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Mechanical Signaling in Reproductive Tissues: Mechanisms and Importance

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Abstract

The organs of the female reproductive system are among the most dynamic tissues in the human body, undergoing repeated cycles of growth and involution from puberty through menopause. To achieve such impressive plasticity, reproductive tissues must respond not only to soluble signals (hormones, growth factors, and cytokines) but also to physical cues (mechanical forces and osmotic stress) as well. Here, we review the mechanisms underlying the process of *mechanotransduction*—how signals are conveyed from the extracellular matrix that surrounds the cells of reproductive tissues to the downstream molecules and signaling pathways that coordinate the cellular adaptive response to external forces. Our objective was to examine how mechanical forces contribute significantly to physiological functions and pathogenesis in reproductive tissues. We highlight how widespread diseases of the reproductive tract, from preterm labor to tumors of the uterus and breast, result from an impairment in mechanical signaling.

Keywords

mechanotransduction, ROCK/Rho kinase, fibroids, extracellular matrix, endometriosis, cervical insufficiency

Introduction

The reproductive system is dynamic and constantly undergoes extensive structural remodeling throughout each reproductive cycle. This is achieved, in part, by changes in the extracellular matrix (ECM), which are regulated by cyclic hormonal cues as well as cytokines and growth factors. Although soluble signaling molecules have long been recognized as important factors in growth, development, differentiation, and function, mechanical signaling (MS) has only been more recently shown to play a critical role in these functions.

Four decades of research have led to an evolution of thought from a simplistic and static view of the role of extracellular structural proteins in gene expression[1] to the concept of mechanotransduction and mechanobiology involving the conversion of mechanical energy into biochemical energy,[2] and further to an understanding of the ECM as a tensionally integrated structural system that conveys information to other systems from the macroscale to the nanoscale (for review[3]). Mechanotransduction refers to the cellular processes that translate mechanical stimuli into biochemical signals, allowing cells to adapt to their changing physical environment, of particular importance in dynamic systems of the reproductive tract. Thus, the ECM is not static but rather a dynamic structure of collagens, enzymes, proteins and glycoproteins, extracellular phosphatases, kinases, cytokines, and growth factors. The ECM senses and responds to cues as subtle and exquisite as moving air molecules in sound waves, to the constant weight bearing of hundreds of pounds in a knee joint, or generation of sufficient force needed to effect vaginal delivery.[3]

Specifically, the ECM signals directly to cells using cell–ECM adhesions, such as integrins and focal adhesions.[4] Additionally, the ECM also transduces information to cells via surface processes (cilia and stereocilia), stress on cellular membranes affecting stretch-sensitive ion channels,[5] caveolae and surface receptors, and tension-dependent enzymes or proteins,[6] and changes in biopolymers.[7] Furthermore, it is clear that fluid in the ECM, the extracellular fluid, can generate intrinsic mechanical force.[8]

Through these mechanisms, cells sense mechanical force based on conformational changes in mechanosensitive proteins that result in the opening of membrane channels, alterations in binding affinities, and increased phosphorylation,[9] which serve to activate intracellular signaling pathways. Changes in intracellular force transmission due to alterations in cellular or extracellular structure and organization can lead to mechanosensitive

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signals that may be attenuated or increased, as compared to the normal state.[10] This extraordinary expansion of the field of matrix biology allowed for the development of new insights into cell–matrix interactions.

An important concept to grasp is the reciprocal nature of mechanotransduction—namely that highly dynamic ECM remodeling, rapid transient cell–cell and cell–matrix occur during these processes, a phenomenon referred to as dynamic reciprocity.[2,3,11] Mutations or modifications that result in abnormal mechanotransduction and cellular sensitivity to mechanical stress can be implicated in the development of many different diseases (reviewed in[12,13]). The predominant mechanism behind disease initiation may be a disruption in force transmission between the ECM, cellular cytoskeleton, and the inside of the nucleus.[10] Alternatively, mechanotransduction can be altered by mutations in proteins involved in the downstream signaling pathways.

The ECM conveys physical and chemical signals to the cell membrane, which in turn leads to signal propagation and amplification via a complex array of intracellular signals.[3,13] As an example, the Rho/Rho-associated protein kinase (ROCK)/mitogen-activated protein kinase (MAPK) signaling pathway is one important pathway in mechanotransduction. RhoA belongs to a family of small guanine triphosphate (GTP)-binding proteins. Both RhoA and ROCK,[14,15] a downstream target of Rho, are involved in the regulation of a wide variety of cellular processes, including changes in cell motility and morphology, focal adhesion formation, and light-chain phosphorylation.[16] As noted previously, reciprocity is a main feature of mechanosensitive cells: not only do cells respond to biophysical and biochemical cues from the ECM but also they help maintain and remodel the ECM through secretion of various ECM components and the molecules that degrade them. Thus, ECM remodeling will produce a matrix that can be either pliable or stiff depending on protein cross-links, content of glycoproteins, and other components of the ECM.

Mechanical signaling has previously been well described in specialized tissues and has also been studied in other tissues such as blood vessels, stem cells, respiratory epithelium, and musculoskeletal tissues. Currently, the expanding study of mechanotransduction is allowing for a wide variety of advancements in reproduction, ranging from improvements in gamete development and stem cells[17] to the development of autologous vaginal tissue grafts now in use clinically.[18,19] Here, we will review recent work describing the significance of MS in various reproductive tissues in both physiological and pathological states, including the uterus, cervix, endometrium, ovary, and breast.

Uterus

Uterine Physiology: Normal Pregnancy

Mechanical signaling is important for the extensive adaptation and remodeling that occurs throughout the course of pregnancy, where the human uterus increases from a weight of approximately 50 g in the nonpregnant state to roughly 1200 g at term to accommodate the growing fetus and products of conception.[20] For the majority of pregnancy, the myometrium accommodates the increasing mechanical stress and remains quiescent as the uterus increases in size. Smooth muscle hypertrophy is mediated by a number of mechanisms, including both hormonal cues, such as estrogen[21] and progesterone,[22] and mechanical stretch.[23,24]

Studies investigating the effects of mechanical stretch on hypertrophy found that passive filling of a nonpregnant uterine horn with a saline balloon was sufficient to produce an increase in uterine size secondary to both cell hypertrophy and hyperplasia.[24] A similar study utilizing wax distention further showed that in addition to increasing uterine size, physical stretch had the added effect of promoting collagen formation and changes in the cell population.[25] To tease out the contributions of endocrine cues versus mechanical stretch, unilaterally pregnant mice were studied. Greater hypertrophy was observed in the pregnant horn as compared to the nonpregnant horn, and regions immediately surrounding a fetus also exhibited more hypertrophy than regions between fetuses.[26,27] These results suggest that given the same hormonal environment, mechanical stretch has the ability to stimulate myometrial hypertrophy, and that the degree of hypertrophy is related to the amount of stretch applied. This finding is further substantiated by the fact that hypertrophy, an increase in the production of ECM proteins, has also been shown to be increased in the second half of gestation, when fetal growth is greatest.[28]

Mechanical stretch results in the increase in a number of labor-associated proteins, as well as activation of MAPK cascades, including extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation, and ultimately increased transcription of various cytoskeleton-related genes (reviewed in[29,30]). The mechanism of signal transduction between stretch and these downstream effectors is unclear but has been proposed to be related to alterations in calcium signaling.[31] Uterine growth that results from hypertrophy may signify a mechanism by which increases in tension on the myometrium can be avoided, such that the uterus can maintain quiescence prior to parturition.

Near the end of pregnancy, at the time of parturition, the myometrium undergoes a dramatic switch to a contractile phenotype and begins to generate powerful, coordinated contractions. The mechanisms underlying this switch are poorly understood, but alterations in the expression of RhoA and focal adhesion kinase (FAK) are currently being investigated as potential contributing factors. The messenger RNA (mRNA) expression of RhoA as well as 2 isoforms of ROCK (α and β) has been found to be elevated in both pregnant rat[32] and human[33] myometrium. Furthermore, using real-time reverse transcription-polymerase chain reaction, it has been shown that RhoA mRNA expression was significantly greater in human pregnant myometrium after labor onset, as compared to the same myometrium before labor onset as well as nonpregnant myometrium.[34] Collectively, these results suggest that differential expression of RhoA could be a key player

in the switch between these myometrial phenotypes. A study by Li et al[35] showed that myometrial stretch results in an increase in FAK-Src signaling, which is necessary for the increase in myometrial ERK, one of the signals that leads to activation prior to the onset of labor. This study also demonstrated that pretreatment of tissue with PP2, a Src inhibitor, significantly suppressed stretch-induced increases in FAK, paxillin, and Src and consequently decreased ERK phosphorylation and myometrial contractility.

Uterine Pathology: Preterm Birth and Leiomyomata

Preterm birth. Preterm birth is the major obstetric cause of perinatal morbidity and mortality.[36] The cause of preterm birth is multifactorial and is associated with a number of different mechanisms, including infection, placental hemorrhage, cervical insufficiency, and uterine overdistention. Spontaneous idiopathic preterm labor is attributed as the cause of 30% to 50% of all preterm deliveries. Although progesterone plays a key role in preventing singleton preterm birth, as evidenced by success of treatment with 17α-hydroxyprogesterone caproate,[37] many studies have failed to demonstrate efficacy of 17α-hydroxyprogesterone caproate for preventing preterm birth in multiple gestation pregnancies, including the recent trial by Lim et al.[38] These results may point to the importance of MS in preterm births, particularly in cases of uterine overdistention, an issue that continues to become more prevalent with advances in the treatment of infertility.

Regardless of the initiating factor, preterm labor can ultimately be linked to 1 of the 2 pathologic states: (1) a state of increased contractility of uterine smooth muscle or (2) cervical insufficiency (see cervix subsequently). Contractility is regulated by intracellular calcium concentration, as well as the sensitivity of the myofilaments within the uterus to calcium.[39] Briefly, increased calcium results in phosphorylation of myosin light chain (MLC), which is catalyzed by MLC kinase and antagonized by MLC phosphatase (MLCP). RhoA and ROCK increase calcium sensitization by inhibiting MLCP and increasing the amount of phosphorylated MLC. In this way, RhoA and ROCK have been implicated in the maintenance of sustained contraction in cells and are key players in disorders featuring increased contractility of smooth muscle,[34] including hypertension, angina, and heart failure. Furthermore, treatment with ROCK inhibitors, such as Y-27632, resulted in potent relaxation of the myometrium.[33] Taken together, these studies point to a potential Rho-mediated mechanism by which preterm labor results from factors other than progesterone deficiency, as well as potential therapeutic strategies to delay labor via attenuation of such contractility-associated proteins.

Leiomyomata. Although hypertrophy may be protective against premature disruption of myometrial quiescence during pregnancy, the uterus must also be able to involute quickly after parturition. When this does not occur, pathologic states arise. It has been proposed that the generation of leiomyomata may be a pathological state in which myometrial cells that are normally stimulated to undergo hypertrophy as a result of certain hormonal and mechanical cues do not receive the signal to involute and continue to grow and secrete an abnormal ECM (Figure 1).

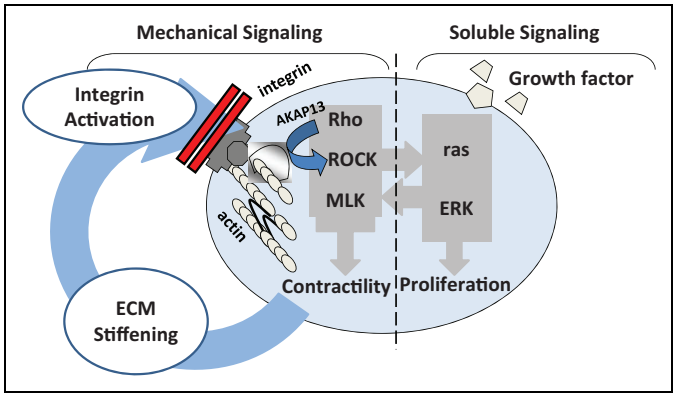

Figure 1. Attenuated signaling in leiomyoma may lead to impaired feedback, excessive ECM deposition, and growth. Mechanical signaling and soluble signaling are shown schematically. Mechanical stress activates integrins, leading to activation of Rho, contraction of myosin, stiffening of the cell, and secretion of ECM. Blockage of mechanical signaling, depicted by double bars, could interrupt the feedback loop, leading to secretion of excessively stiff and abundant ECM and leiomyoma growth. ECM indicates extracellular matrix.

Uterine fibroids are characterized by excessive, abnormal, and disorganized extracellular components.[40] One such component is collagen, which has been shown to contain an abnormal fibril structure and orientation in uterine fibroids, suggesting that the well-regulated fibril formation in myometrium is altered.[41] Additionally, early studies have shown that leiomyomata and myometrium possess different percentages of the glycosaminoglycans chondroitin sulfate and dermatan sulfate (78% in myometrium vs 95% in leiomyoma).[42] The main glycosaminoglycan (GAG) is a D-glucuronic acid-rich dermatan sulfate whose presence has been shown to directly correlate with leiomyoma size.[43] Furthermore, decorins, which interact with specific regions on the surface of collagens type I and II and play a role in organization and assembly of collagen fibrils, were shown to exist in a higher molecular weight form as compared to normal myometrium.[44] Distribution patterns of decorin and collagen were completely different in leiomyoma as compared to normal myometrium when observed by immunofluorescence, and the ratio of decorin to type I collagen was also increased in leiomyoma.[44] Given that uterine fibroids are primarily composed of this abnormal cell matrix, dissolution of the ECM is critical for resolution of the bulk symptoms imposed by these tumors.

In addition to alterations in ECM components, the characteristics of uterine fibroids are consistent with altered response to mechanical forces. Uterine fibroids exist in an environment of increased mechanical stress but have a decreased response to mechanical cues.[45,46] Upon application of stress, it was observed that myometrial cells reoriented their actin cytoskeleton perpendicular to the axis of applied strain, whereas fibroid cells did not do so as readily.[46] Moreover, upon application of

mechanical stress in vitro, uterine fibroid cells had decreased activation of RhoA as compared to myometrium.[46] Unconfined compression in vitro demonstrated increased stiffness of leiomyomata as compared to surrounding myometrium.[45] It has been postulated that ECM stiffness may play a role in tumorigenesis,[47,48] potentially through the RhoA pathway.[49,50]

Interestingly, several studies implicate a role for the activation of the mammalian target of rapamycin (mTOR) by dysregulated phosphoinositide 3-kinase–protein kinase (P13K/AKT) pathway in the pathogenesis of leiomyomas.[51,52] Activation of mTOR promotes cell growth, differentiation, and proliferation. A recent report suggests that the loss of the RE1 silencing transcription factor (TF)/neuron-restrictive silencing factor along with aberrant G-protein coupled receptor 10 expression activates the mTOR pathway and is a major factor in leiomyoma pathogenesis.[53] It is intriguing that mTOR activation is induced by mechanical stimuli through P13K/Akt/mTOR.[54]

Notably, leiomyomata also have increased fluid content relative to myometrium and an abnormal response to osmotic stress.[55,56] Studies have shown that baseline levels of nuclear factor of activated T cell 5 (NFAT5), a TF that responds to osmotic stress, were increased in leiomyomata.[57] Moreover, increases in extracellular osmolarity further upregulated expression of NFAT5 and its downstream targets, aldose reductase and sodium myoinositol transporter 1 in fibroid cells, leading to an increase in the intracellular osmolytes sorbitol and inositol.[57] Thus, fibroid cells were capable of maintaining osmotic homeostasis and of preserving their cellular architecture in the face of a hyperosmolar extracellular milieu. Such tolerance to hyperosmolarity may support the increased viscoelastic stiffness characteristic of leiomyomata.

A number of medical therapies for fibroids have been investigated as alternatives to hysterectomy for women who desire to preserve fertility. A GnRH analog, leuprolide acetate, has been shown to cause a rapid regression in leiomyoma size; however, tumors quickly rebound after treatment is discontinued.[58] Given the low mitotic index exhibited by leiomyomata, this rebound effect is believed to be due to alterations in regulation of leiomyoma ECM rather than cell proliferation. Although it was previously believed that GnRH analogs promoted uterine fibroid regression primarily through regulation of gonadal hormone exposure, a recent study by Britten et al[59] showed that these analogs can also act directly on leiomyoma. In vitro experiments on leiomyoma cells showed that leuprolide acetate results in an initial increase in the expression of ECM genes *COL1A1*, fibronectin, and versican variant 0, with levels beginning to normalize after 5 days of treatment. Interestingly, recent data suggest that leuprolide may also contribute to a decrease in fibroid volume by downregulating NFAT5 signaling,[57] leading to a breakdown in cellular osmotic integrity and a subsequent efflux of water from the fibroid. These findings are further substantiated by earlier reports of the presence of GnRH receptors in surgical specimens of uterine leiomyomas as well as patient-matched myometrium.[60] Thus, as our understanding of the importance of mechanical and osmotic signaling in fibroid pathogenesis increases, this may suggest new approaches to fibroid therapy.

Cervix

Cervix Physiology: Pregnancy

The cervix serves a dual structural function: prior to term, the cervix is responsible for load bearing and must stay closed to allow for normal fetal development to occur and at term, the cervix must dilate to allow for parturition. Cervical mechanical properties are derived from its ECM. Although the rest of the uterus is mainly composed of smooth muscle with ECM between the cells, the cervix contains only 10% to 15% of smooth muscle and 85% to 90% of ECM.[61]

The cervix is primarily composed of fibrillar collagen types I and III.[61] Throughout the process of pregnancy, the ratio of type I to type III collagen, as well as changes in posttranscriptional processing of collagen monomers and assembly of these monomers into collagen fibrils all affect the mechanical strength of cervical tissue.[62,63] The cervix is composed of 3 zones of structured collagen that are seamlessly blended.[64] The outermost and innermost zones contain fibers that are primarily longitudinal or parallel to the canal.[64] The middle zone contains fibrils that are primarily oriented circumferentially.[64] Magnetic resonance imaging[65] and x-ray studies[64] have shown that the cervical collagen is oriented in a pattern that resists premature cervical deformation. Alterations in collagen structure and orientation are influenced by the composition of GAGs within the ECM.

The cervix undergoes extensive remodeling during pregnancy to prepare for childbirth. This remodeling can be divided into 4 phases: softening, ripening, dilation, and postpartum repair.[66,67] Cervical tissue compliance progressively increases beginning in early pregnancy and ends with maximal loss of tensile strength at the time of parturition.[68] The gradual changes in mechanical properties during normal pregnancy are counteracted with an increase in tissue volume that allows the cervix to soften while remaining closed.[69,70] The properties of human cervical tissue vary greatly depending on several factors, which include biochemical content, obstetric history, anatomical site, and the direction of loading (reviewed in[62]). During gestation, there is active synthesis of type I collagen, and this remains high throughout pregnancy.[71] However, due to an increase in cervical water and a marked increase in noncollagen proteins and proteoglycans, the collagen density decreases—a fact that initially caused confusion as it was interpreted as collagen degradation. A study by Read et al[66] showed that although collagen solubility increases during cervical softening, there is no change in the total collagen content, suggesting that alterations in structure, rather than amount, are central to cervical remodeling. Others have proposed that in late pregnancy, the cervix responds to mechanical stress imposed by the fetal presenting part, which causes muscle fibers, fibroblasts, and collagenous/elastic tissues to align parallel to one another.[72]

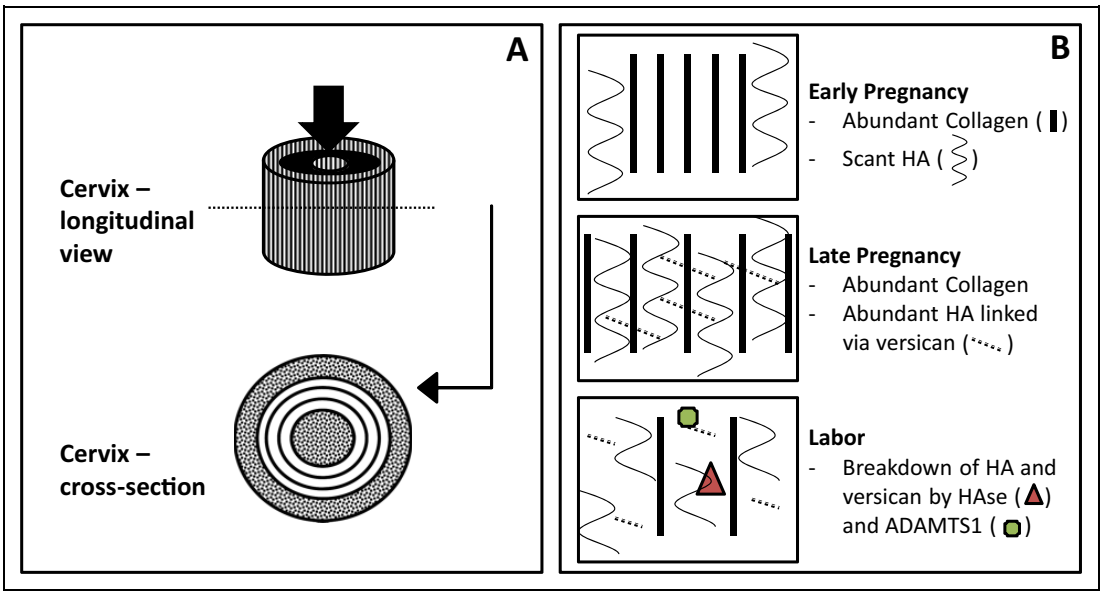

Figure 2. The cervical ECM during pregnancy and parturition. A, The cervix is composed of 3 zones of structured collagen: the outermost and innermost zones contain fibers arranged longitudinally (parallel to the canal), while the middle zone contains fibrils that are oriented circumferentially. This arrangement is designed to withstand the weight imposed by the gravid uterus. B, The ECM remodeling is essential for cervical dilation during labor and is initiated by the concerted action of soluble mediators and extrinsic forces imposed by the fetal presenting part. The increase in HA (cross-linked to versican) promotes tissue hydration through osmosis and spaces out collagen fibers, thus allowing the cervix to soften while remaining closed. Breakdown of HA and versican by HAse and ADAMTS1 facilitates cervical effacement and dilation during labor. HA indicates hyaluronic acid; HAse, hyaluronidase; ADAMTS 1, a disintegrin and metalloproteinase with thrombospondin-like repeats 1; ECM indicates extracellular matrix.

In addition to changes in collagen, alterations in GAGs such as hyaluronic acid (HA) have been shown to play a role in the process of parturition. Cervical HA increases from a baseline of 19% of vertical GAGs in early pregnancy to a total of 71% at term.[73] Hyaluronic acid has been shown to exist in a high-molecular weight form, which is a hydrophilic, space-filling molecule that increases tissue viscoelasticity before birth and a low-molecular weight form shortly after birth.[74] This high-molecular weight form helps to increase spacing between collagen fibers and may contribute to the increase in tissue hydration seen during this time.[74] Furthermore, cyclic mechanical stretch will increase HA expression in cultured human cervical fibroblasts.[75] Timmons et al[70] hypothesize that either prior to or during the onset of labor, there is an increase in the expression of hyaluronidase and a disintegrin and metalloproteinase with thrombospondin-like repeats 1 (ADAMTS1), which may lead to a disruption of hyaluronidase-versican cross-links and promote cervical dilation (Figure 2).

Cervix Pathology: Cervical Incompetence

Cervical insufficiency occurs when cervical tissue prematurely softens, resulting in preterm spontaneous birth. Despite an alarming rate of preterm births, accounting for 12.5% of all pregnancies, the etiology of cervical insufficiency is not well understood. Preterm cervical ripening may occur due to defects with regulation in timing of the same physiologic process that occurs at term or from entirely distinct molecular mechanisms. Acceleration of cervical ripening, at term and preterm, is associated with a decline in progesterone synthesis, which may explain why treatment with 17α-hydroxyprogesterone has been shown to be an effective means of preventing preterm labor in singleton pregnancies.[37]

Although the process of labor has long been considered an inflammatory process, new data from a number of groups have suggested that immune cells are present but not activated during physiological ripening and may be sufficient but not necessary for cervical ripening.[76-78] Recent studies using a mouse model that is administered lipopolysaccharide, found in the outer membrane of gram-negative bacteria, to mimic a proinflammatory response similar to intra-amniotic infection resulted in a preterm labor without a decrease in P4.[79] Thus, in the case of intra-amniotic infection, it has been hypothesized that the loss of cervical competency may lead to a compromise of protection against invading microorganisms, which may serve to predispose these women to ascending infection, which is then sufficient to trigger premature cervical ripening. However, this is not the sole mechanism behind preterm labor, as administration of a progesterone antagonist, RU286/mifepristone, also resulted in a premature onset of labor, without activation of proinflammatory cascades.[76]

In addition to inflammation and withdrawal of progesterone, defects in mechanical properties or signaling leading to modifications in collagen structure and organization may play a role in preterm labor. This hypothesis is supported by the increased incidence of preterm birth in women with inherited defects in collagen synthesis or assembly.[80] Finally, one study noted that there is a decrease in elastin in the incompetent cervix.[81]

Elastin is an ECM protein that in its coiled state during gestation helps retain a growing fetus. As the presenting part of the fetus descends into the lower uterine segment and into the inner os of the cervical canal during normal labor, the mechanical forces generated cause the elastin to stretch. The decreased elastin in incompetent cervices would thus hinder the ability of cervix to retain a fetus until term.

Endometrium

Endometrial Physiology: Embryo Implantation

Mechanical signaling is involved in the successful establishment of pregnancy. Embryo implantation is initiated by embryo attachment to endometrial epithelium followed by a "decidualization" process in the stroma beneath the epithelium into large, round, or polygonal decidual cells. This process can be induced in rodents, even in the absence of an embryo, by diverse mechanical stimuli, including intraluminal injections of oil and air,[82] and endometrial scratching.[83] Clinically, studies have shown that endometrial biopsies—a form of endometrial injury similar to scratching performed in rodents—promoted implantation in women undergoing in vitro fertilization.[84]

The mechanism by which such mechanical stimuli promote decidualization may involve the epithelial sodium channel (ENaC). The ENaC is a mechanosensitive channel, previously shown to be activated by shear force,[85] in addition to ovarian hormones,[86] and serine proteases[87,88] in various tissues. A recent study by Ruan et al[89] found that activation of ENaC triggered downstream production and release of prostaglandin E2 (PGE2), which was required for embryo implantation. Consistent with a role of ENaC in implantation, these authors found that the endometrium of women with failed IVF cycles expressed significantly lower levels of ENaC as compared to those from women with successful cycles.

Recent studies have also implicated lysophosphatidic acid (LPA), a small phospholipid, as a potential mediator of on-time implantation and proper embryo spacing. Lysophosphatidic acid acts on its cognate receptor LPA3, a G protein-coupled receptor found on the plasma membrane of endometrial decidual cells.[90] Downstream of LPA receptor activation, the small GTPase Rho, can be activated, subsequently activating ROCK. Activation of Rho/ROCK leads to the formation of actin–myosin stress fibers (the contractile apparatus of nonmuscle cells) and increases cellular contractility through the inhibition of myosin light-chain phosphatase, which allow for stromal cell decidualization. Expression of LPA3 itself is upregulated by the action of progesterone in the mouse uterus.[91] Mice deficient in LPA3 exhibited delayed implantation and crowding of monochorionic embryos near the cervix. This, in turn, was associated with placental hypertrophy, delayed embryonic development, and increased embryonic death, leading to a 50% reduction in litter size.[92] In a follow-up study, LPA agonists were found to induce peri-implantation uterine contractions in wild-type mice but not in LPA3 knockout mice. This decrease in uterine contractions may explain the aberrant embryo spacing seen in LPA3 knockouts. Additionally, timing of implantation, but not proper embryo spacing, could be rescued by treatment of the dames with PGE2 or carbaprostacyclin, suggesting that the effects of LPA may also be mediated by downstream activation of PGs. This effect of propagation is also seen in the fallopian tubes. Transmembrane member 16a leads to the Ca efflux, resulting in oviduct pacemaker activity of the smooth muscle.[93] A model for embryo implantation that accounts for both mechanical and soluble signals, acting via ENaC and LPA3 receptors, and resulting in downstream PG production as well as Rho-mediated stress fiber formation is proposed in Figure 3.

Endometrial Pathology: Endometriosis

Endometriosis is a prevalent, chronic condition of reproductive-aged women, characterized by extrauterine implants of endometrial epithelial and stromal cells, which can result in significant pain and are associated with infertility. Accumulating evidence underscores the significance of mechanical stress, such as from myometrial movement during menstruation, in the pathophysiology of endometriosis. Using an experimental device to stretch cultured endometrial stromal cells, Harada et al[94] showed that mechanical stress induced expression and secretion of the inflammatory mediator interleukin 8 (IL-8) from these cells. Stretch also induced ERK1/2 phosphorylation, and PD98059, an ERK1/2-specific inhibitor, significantly inhibited the stimulatory effect of stretch on IL-8 secretion, suggesting that stretch may work via phosphorylation pathways to promote inflammation. Additionally, progesterone significantly suppressed the stretch-induced secretion of IL-8 from endometrial stromal cells, whereas estradiol did not have any significant effect. These findings imply that mechanical movement of the uterus may induce biochemical signals in the endometrium leading to the creation of a proinflammatory environment, which is counteracted by progesterone.

Since endometriosis is an inflammatory condition, uterine contraction-induced IL-8 secretion from the endometrium may be involved in the pathogenesis of this disease. In fact, various investigators have observed IL-8 elevation in the peritoneal fluid of women with endometriosis, and the levels correlate with severity of disease.[95,96] Interleukin 8 is a cytokine that induces chemotaxis of neutrophils, is a potent angiogenic agent, stimulates cellular proliferation, and promotes endometrial cell adherence via an integrin-mediated mechanism (reviewed in[97]). Interestingly, IL-8 receptor expression is higher in the eutopic endometrium of women with endometriosis compared to the endometrium of women without endometriosis,[98] suggesting that there may be a difference in the endometrial cells themselves which predisposes a woman to endometriosis. Progesterone, on the other hand, suppresses uterine contraction-induced inflammatory responses in the endometrium, which may explain 1 mechanism behind the protective role of this hormone in endometriosis.

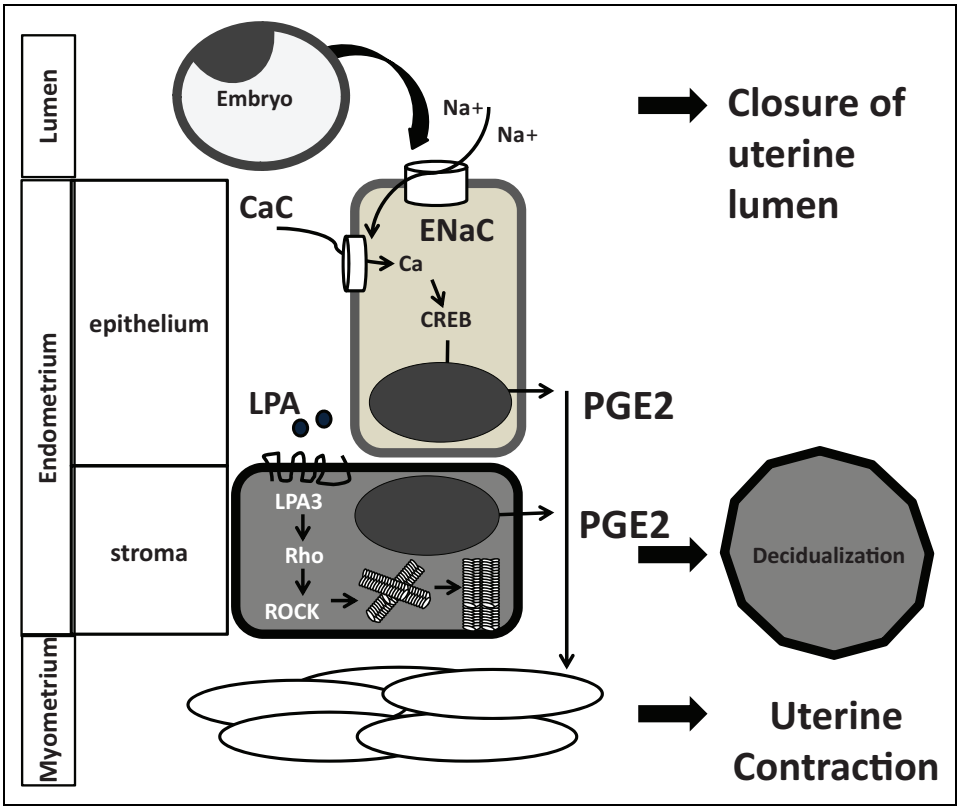

Figure 3. The role of mechanical signaling pathways in embryo implantation. Mechanical stress caused by the presence of the embryo, as well as by uterine contractions, leads to the activation of epithelial sodium channels (ENaCs) and downstream production of prostaglandin E2 (PGE2). Soluble LPA molecules signal via LPA3 receptors and downstream Rho/Rho-associated protein kinase (ROCK)/MLP to direct actin organization into stress fibers, promoting stromal cell decidualization. Additionally, LPA signaling also directs prostaglandin production and release. Prostaglandins promote stromal cell decidualization, further uterine contraction, and closure of the uterine lumen, all of which are necessary for successful implantation. LPA indicates lysophosphatidic acid.

Ovary

Ovarian Physiology: Follicular Development and Ovulation

Folliculogenesis. While the hormonal regulation of folliculogenesis has been widely investigated, the role of MS in this process has received less attention. There is growing evidence that the 3-dimensional architecture and physical properties of the ovarian ECM, such as its mechanical stiffness, play a critical role in follicle development by directly impacting cellular proliferation, differentiation, and growth factor production and diffusion.

Primordial (dormant) follicles are present at birth and are composed of a meiotically arrested oocyte surrounded by nondividing, squamous pregranulosa cells, encased in a basal lamina. The follicles are confined to the collagen-rich ovarian cortex, which offers a rigid physical environment in comparison to the medulla.[99] In vitro studies have shown that this rigid physical environment supports follicular architecture, leading to increased survival: primate primordial follicles cultured in 2% alginate maintained their integrity for longer and exhibited optimal morphology compared with follicles cultured in 0.5% alginate.[99] On the other hand, the rigidity of the cortical ECM may also limit expansion of the follicle and consequently oocyte maturation, maintaining the follicle in its quiescent state.[100] Thus, the ovarian cortex may be characterized as a "less permissive" region of the ovary.

Throughout a woman's reproductive life span, a subset of follicles is recruited each cycle and enters the growing follicle pool. Little is known about what triggers a dormant follicle to enter the follicular cycle. Follicle-stimulating hormone and luteinizing hormone (LH) are unlikely candidates for this role, as primordial follicles lack gonadotropin receptors.[101] Rather, signals intrinsic to the ovary may trigger follicle activation. It has been demonstrated that genes involved in the PTEN/P13K/Akt/FOXO3 signaling pathway are important for regulating primordial follicle activation.[102] Specifically, the TF Foxo3 may function as a suppressor of follicular activation; its conditional deletion in knockout mice led to increased follicular activation and premature ovarian failure.[103] Since expression of P13K and Akt is mediated by mechanical loading,[49] it is interesting to speculate whether mechanotransduction may contribute to the initial trigger for follicular activation.

Whatever the initial signals that activate growth, as a follicle migrates to the medulla of the ovary, it encounters a softer, more permissive biomechanical environment. Secondary and

larger follicles encapsulated and cultured in 0.25% alginate grow larger, produce more hormones, and have better quality gametes compared with those grown in higher alginate concentrations.[104,105] Although a stiff environment favored high progesterone and androgen secretion, decreasing alginate stiffness resulted in estrogen production, which exceeded progesterone and androgen accumulation.[105] This is more reminiscent of the hormonal milieu of a healthy, growing secondary follicle.

In an effort to recreate the complexities ovarian microenvironment, researchers have developed artificial matrices, or hydrogels, made up of interpenetrating networks of fibrin and alginate (FA-IPN) with dynamic, cell-responsive mechanical properties.[106] Although older alginate-only hydrogels, which are nondegradable, became too rigid to support follicle growth as the follicle expanded, the fibrin component in FA-IPN hydrogels degrades over time, thereby changing the matrix to a softer, more permissible substrate for follicular growth. Thus, FA-IPN hydrogels mimic, in a temporal fashion, the spatial migration of a follicle from stiff ovarian cortex to soft medulla. The FA-IPN gels also allow for bidirectional signaling between matrix and cell. Cells themselves produce their own ECM components, which are incorporated into the alginate scaffold. Additionally, the very process of fibrin degradation is driven by soluble factors released by granulosa and thecal stromal cells, notably plasminogen activator (PA).[107,108] The results of implementation of the FA-IPN matrix are promising: the rate of meiotically competent oocytes produced by culture in FA-IPNs was between 75% and 82%,[106] in comparison to 67% with the prior alginate-only system.[109] Thus, the growing understanding of the importance of MS in follicular development has already began to be translated into advances in tissue bioengineering, with important implications for the field of reproductive technology.[110]

Ovulation. Ovulation—the expulsion of an egg from a mature follicle—is a complex process. It is triggered by the LH surge, which in turn stimulates multiple biochemical, biophysical, and morphological changes in the follicle that ultimately result in rupture. For rupture to occur, there must either be a decrease in the tensile strength of the follicle wall or an increase in the pressure inside the follicle—or, as is the case, a combination of these events. Follicular rupture is caused in part by decreased tensile strength at the follicular apex due to degradation of collagen fibers of the ECM. In addition, changes in intrafollicular pressure occur during the ovulatory process and such changes facilitate ultimate rupture of the weakened follicle.[111]

The surge in LH that occurs prior to ovulation induces a wide variety of biochemical cascades, which include synthesis and secretion of progesterone, PGs, cytokines, and proteolytic enzymes.[112] Under the influence of these mediators, the ECMs of the tunica albuginea and theca externa, especially at the follicular apex, as well as the basement membrane (BM) separating the granulosa and thecal cell layers, become fragmented and disorganized as collagen disintegrates. The process of ECM degradation is accomplished by a variety of remodeling enzymes, including matrix metalloproteinases (MMPs), PAs and plasmin, and ADAMTS.

Inflammatory mediators promote these apical morphological changes. Studies have shown that inhibition of PGs with indomethacin block ovulation,[113] and ovulation in cyclooxygenase 2 deficient, anovulatory mice can be restored by treatment with PGE2.[114] Additionally, tumor necrosis factor α is a cytokine that is synthesized as an integral membrane protein and can be liberated by plasmin or an MMP. Tumor necrosis factor α may have a dual role of promoting breakdown of collagen fibrils and apoptosis of ovarian superficial epithelial cells in the apical region of preovulatory follicle.[115]

As the follicular wall beings to thin, the pressure inside the follicle rises. Recent studies in equine chorionic gonadotropin–human chorionic gonadotropin primed rats using highly sensitive measurements of very minute, and rapid changes have enabled researchers to accurately assess intrafollicular pressure. In these studies,[111] the basal intrafollicular pressure at the preovulatory phase was shown to increase gradually throughout the ovulatory process, peaking at the late ovulatory phase—this difference was significant. In parallel, the wall tension index, which is calculated taking into account follicular size and wall thickness, increased even more markedly from preovulatory to mid and late ovulatory phases. Additionally, short-term pressure peaks were evident during the mid and late ovulatory phases. These peaks are thought to represent ovarian contractility, and the structures responsible for this spontaneous contractility are most likely the smooth muscle cells (SMCs) found in the theca externa.[116,117]

There is evidence that PGF-2α and bradykinin may be involved in the mechanism underlying the increase in intrafollicular pressure. Increases in these 2 mediators coincide with increases in intrafollicular pressure and contractions.[111] Mechanistically, inflammatory mediators are vasodilatory and thus promote fluid transudation from leaky capillaries, increasing levels of follicular fluid and ultimately pressure. Additionally, the increased colloid osmotic pressure in the enzymatic degradation of follicular fluid mucopolysaccharides further promotes fluid entry into the follicle.

Finally, endothelin 2 (EDN-2)-mediated contraction may be a final mechanical signal facilitating ovulation. Endothelin 2 is transiently expressed in granulosa cells immediately prior to ovulation.[118] Endothelin 2 from granulosa cells diffuses to theca and SMC layer upon degradation of the BM, where it binds to EDN receptors (specifically subtype A[119]) on follicular wall SMCs. Contraction of individual SMCs results in follicular constriction, which increases follicular pressure and generates tension in the follicle wall. Eventually, the follicle ruptures at the apex where the tensile force is weakest due to the lack of SMCs and low structural integrity. This process is summarized in Figure 4.

Ovarian Pathology: Polycystic Ovarian Syndrome

Polycystic ovarian syndrome (PCOS) is characterized by insulin resistance, clinical or biochemical signs of androgen excess,

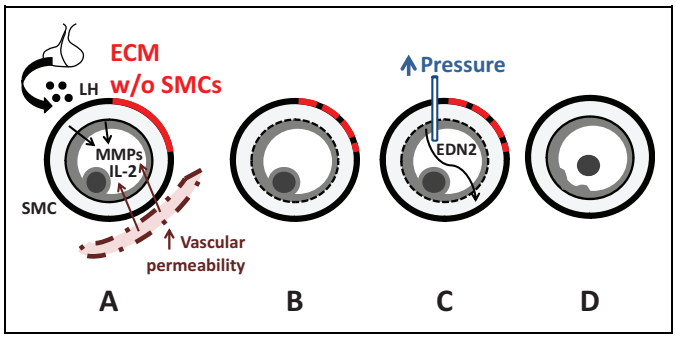

Figure 4. Mechanical signaling processes involved in follicle development and oocyte maturation. A, The luteinizing hormone (LH) surge results in increased transcription of genes involves in proteolysis, inflammation, vascular permeability, and contraction. B, Weakening of follicular wall and of basement membrane (BM) separating granulosa and theca layers. C, Diffusion of endothelin 2 (EDN2) to theca externa layer results in contraction of smooth muscle cell (SMC) and further increases tension in follicle wall. D, Rupture of the follicle and expulsion of the oocyte ensues.

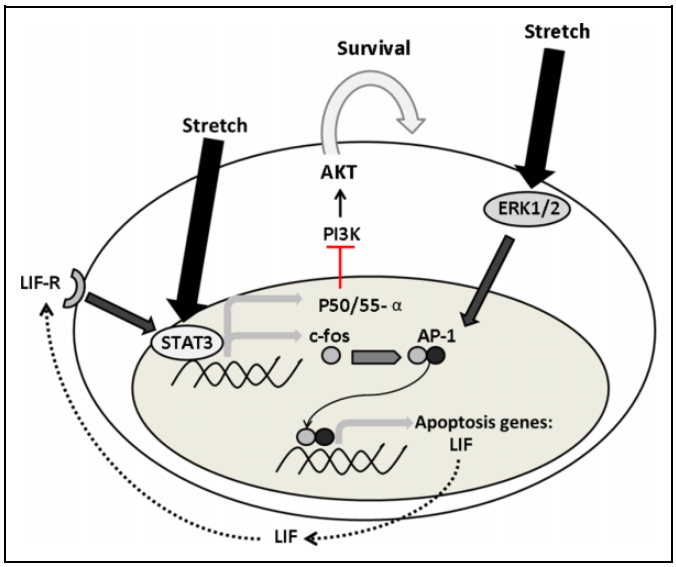

Figure 5. Mammary cell involution in response to stretch. Mechanical stretch imposed on mammary alveolar cells by milk stasis secondary to weaning activates the mitogen-activated protein kinase extracellular signal-regulated kinase 1/2 (ERK1/2) and induces expression of the mechanosensitive transcription factor STAT3. Downstream of these molecules, increased expression of the transcription factor c-fos and its association into AP-1 dimers results in the expression of proapoptosis genes. One such gene is leukemia inhibitory factor (LIF), which feedbacks on the cell to promote further activation of STAT3, thereby perpetuating the apoptotic signaling pathway. Moreover, STAT3 activates 2 regulatory subunits of PI3K (p50-α and p55-α), which in turn inhibits AKT phosphorylation. Phosphorylated AKT provides a critical cell survival signal that must be silenced during mammary cell involution. AKT indicates protein kinase; STAT3, signal transducers and activators of transcription 3.

and polycystic ovaries. Histologically, PCOS ovaries possess a thick cortex that is densely collagenized.[120] Consistent with the importance of the physical properties of the ovarian ECM in follicular development, it has been hypothesized that this relatively dense cortex may create a biomechanically nonpermissive environment that could contribute to the disease phenotype.[100] Additionally, the follicles found in a PCOS ovary are usually small, accumulate in the cortex, and secrete high levels of androgen and relatively low levels of estrogen.[100] This finding is paralleled in in vitro follicular cultures, where follicles grown in stiffer and denser substrates secrete higher levels of androgen and progesterone and relatively low levels of estrogen,[105] further supporting a role of MS in PCOS pathogenesis.

In addition to impaired folliculogenesis, PCOS is also associated with anovulation. D'Ascenzo et al[121] observed reduced levels of MMPs in women undergoing assisted reproductive technology compared with levels from normally ovulating women. On the other hand, Tarkun et al[122] examined levels of the plasmin activator inhibitor 1 (PAI-1), which counteracts plasmin-induced proteolysis by blocking plasminogen conversion to plasmin, in women with PCOS and found increased concentration and activity of PAI-1 in these women. In combination, these results suggest that women with anovulatory infertility, including PCOS women, may have a relative inability to degrade the ECM due to deficiency in proteolytic enzymes and/or excess of proteolysis inhibitors.

Breast

Breast Physiology: Mammary Gland Involution

The mammary gland must be able to respond to multiple biochemical and biophysical stimuli in order to fulfill its reproductive functions during pregnancy, lactation, and weaning. Postlactational involution, in particular, is divided into 2 phases. The first begins hours after weaning, when milk stasis induces the expression of local factors in the alveolar epithelium, such as leukemia inhibitory factor (LIF), that trigger apoptosis.[123,124] Several days later, there is a decrease in the circulating levels of the hormone prolactin, which initiates tissue remodeling of the mammary gland. It has been proposed that cell stretching caused by milk stasis and accumulation secondary to weaning might be the earliest stimulus that triggers the expression and release of these phase I local factors, thereby initiating the remodeling and involution of the mammary gland, well before prolactin levels decrease.[125] HC11 nontumorigenic mammary epithelial cells cultured in flexible silicone membranes and subjected to radial mechanical strain exhibited a phenotype associated with involution, including ERK1/2 phosphorylation, increased levels of c-fos mRNA and protein expression, LIF expression, signal transducers and activators of transcription 3 (STAT3) activation, and AKT phosphorylation inhibition.[126]

The mechanism by which these diverse molecules promote apoptosis is dynamic and complex (Figure 5). The MAPK ERK1/2 is phosphorylated by mechanical stretch and, in turn, translocates to the nucleus to phosphorylate TFs, notably c-fos—a monomeric component of the AP-1 family of dimeric

TFs.[127,128] By controlling the expression of genes involved in cell fate decisions (apoptosis vs cell survival), AP-1 proteins can be either oncogenic or antioncogenic, depending on dimer composition as well as on the cellular and genetic context.[129] In mammary cells undergoing involution, AP-1 regulates the expression of apoptosis genes.

The mechanosensitive TF STAT3 may also promote involution through at least 2 mechanisms. First, STAT3 also upregulates transcription of c-fos, leading to AP-1 generation and downstream expression of proapoptosis genes.[130] Additionally, STAT3 induces expression of the PI3K-regulated subunits p55-α and p50-α, which function to inhibit activation of PI3K/AKT.[131] Phosphorylated AKT provides a critical cell survival signal that must be silenced during mammary cell involution. Consistent with a role for AKT downregulation in mammary gland involution, activated AKT transgenic mice showed significant delay in the involution process.[132] Finally, a second wave of STAT3 activation may also be due to LIF secreted by the stretched cells, thereby perpetuating the apoptotic signaling pathway.[126]

Breast Pathology: Breast Cancer

Extracellular matrix rigidity induces a malignant phenotype in mammary epithelial cells.[50] Rather than arranged in relaxed nonoriented fibrils, the collagen in breast tumors is often highly linearized and either oriented adjacent to the epithelium or projecting perpendicularly into the tissues.[133,134] Such stiffness of the ECM upregulates integrin signaling and promotes focal adhesion assembly. Focal adhesion kinase is recruited to and activated by the adhesion complex and in turn activates Rho/ROCK, ERK, and P13K/Akt signaling pathways. These pathways act to rearrange the actin cytoskeleton into stress fibers, which promote cell contractility, migration, and proliferation.[50,134,135]

Thus, the breast cancer cell is able to sense and respond to the stiffness of its substrate via mechanotransduction pathways involving integrins, focal adhesion molecules, and downstream activation of phosphorylation pathways. But what makes the tumor ECM stiff? Several lines of research have indicated that cancer cell hypoxia may be partly responsible for ECM remodeling in tumors. Hypoxia increases expression of lysyl oxidase (LOX) enzyme, which cross-links collagen, thereby increasing tissue stiffness and angiogenesis.[136] Importantly, LOX upregulation is seen in breast cancers and correlates with poor prognosis.[137]

Endocrine signals mediate breast cancer cell behavior through similar transduction pathways. For instance, hormone-bound progesterone receptor activates the tyrosine kinase c-Src, which in turn activates PI3K/Akt and RhoA/ROCK pathways, and leads to FAK phosphorylation and increases the formation of FA complexes, thereby initiating the same cycle of actin cytoskeletal rearrangement and enhancement of cell migration.[138] Additionally, progesterone receptor themselves may be phosphorylated to an overactive form by MAPKs, further connecting the biophysical and biochemical signaling pathways that underlie cancer cell biology.[139]

Concluding Remarks

Mechanical signaling is critically important for the essential, impressive plasticity of the female reproductive tract. Myometrium, cervix, endometrium, ovary, and breast tissues are all subject to a myriad of mechanical forces—whether from fetus, embryo, follicle, or milk accumulation—that they must sense and respond to in order to ensure proper reproductive functioning. We have reviewed many of the essential players in the process of *mechanotransduction*: forces received by the ECM are conveyed to cells via the activation of integrins and associated focal adhesions, G-protein-coupled receptors, and ion channels on the plasma membrane, leading to downstream activation of signaling cascades (prominently Rho/ROCK, MAPK, and P13K/Akt pathways) and ultimately changes in cytoskeletal organization and gene transcription, which produce the biologic cellular responses. Notably, rearrangement of the actin–myosin cytoskeleton into stress fibers in response to mechanical forces produces cellular tension and promotes contractility, motility, and survival. Increased expression and secretion of inflammatory mediators, especially PGs, also promote a contractile phenotype.

A key concept in MS is the importance of ECM–cellular interactions, which are bidirectional. The ECM generates and conveys physical and chemical signals to cells. The cells themselves respond to these cues by producing the major proteins that make up the ECM and the enzymes that degrade it. Another point of integration involves cross-talk between soluble and MS. In particular, MS pathways are subject to modulation by sex steroids, which, of course, are critical players in reproductive tissues. One, for example, is AKAP13, which mediates sex steroid receptor activity and is essential for osmotic stress-stimulated NFAT5, a TF, needed for intracellular osmolarity.[140] Overall, the mechanisms that integrate biophysical and biochemical signals are interrelated and conserved across these tissues.

Finally, changes in the ECM and/or in the signaling pathways that sense and respond to it are important pathologically. Alterations in mechanotransduction are involved in a wide range of obstetric and gynecologic pathologies, among which are featured preterm birth, cervical incompetence, endometriosis, PCOS, and reproductive tract neoplasms including leiomyomata and breast cancer.

Biologists are beginning to understand the complexities of cell–matrix interactions in the reproductive tract that are responsible for mechanical forces that lead to chemical signaling within and without the cell. As the biochemical cascades triggered by mechanical stimuli in these tissues are studied in the near future, our understanding of normal reproductive physiology and of reproductive tract pathogenesis will increase and enable the development of new therapeutic approaches to disease and new means to enhance normal physiology.

Authors' Note

Soledad Jorge and Sydney Chang contributed equally to this article.

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