thyroid carcinoma cells. *Cell Tissue Res.* 324:267–277, 2006.

[58]Ingber, D. E. Can cancer be reversed by engineering the tumor microenvironment? *Semin. Cancer Biol.* 18:356–364, 2008.

[59]Jaalouk, D. E., and J. Lammerding. Mechanotransduction gone awry. *Nat. Rev. Mol. Cell Biol.* 10:63–73, 2009.

[60]Jian, B., N. Narula, Q. Y. Li, E. R. Mohler, and R. J. Levy. Progression of aortic valve stenosis: TGF-β1 is present in calcified aortic valve cusps and promotes aortic valve interstitial cell calcification via apoptosis. *Ann. Thorac. Surg.* 75:457–465, 2003.

[61]Jordan, M. A., and L. Wilson. Microtubules and actin filaments: dynamic targets for cancer chemotherapy. *Curr. Opin. Cell Biol.* 10:123–130, 1998.

[62]Jurasz, P., D. Courtman, S. Babaie, and D. J. Stewart. Role of apoptosis in pulmonary hypertension: from experimental models to clinical trials. *Pharmacol. Ther.* 126:1–8, 2010.

[63]Kaiser, D., M.-A. Freyberg, and P. Friedl. Lack of hemodynamic forces triggers apoptosis in vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 231:586–590, 1997.
[64]Kannan, K., and S. K. Jain. Oxidative stress and apoptosis. *Pathophysiology* 7:153–163, 2000.
[65]Kavallaris, M. Microtubules and resistance to tubulin-binding agents. *Nat. Rev. Cancer* 10:194–204, 2010.
[66]Kerr, J. F. R., A. H. Wyllie, and A. R. Currie. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *J. Intern. Med.* 258:479–517, 1972.
[67]Kilian, K. A., B. Bugarija, B. T. Lahn, and M. Mrksich. Geometric cues for directing the differentiation of mesenchymal stem cells. *Proc. Natl. Acad. Sci. USA* 107:4872–4877, 2010.
[68]Kim, N. H., and P. M. Kang. Apoptosis in cardiovascular diseases: mechanism and clinical implications. *Korean Circ. J.* 40:299–305, 2010.
[69]Kim, D. H., S. B. Khatau, Y. Feng, S. Walcott, S. X. Sun, G. D. Longmore, and D. Wirtz. Actin cap associated focal adhesions and their distinct role in cellular mechanosensing. *Sci. Rep.* 2:1–13, 2012.
[70]Klein, E. A., L. Yin, D. Kothapalli, P. Castagnino, F. J. Byfield, T. Xu, I. Levental, E. Hawthorne, P. A. Janmey, and R. K. Assoian. Cell-cycle control by physiological matrix elasticity and *in vivo* tissue stiffening. *Curr. Biol.* 19:1511–1518, 2009.
[71]Konduri, S., Y. Xing, J. N. Warnock, Z. He, and A. P. Yoganathan. Normal physiological conditions maintain the biological characteristics of porcine aortic heart valves: an ex vivo organ culture study. *Ann. Biomed. Eng.* 33:1158–1166, 2005.
[72]Kong, D., T. Zheng, M. Zhang, D. Wang, S. Du, X. Li, J. Fang, and X. Cao. Static mechanical stress induces apoptosis in rat endplate chondrocytes through MAPK and mitochondria-dependent caspase activation signaling pathways. *PLoS ONE* 8:1–10, 2013.
[73]Kumar, S. Caspase function in programmed cell death. *Cell Death Differ.* 14:32–43, 2007.
[74]Kusano, H., S. Shimizu, R. C. Koya, H. Fujita, S. Kamada, N. Kuzumaki, and Y. Tsujimoto. Human gelsolin prevents apoptosis by inhibiting apoptotic mitochondrial changes via closing VDAC. *Oncogene* 19:4807–4814, 2000.
[75]Lagadic-Gossmann, D., L. Huc, and V. Lecureur. Alterations of intracellular pH homeostasis in apoptosis: origins and roles. *Cell Death Differ.* 11:953–961, 2004.
[76]Leadsham, J. E., V. N. Kotiadis, D. J. Tarrant, and C. W. Gourlay. Apoptosis and the yeast actin cytoskeleton. *Cell Death Differ.* 17:754–762, 2010.
[77]Leight, J. L., M. A. Wozniak, S. Chen, M. L. Lynch, and C. S. Chen. Matrix rigidity regulates a switch between TGF-β1-induced apoptosis and epithelial-mesenchymal transition. *Mol. Biol. Cell* 23:781–791, 2012.
[78]Leopold, J. A. Cellular mechanisms of aortic valve calcification. *Circ. Cardiovasc. Interv.* 5:605–614, 2012.
[79]Li, Q., A. Kumar, E. Makhija, and G. V. Shivashankar. The regulation of dynamic mechanical coupling between actin cytoskeleton and nucleus by matrix geometry. *Biomaterials* 35:961–969, 2014.
[80]Li, B., F. Li, K. M. Puskar, and J. H. C. Wang. Spatial patterning of cell proliferation and differentiation depends on mechanical stress magnitude. *J. Biomech.* 42:1622–1627, 2009.
[81]Liao, X., X. Wang, Y. Gu, Q. Chen, and L. Y. Chen. Involvement of death receptor signaling in mechanical stretch-induced cardiomyocyte apoptosis. *Life Sci.* 77:160–174, 2005.
[82]Liu, X. M., D. Ensenat, H. Wang, A. I. Schafer, and W. Durante. Physiologic cyclic stretch inhibits apoptosis in vascular endothelium. *FEBS Lett.* 541:52–56, 2003.
[83]Locksley, R. M., N. Killeen, and M. J. Lenardo. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104:487–501, 2001.
[84]Loreto, C., G. Musumeci, A. Castorina, C. Loreto, and G. Martinez. Degenerative disc disease of herniated intervertebral discs is associated with extracellular matrix remodeling, vimentin-positive cells and cell death. *Ann. Anat.* 193:156–162, 2011.
[85]Lotem, J., and L. Sachs. Cytokines as suppressors of apoptosis. *Apoptosis* 4:187–196, 1999.
[86]Lunova, M., V. Zablotskii, N. M. Dempsey, T. Devillers, M. Jirsa, E. Sykova, S. Kubinova, O. Lunov, and A. Dejneka. Modulation of collective cell behaviour by geometrical constraints. *Integr. Biol.* 8:1093–1196, 2016.
[87]Malek, A. M., S. L. Alper, and S. Izumo. Hemodynamic shear stress and its role in atherosclerosis. *Jama* 282:2035–2042, 1999.
[88]Mana-Capelli, S., M. Paramasivam, S. Dutta, and D. McCollum. Angiomotins link F-actin architecture to Hippo pathway signaling. *Mol. Biol. Cell* 25:1676–1685, 2014.
[89]Mayr, M., Y. Hu, P. Hainaut, and Q. Xu. Mechanical stress-induced DNA damage and rac- p38MAPK signal pathways mediate p53-dependent apoptosis in vascular smooth muscle cells. *FASEB J* 16:1423–1425, 2002.
[90]Mayr, M., C. Li, Y. Zou, U. Huemer, Y. Hu, and Q. Xu. Biomechanical stress-induced apoptosis in vein grafts involves p38 mitogen-activated protein kinases. *FASEB J* 14:261–270, 2000.
[91]McIlwain, D. R., T. Berger, and T. W. Mak. Caspase functions in cell death and disease. *Cold Spring Harb. Perspect. Biol.* 7:1–28, 2015.
[92]Mleynek, T., D. Li, J. Rosenblatt, M. J. Redd, A. Chan, and Y. Gu. Endothelia extrude apoptotic cells to maintain a constant barrier. *BioRix2* 2018. https://doi.org/10.1101/268946.
[93]Mohler, E. R., F. Gannon, C. Reynolds, R. Zimmerman, M. G. Keane, and F. S. Kaplan. Bone formation and inflammation in cardiac valves. *Circulation* 103:1522–1528, 2001.
[94]Morbidelli, L., M. Monici, N. Marziliano, A. Cogoli, F. Fusi, J. Waltenberger, and M. Ziche. Simulated hypogravity impairs the angiogenic response of endothelium by up-regulating apoptotic signals. *Biochem. Biophys. Res. Commun.* 334:491–499, 2005.
[95]Musumeci, G., C. Loreto, M. L. Carnazza, I. Strehin, and J. Elisseeff. OA cartilage derived chondrocytes encapsulated in poly(ethylene glycol) diacrylate (PEGDA) for the evaluation of cartilage restoration and apoptosis in an *in vitro* model. *Histol. Histopathol.* 26:1265–1278, 2011.
[96]Narula, J., N. Haider, R. Virmani, T. G. Disalvo, F. D. Kolodgie, R. J. Hajjar, U. Schmidt, M. J. Semigran, G. W. Dec, and B. Khaw. Apoptosis in myocytes in end-stage heart failure. *N. Engl. J. Med.* 335:1182–1189, 1996.
[97]Nelson, C. M., R. P. Jean, J. L. Tan, W. F. Liu, N. J. Sniadecki, A. A. Spector, and C. S. Chen. Emergent patterns of growth controlled by multicellular form and

mechanics. *Proc. Natl. Acad. Sci. USA* 102:11594–11599, 2005.
[98]Norbury, C. J., and B. Zhivotovsky. DNA damage-induced apoptosis. *Oncogene* 23:2797–2808, 2004.
[99]Odaka, C., M. L. Sanders, and P. Crews. Jasplakinolide induces apoptosis in various transformed cell lines by a caspase-3-like protease-dependent pathway. *Clin. Diagn. Lab. Immunol.* 7:947–952, 2000.
[100]Ohsawa, S., J. Vaughen, and T. Igaki. Cell extrusion: a stress-responsive force for good or evil in epithelial homeostasis. *Dev. Cell* 44:284–296, 2018.
[101]Ohtsu, M., N. Sakai, H. Fujita, M. Kashiwagi, S. Gasa, S. Shimizu, Y. Eguchi, Y. Tsujimoto, Y. Sakiyama, K. Kobayashi, and N. Kuzumaki. Inhibition of apoptosis by the actin-regulatory protein gelsolin. *EMBO J.* 16:4650–4656, 1997.
[102]Olivetti, G., R. Abbi, and F. Quaini. Apoptosis in the failing human heart. *N. Engl. J. Med.* 336:1131–1141, 1997.
[103]Olson, E. N., and A. Nordheim. Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat. Rev. Mol. Cell Biol.* 11:353–365, 2010.
[104]Paoli, P., E. Giannoni, and P. Chiarugi. Anoikis molecular pathways and its role in cancer progression. *Biochim. Biophys. Acta Mol. Cell Res.* 3481–3498:2013, 1833.
[105]Parlato, S., A. M. Giammarioli, M. Logozzi, F. Lozupone, P. Matarrese, F. Luciani, M. Falchi, W. Malorni, and S. Fais. CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. *EMBO J.* 19:5123–5134, 2000.
[106]Paul, C., F. Manero, S. Gonin, C. Kretz-Remy, S. Virot, and A.-P. Arrigo. Hsp27 as a negative regulator of cytochrome c release. *Mol. Cell. Biol.* 22:816–834, 2002.
[107]Peacock, A. J., N. F. Murphy, J. J. McMurray, L. Caballero, and S. Stewart. An epidemiological study of pulmonary arterial hypertension. *Eur. Respir. J.* 30:104–109, 2007.
[108]Peitsch, M. C., B. Polzar, H. Stephan, T. Crompton, H. R. MacDonald, H. G. Mannherz, and J. Tschopp. Characterization of the endogenous deoxyribonuclease involved in nuclear DNA degradation during apoptosis (programmed cell death). *EMBO J.* 12:371–377, 1993.
[109]Perry, S. W., J. P. Norman, J. Barbieri, E. B. Brown, and H. A. Gelbard. Mitochondrial membrane potential probes and the proton gradient: a practical usage guide. *Biotechniques* 50:98–115, 2011.
[110]Peyronnet, R., J. M. Nerbonne, and P. Kohl. Cardiac mechano-gated ion channels and arrhythmias. *Circ Res.* 118:239–311, 2016.
[111]Phrommintikul, A., L. Tran, A. Kompa, B. Wang, A. Adrahtas, D. Cantwell, D. J. Kelly, and H. Krum. Effects of a Rho kinase inhibitor on pressure overload induced cardiac hypertrophy and associated diastolic dysfunction. *Am. J. Physiol. Hear. Circ. Physiol.* 294:1804–1814, 2008.
[112]Pickup, M. W., J. K. Mouw, and V. M. Weaver. The extracellular matrix modulates the hallmarks of cancer. *EMBO Rep.* 15:1243–1253, 2014.
[113]Porter, A. G., and R. U. Jänicke. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ.* 6:99–104, 1999.
[114]Puthalakath, H., A. Villunger, L. A. O'Reilly, J. G. Beaumont, L. Coultas, R. E. Cheney, D. C. S. Huang, and A. Strasser. Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. *Science* 293:1829–1832, 2001.
[115]Qiao, L., and G. C. Farrell. The effects of cell density, attachment substratum and dexamethasone on spontaneous apoptosis of rat hepatocytes in primary culture. *Vitro. Cell. Dev. Biol. Anim.* 35:417–424, 1999.
[116]Rikitake, Y., and J. K. Liao. Rho GTPases, statins, and nitric oxide. *Circ. Res.* 97:1232–1235, 2005.
[117]Rodriguez, K. J., L. M. Piechura, A. M. Porras, and K. S. Masters. Manipulation of valve composition to elucidate the role of collagen in aortic valve calcification. *BMC Cardiovasc. Disord.* 14:1–10, 2014.
[118]Roos, W. P., and B. Kaina. DNA damage-induced cell death by apoptosis. *Trends Mol. Med.* 12:440–450, 2006.
[119]Rowe, V. L., S. L. Stevens, T. T. Reddick, M. B. Freeman, R. Donnell, R. C. Carroll, and M. H. Goldman. Vascular smooth muscle cell apoptosis in aneurysmal, occlusive, and normal human aortas. *J. Vasc. Surg.* 31:567–576, 2000.
[120]Roy, S., and D. W. Nicholson. Cross-talk in cell death signaling. *J. Exp. Med.* 192:F21–F25, 2000.
[121]Sendoel, A., and M. O. Hengartner. Apoptotic cell death under hypoxia. *Physiology* 29:168–176, 2014.
[122]Solon, J., I. Levental, K. Sengupta, P. C. Georges, and P. A. Janmey. Fibroblast adaptation and stiffness matching to soft elastic substrates. *Biophys. J.* 93:4453–4461, 2007.
[123]Song, A. S. Thermally induced apoptosis, necrosis, and heat shock protein expression in 3D culture. *J. Biomech. Eng.* 136:1–10, 2014.
[124]Song, Y., J. Fu, M. Zhou, L. Xiao, X. Feng, H. Chen, and W. Huang. Activated Hippo/Yes-associated protein pathway promotes cell proliferation and anti-apoptosis in endometrial stromal cells of endometriosis. *J. Clin. Endocrinol. Metab.* 101:1552–1561, 2016.
[125]Sotoudeh, M., Y.-S. Li, N. Yajima, C.-C. Chang, T.-C. Tsou, Y. Wang, S. Usami, A. Ratcliffe, S. Chien, and J. Y.-J. Shyy. Induction of apoptosis in vascular smooth muscle cells by mechanical stretch. *Am. J. Physiol. Circ. Physiol.* 282:H1709–H1716, 2002.
[126]Steen, R. L., and P. Collas. Mistargeting of B-type lamins at the end of mitosis: implications on cell survival and regulation of lamins A/C expression. *J. Cell Biol.* 152:621–626, 2001.
[127]Stournaras, C., E. Stiakaki, S. B. Koukouritaki, P. A. Theodoropoulos, M. Kalmanti, Y. Fostinis, and A. Gravanis. Altered actin polymerization dynamics in various malignant cell types: Evidence for differential sensitivity to cytochalasin B. *Biochem. Pharmacol.* 52:1339–1346, 1996.
[128]Streichan, S. J., C. R. Hoerner, T. Schneidt, D. Holzer, and L. Hufnagel. Spatial constraints control cell proliferation in tissues. *Proc. Natl. Acad. Sci. USA* 111:5586–5591, 2014.
[129]Su, B. Y., K. M. Shontz, N. A. Flavahan, and P. T. Nowicki. The effect of phenotype on mechanical stretch-induced vascular smooth muscle cell apoptosis. *J. Vasc. Res.* 43:229–237, 2006.
[130]Suria, H., L. Chau, E. Negrou, D. Kelvin, and J. Madrenas. Cytoskeletal disruption induces T cell apoptosis by a caspase-3 mediated mechanism. *Life Sci.* 65:2697–2707, 1999.
[131]Suzanne, M., and H. Steller. Shaping organisms with apoptosis. *Cell Death Differ.* 20:669–675, 2013.
[132]Tamada, M., M. P. Sheetz, and Y. Sawada. Activation of a signaling cascade by cytoskeleton stretch. *Dev. Cell* 7:709–718, 2004.
[133]Tanaka, K., M. Sata, D. Fukuda, Y. Suematsu, N. Motomura, S. Takamoto, Y. Hirata, and R. Nagai. Age-

associated aortic stenosis in apolipoprotein E-deficient mice. *J. Am. Coll. Cardiol.* 46:134–141, 2005.
[134]Tang, H. L., K. L. Yuen, H. M. Tang, and M. C. Fung. Reversibility of apoptosis in cancer cells. *Br. J. Cancer* 100:118–122, 2009.
[135]Tilghman, R. W., C. R. Cowan, J. D. Mih, Y. Koryakina, D. Gioeli, J. K. Slack-Davis, B. R. Blackman, D. J. Tschumperlin, and J. T. Parsons. Matrix rigidity regulates cancer cell growth and cellular phenotype. *PLoS ONE* 5:1–13, 2010.
[136]Tomasek, J. J., G. Gabbiani, B. Hinz, C. Chaponnier, and R. A. Brown. Myofibroblasts and mechanoregulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* 3:349–363, 2002.
[137]Tsujimoto, Y. Cell death regulation by the Bcl-2 protein family in the mitochondria. *J. Cell. Physiol.* 195:158–167, 2003.
[138]Ulrich, T. A., E. M. de Juan Pardo, and S. Kumar. The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells. *Cancer Res.* 69:4167–4174, 2009.
[139]Vidyasekar, P., P. Shyamsunder, R. Arun, R. Santhakumar, N. K. Kapadia, R. Kumar, and R. S. Verma. Genome wide expression profiling of cancer cell lines cultured in microgravity reveals significant dysregulation of cell cycle and MicroRNA gene networks. *PLoS ONE* 10:1–20, 2015.
[140]Virani, S. S., *et al.* Heart disease and stroke statistics—2020 update. *Circulation* 141:139–596, 2020. https://doi.org/10.1161/cir.0000000000000757.
[141]Wada, T., and J. M. Penninger. Mitogen-activated protein kinases in apoptosis regulation. *Oncogene* 23:2838–2849, 2004.
[142]Wang, K., J. J. Brems, R. L. Gamelli, and J. Ding. Reversibility of caspase activation and its role during glycochenodeoxycholate-induced hepatocyte apoptosis. *J. Biol. Chem.* 280:23490–23495, 2005.
[143]Wang, G., W. Chen, H. Qu, and X. Li. The effect of cyclic stretch on apoptosis of human squamous carcinoma of tongue cell line Tca8113. *Biomed. Eng. Inform.* 2009. https://doi.org/10.1109/BMEI.2009.5303118.
[144]Wang, H.-B., M. Dembo, and Y.-L. Wang. Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am. J. Physiol. Physiol.* 279:C1345–C1350, 2000.
[145]Wang, Y. X., B. Martin-McNulty, V. Da Cunha, J. Vincelette, X. Lu, Q. Feng, M. Halks-Miller, M. Mahmoudi, M. Schroeder, B. Subramanyam, J. L. Tseng, G. D. Deng, S. Schirm, A. Johns, K. Kauser, W. P. Dole, and D. R. Light. Fasudil, a Rho-kinase inhibitor, attenuates angiotensin II-induced abdominal aortic aneurysm in apolipoprotein E-deficient mice by inhibiting apoptosis and proteolysis. *Circulation* 111:2219–2226, 2005.
[146]Wang, P., A. J. Valentijn, A. P. Gilmore, and C. H. Streuli. Early events in the anoikis program occur in the absence of caspase activation. *J. Biol. Chem.* 278:19917–19925, 2003.
[147]Watters, D. Molecular mechanisms of ionizing radiation-induced apoptosis. *Immunol. Cell Biol.* 77:263–271, 1999.
[148]Wencker, D., M. Chandra, K. Nguyen, W. Miao, S. Garantziotis, S. M. Factor, J. Shirani, R. C. Armstrong, and R. N. Kitsis. A mechanistic role for cardiac myocyte apoptosis in heart failure. *J. Clin. Invest.* 111:1497–1504, 2003.
[149]Wernig, F., M. Mayr, and Q. Xu. Mechanical stretch-induced apoptosis in smooth muscle cells is mediated by β1-integrin signaling pathways. *Hypertension* 41:903–911, 2003.
[150]Wernig, F., and Q. Xu. Mechanical stress-induced apoptosis in the cardiovascular system. *Prog. Biophys. Mol. Biol.* 78:105–137, 2002.
[151]Wu, C.-C., Y.-S. Li, J. H. Haga, R. Kaunas, J.-J. Chiu, F.-C. Su, S. Usami, and S. Chien. Directional shear flow and Rho activation prevent the endothelial cell apoptosis induced by micropatterned anisotropic geometry. *Proc. Natl. Acad. Sci. USA* 104:1254–1259, 2007.
[152]Wu, Y., D. Zhao, J. Zhuang, F. Zhang, and C. Xu. Caspase-8 and caspase-9 functioned differently at different stages of the cyclic stretch-induced apoptosis in human periodontal ligament cells. *PLoS ONE* 11:1–15, 2016.
[153]Yamazaki, T., I. Komuro, S. Kudoh, Y. Zou, R. Nagai, R. Aikawa, H. Uozumi, and Y. Yazaki. Role of ion channels and exchangers in mechanical stretch-induced cardiomyocyte hypertrophy. *Circ. Res.* 82:430–437, 1998.
[154]Yeung, T., P. C. Georges, L. A. Flanagan, B. Marg, M. Ortiz, M. Funaki, N. Zahir, W. Ming, V. Weaver, and P. A. Janmey. Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motil. Cytoskeleton* 60:24–34, 2005.
[155]Zhang, T., F. Tian, J. Wang, J. Jing, S. S. Zhou, and Y. D. Chen. Atherosclerosis-associated endothelial cell apoptosis by MiR-429-mediated down regulation of Bcl-2. *Cell. Physiol. Biochem.* 37:1421–1430, 2015.
[156]Zhang, Y. H., C. Q. Zhao, L. S. Jiang, and L. Y. Dai. Substrate stiffness regulates apoptosis and the mRNA expression of extracellular matrix regulatory genes in the rat annular cells. *Matrix Biol.* 30:135–144, 2011.
[157]Zhao, T., X. Tang, C. S. Umeshappa, H. Ma, H. Gao, Y. Deng, A. Freywald, and J. Xiang. Simulated microgravity promotes cell apoptosis through suppressing Uev1A/TICAM/TRAF/NF-κB-regulated anti-apoptosis and p53/PCNA- and ATM/ATR-Chk1/2-controlled DNA-damage response pathways. *J. Cell. Biochem.* 2148:2138–2148, 2016.