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Minireview

Dynamic Reciprocity Between Cells and Their Microenvironment in Reproduction[1]

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ABSTRACT

Dynamic reciprocity (DR) refers to the ongoing, bidirectional interaction between cells and their microenvironment, specifically the extracellular matrix (ECM). The continuous remodeling of the ECM exerts mechanical force on cells and modifies biochemical mediators near the cell membrane, thereby initiating cell-signaling cascades that produce changes in gene expression and cell behavior. Cellular changes, in turn, affect the composition and organization of ECM components. These continuous interactions are the fundamental principle behind DR, and its critical role throughout development and adult tissue homeostasis has been extensively investigated. While DR in the mammary gland has been well described, we provide direct evidence that similar dynamic interactions occur in other areas of reproductive biology as well. In order to establish the importance of DR in the adaptive functioning of the female reproductive tract, we present our most current understanding of DR in reproductive tissues, exploring the mammary gland, ovary, and uterus. In addition to explaining normal physiological function, investigating DR may shed new light into pathologic processes that occur in these tissues and provide an exciting opportunity for novel therapeutic intervention.

breast, dynamic reciprocity (DR), extracellular matrix (ECM), fibroids, folliculogenesis, mechanotransduction (MT), ovary, ovulation, pathogenesis, uterine leiomyoma

INTRODUCTION

Throughout each reproductive cycle, as well as throughout life, the female reproductive system undergoes extensive and dynamic structural remodeling [1–4]. There are complex biochemical signals that initiate and regulate remodeling that affect both the extracellular matrix (ECM) and the cell, resulting in tissue organization. This impressive dynamism is achieved by changes in the ECM that lead to mechanotransduction (MT), the cellular processes that translate mechanical stimuli into biochemical signals, as well as soluble biochemical signaling through hormones and cytokines, allowing cells to adapt to their changing physical environment. These rapid, transient cell-cell and cell-matrix interactions are bidirectional and referred to as dynamic reciprocity (DR) [5–7]. Mammoto and Ingber [8] as well as Mammoto et al. [9] substantiated this concept in developmental biology, proving the role of mechanical force is as critical as biochemical signaling in embryogenesis, thereby transforming how we view the extracellular environment. Mechanical signals are relayed through the membrane and cytoskeleton to the nucleus by integrins, cell adhesion molecules, cytoskeletal filaments, and signaling cascades resulting in changes in gene transcription and chromatin remodeling [10]. Furthermore, cell-cell and cell-matrix interactions create intracellular contractile forces that place the cell in a state of tension and can act to modify cell form and function [11]. In this manner, cellular mechanochemical processes and changes in the ECM microenvironment govern tissue morphogenesis and adult organ homeostasis.

Bissell and colleagues [5, 12, 13] extrapolated a role for DR in reproductive biology by studying normal mammary gland development and the progression of cell events leading to malignancy. In addition, a number of earlier studies indicated that stretching uterine tissues induced protein synthesis and changes in cellular function and was significant in parturition, validating a role for mechanical signaling in these tissues and demonstrating how it contributed to cell form and behavior [14–16]. More recently, there has been extraordinary expansion in the field of matrix biology that has led to new insights into DR and reproduction [9, 17–20]. Biochemical signaling alone is not sufficient to explain the complexities that occur in development and function of the breast, ovary, and uterus, and there is now persuasive evidence for the critical role of mechanical signaling in these tissues [21, 22]. Through further advancement and integration of our understanding of mechanochemical transduction events, we can gain valuable insights into both normal and tumorigenic behavior of cells, tissues, and organs and develop effective interventions for reproductive tract functions and disease. Toward this goal, this is the first review to focus on the significance of DR in the reproductive

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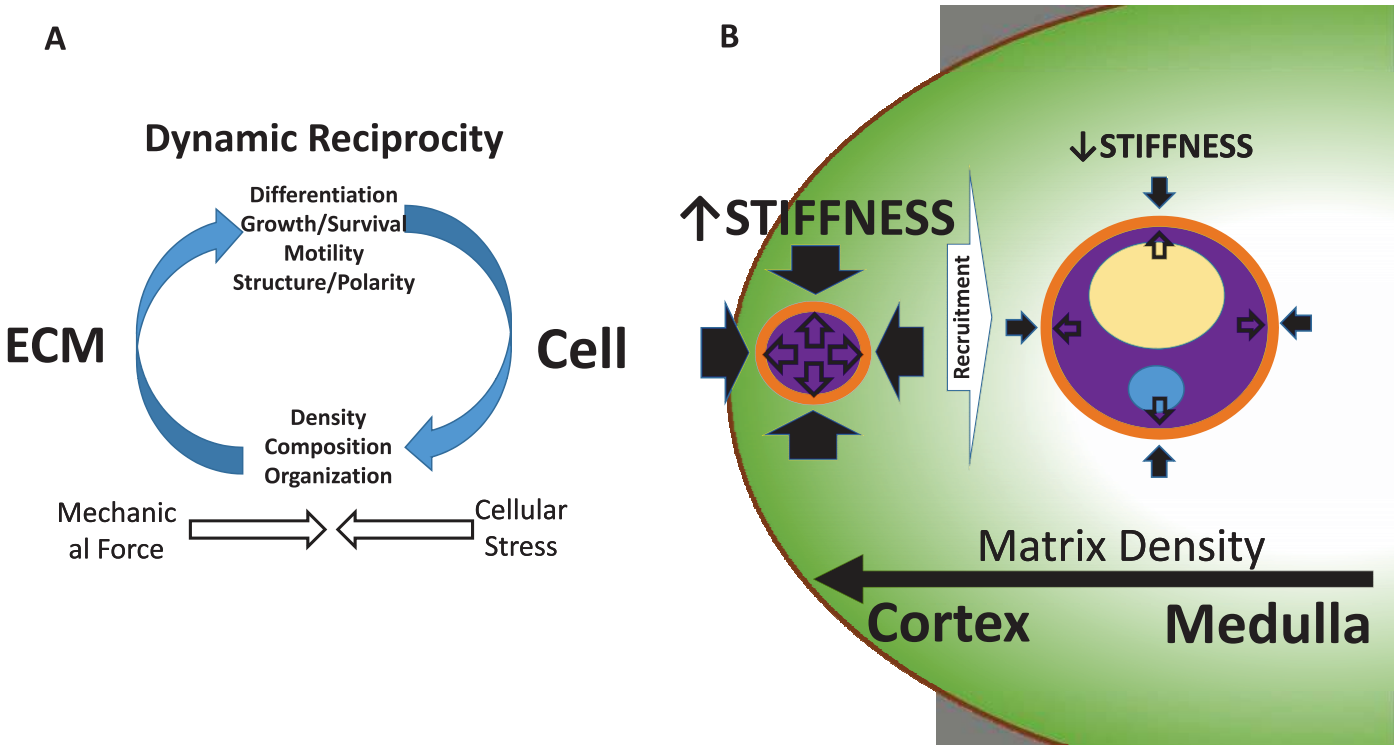

FIG. 1. **A**) Schematic diagram of the bidirectional interaction between the cell and its environment, specifically the ECM, demonstrating the concept of DR. Mechanical force from the ECM is sensed by the cell and leads to changes in cell structure and function. These changes, in combination with mechanical signaling, can alter gene expression and epigenetic remodeling of the cell nucleus, leading to changes in ECM content, composition, and organization and an overall remodeling of the matrix. Cells sense the mechanical force and counter it with intracellular contractile tension, creating cellular stress. In this manner, force-generated mechanochemical signaling affects both the cell and its environment. **B**) An example of DR in folliculogenesis. Mechanical forces (closed arrows) in the stiff outer ovarian cortex act on the primordial follicle, contributing to its quiescent state. Tensional forces (open arrows) within the follicle counter this. Recruitment to the more pliable inner medulla relieves the mechanical strain and permits the follicle to proceed through folliculogenesis. Factors that determine progression from the cortex to the medulla are unknown. Arrow size is proportional to the amount of perceived force.

system. An overview of MT, a concept critical to appreciating DR, will be discussed first. We next explore the mammary gland where a more comprehensive picture of DR has been demonstrated and highlight those studies that have contributed most to our appreciation of this concept. We then review other reproductive organs where an appreciation of DR is emerging, focusing on the processes of folliculogenesis, ovulation, and uterine fibroids (leiomyomas).

THE PROCESS OF MT

In the broader field of cell biology, Ingber and colleagues [23, 24] described the importance of the cell cytoskeleton in mechanical signaling and developed the concept of tensegrity (tensorial integrity) to describe how this signaling exhibits the properties of an integrated system. They demonstrated that mechanical strain placed on integrins, located in the cell membrane, immediately changed the shape and organization of the nuclei, demonstrating a MT process dependent on intermediate filaments and microfilaments [25, 26]. Integrins are not static proteins but are expressed transiently on the cell surface in a dynamic fashion and are involved in bidirectional signaling from the cell to its microenvironment [27, 28]. Mechanical forces drive tissue homeostasis through cell-cell junctions and cell-matrix adhesions mediated by integrins [8]. These forces can be quite small, resulting in compression or stretch, external to the cell, such as movement or gravity or generated through changes in cell contractility or shape. Force changes are sensed or perceived by the cells as signals (transduction) that produce changes in intracellular biochemistry and ultimately gene expression and chromatin remodeling [9].

Many molecular pathways are signaled by the mechanical forces exerted on the cell. Force transmitted across cell surfaces by integrins is relayed to the cell cytoskeleton by focal adhesions, an anchoring complex that functions as a mechanochemical-signaling center [26]. This signaling complex can transmit both internal and external forces and will assemble or separate depending on the presence of stress [29, 30]. Other integrin interacting-signaling molecules, including TRPV4 and talin, undergo conformational changes to mediate downstream signals [31, 32]. Mechanical stress acting through focal adhesion kinase (FAK), a nonreceptor kinase in the cytoplasm, activates the mitogen-activated protein kinase (MAPK) pathway, leading to upregulation of collagen type I and other critical ECM proteins that are involved in matrix composition and remodeling [33–35]. Annexin A2, a multifunctional bridging protein, conducts bidirectional informational flow and is regulated by changes in the ECM and intracellular calcium flux [36]. Thus MT, one aspect of DR, is a dynamic process with many critical signaling factors responding to mechanical force that play a role in the cell. In this manner, all components of the system, both biomechanical and biochemical, and not merely one paramount molecule, influence cell behavior (Fig. 1A).

DYNAMIC RECIPROCITY IN THE MAMMARY GLAND: INSIGHTS INTO NORMAL ANATOMY AND PHYSIOLOGY

The mammary gland is an excellent model organ for the study of how ECM remodeling contributes to tissue morphogenesis and functional differentiation [37, 38]. The gland's function is regulated by reproductive hormones during pubertal development and again in gestation and parturition. Then during lactation and involution, the mammary gland undergoes cycles of branching and formation of acini (also called alveoli). In addition, the surrounding epithelium and myoepithelium ECM is subject to continual assembly and degradation. A cell's cytoskeleton mediates a dynamic and reciprocal integration of tissue architecture and function as well as directs mammary gland development, tissue polarity, and tissue-specific gene expression. Pathology and abnormal development occurs when this interaction is dysregulated [19].

The mammary gland is a complex organ with many cell types from fibroblasts to adipocytes to epithelial cells. The epithelial cells are characterized as being of two types: luminal epithelial cells and myoepithelial cells, both embedded in an interstitial collagen network [38]. These epithelial cells form a branching network of ducts terminating in many spherical small lobules called acini. Polarized luminal epithelial cells generate a continuous layer lining each duct and acini and will eventually make and secrete milk proteins. The basal layer of luminal epithelium is composed of a discontinuous layer of myoepithelial cells and a thin basement membrane consisting of laminin [38], structural proteins that connect to cytoskeletal filaments and the nucleus through intermediate filaments and microfilaments [25].

During mammary gland development, ECM proteins are tightly regulated and expressed [39, 40]. In addition to laminin, collagen, entactin, and proteoglycans constitute the basal lamina and establish cell polarity in acini differentiation [41]. Collagen type I is observed along mammary ducts while collagen type IV and the laminins type I and type V are expressed around acini [42, 43]. Upon weaning, involution results in degradation and remodeling of these proteins and leads to a decrease in milk protein production [44]. Fibronectin content increases during ductal morphogenesis as well as expression of the fibronectin binding $\alpha5\beta1$-integrin in mammary epithelial cells [45, 46]. Loss of fibronectin expression prevents proper gland development [47]. Fiber alignment is also important for appropriate development. Spatially aligned collagen fibers are observed in the terminal end buds prior to fat pad invasion, and a recent study using live cell imaging demonstrated how fiber alignment can affect epithelial cell morphology [48–50].

Remodeling of the external environment requires proper spatiotemporal expression of matrix metalloproteinases (MMPs) [51]. During development, MMP-2 plays a role in the initial invasion of epithelial cells into the stromal fat pad and suppresses lateral ductal branching [52]. In contrast, MMP-3 promotes branching and MMP-3 knockout mice revealed a diminished branching pattern compared to wild type [52]. MMP-14 is highly expressed in the terminal end bud and may assist MMP-2 in ductal invasion [53, 54]. Interestingly, in three-dimensional (3D) culture, there was higher recruitment activity of MMP-14 in a stiffer collagen environment, demonstrating cell-matrix cross talk; furthermore, MMP-14 may activate MAPK signaling [53, 54]. In addition, MMP activity can directly affect intracellular signaling by producing degradation fragments of ECM proteins that act as growth modulators, for example, EGFR is activated by laminin-5 following cleavage by MMP-2 [55].

In the human breast, integrins play major roles in development and function as well as in cancer progression [17, 41, 56, 57]. The $\beta1$-integrin, in conjunction with prolactin signaling, is necessary for mammary cell differentiation [58, 59]. The $\alpha6\beta4$-integrin associates with hemi-desmosomes linking the plasma membrane with intracellular intermediate filaments that form a network along the basolateral aspect of the cells, establishing cell polarity [60, 61]. The $\alpha6\beta4$-integrin has further been demonstrated to affect matrix remodeling by promoting SPARC expression [62]. In addition, both $\beta1$ and $\alpha6\beta4$ have also been linked to β-casein production [63]. Loss of $\beta4$ signaling has been shown to lead to an increase in apoptosis, demonstrating a role in cell survival. In support of this, certain integrins, including $\alpha2$, may also serve as tumor suppressors, and levels of the $\alpha2\beta1$-integrin are reduced in aggressive breast cancers [64].

Obviously, estrogen and progesterone are key players in mammary gland development. Investigations in transgenic mice clearly show that the estrogen receptor (ER) is necessary for elongation of the mammary ducts during puberty [65]. Other studies demonstrate that the progesterone receptor (PR) is required for the growth of acini [66]. In the adult rodent and in human glands, the distribution of ER and PR fluctuates due to changes in estrogen and progesterone during reproductive cycles, pregnancy, lactation as well as age [67]. ER is present in the mammary gland in both isoforms, ERα and ERβ [68]. Basement membrane laminin type 1 and collagen type IV are involved in the maintenance of ERα expression, and in malignant breast cells this becomes disrupted, and the cells are no longer responsive to the ECM [69].

Over the last several decades, scientists have studied the interactions between the mammary cells and the ECM in 3D cultures that successfully mimic the in situ mammary gland [70–72]. When grown on 3D gels, murine and human epithelial cells are able to form aggregates and reorganize into structures of morphologically polarized cells that form acini-like hollow spheres surrounded by basal lamina [73, 74]. This apical-basal polarity is established by the ECM component laminin [75]. The presence of fibronectin can stimulate epithelial cell proliferation and increase acini size [76–78]. Strikingly, in the presence of lactogenic hormones, these mammary cells secrete caseins into the lumina [73, 74]. Furthermore, these interactions can occur in the absence of surrounding myoepithelial cells [70]. In contrast, cells grown in 2D monolayers or 3D collagen cultures without lactogenic hormones do not form acini-like structures and fail to secrete milk proteins [72, 79]. Interestingly, β-casein expression was also inhibited when cells were grown on stiffer gel substrates [75]. In addition, prolactin signaling is not enough to sustain milk protein production without the interaction of a laminin substrate binding the $\beta1$-integrin, allowing the necessary chromatin remodeling needed for tissue-specific gene expression [75, 80, 81]. Taken together, these studies validate that the ECM can direct tissue polarity and morphogenesis and even affect gene expression and nuclear remodeling. This provides unequivocal evidence that the ECM and the surrounding cells behave as a unit and firmly demonstrates the ECM and its cellular interactions are necessary for mammary gland development and function, substantiating a role for DR in the breast.

CONTRIBUTION OF DR TO MAMMARY TUMORIGENESIS

Dynamic reciprocity typically functions to maintain homeostasis in adult cells; however, an imbalance in the mechano-chemical-signaling network can lead to tumorigenesis. Carcinogenesis in breast correlates with collagen cross-linking and ECM stiffening, creating a firm tumor. This creates mechanical force that the cell senses through integrin activity, leading to focal adhesion formation and activation of RhoA that ultimately alters gene expression patterns and can induce tumor invasion [82, 83]. Mammary cells grown in 3D culture on a stiff collagen matrix lose their normal cell polarity, increase proliferation, and adopt an invasive phenotype [77, 84]. Also, an increase in cellular tension by Rho-mediated cellular contractility leads to changes in matrix content and organization [85, 86]. Interestingly, inhibiting the β1-integrin reversed this phenotype [61, 87]. When lysyl oxidase, the collagen cross-linking enzyme is inhibited, metastatic potential of circulating breast cells was reduced [82]. Increased tumor stiffness led to activation of the micro-RNA miR-18a, which decreased levels of HOXA9-dependent PTEN transcription and promoted the malignant phenotype [88]. In breast cancer biopsies, miR-18a expression was correlated with increasing ECM tumor stiffness and inversely related to levels of PTEN and HOXA9 [88]. Therefore, the tumor matrix may have a profound effect on tumor cell behavior and provides an intriguing therapeutic target to prevent metastasis.

Remarkably, multicellular tissues are capable of biopolymer reorganization by mechanical signals and create very long, highly directional fibers, such as collagen lines, that may influence the location and time of tumor invasion [89–91]. Cultured mammary acini that have disrupted architecture will interconnect by forming these long collagen lines that somehow coordinate and even accelerate a transition to an invasive phenotype [92]. When investigators isolated these acini by laser cuts in the 3D culture, effectively disrupting the collagen lines, the acini reverted to a less invasive cell type [92]. Therefore, pairs and groups of acini can interact mechanically through the collagen matrix, and this matrix, in turn, can influence cell behavior.

MMP activity is linked with breast cancer invasion and metastasis and associated with a poor prognosis [93]. In dense matrices, MMPs will cleave fibers around integrin attachment sites allowing space for cell motility [94, 95]. Aberrant expression of MMP-3 prevented normal cell differentiation and led to adoption of an invasive phenotype [96, 97]. The integrin α3β1 has been linked to MMP-9 activity [98]. Induction of MMP-9 through increased activity of the Raf/MEK/ERK pathway led to targeted degradation of laminin type 1, destroying the basement membrane [99]. This resulted in altered tissue polarity and growth, leading the cells to exhibit a cancer phenotype. This phenotype was reversed by the inhibition of MMP-9 or MEK, and an increase in laminin type 1 was noted in a murine xenograft model [99]. Therefore, cell-generated destruction of the ECM, potentially through integrin activation by the matrix itself, leads to distorted tissue polarity and cell proliferation, mimicking an invasive cancer phenotype. When tissue architecture is continuously perturbed, mammary epithelial cells produce reactive oxygen species and ultimately undergo an epithelial-to-mesenchymal transition [100].

PTEN, a known tumor suppressor gene, colocalizes with the E-cadherin/β-catenin complex in 3D culture and supports acini formation [101]. E-cadherin-blocking antibodies reduce endogenous PTEN protein levels and inhibit cell-cell contact accumulation leading to a loss of cell polarity and growth control [101]. The addition of exogenous E-cadherin to cancer cells lacking the protein induced PTEN expression, supporting a role for cell-cell signaling in mammary gland homeostasis [101].

HoxA1 has been identified as a candidate gene that may serve as a possible driver of early breast cancer, confirmed by its overexpression in human breast lesions [102]. Delivery of lipidoid nanoparticles containing *HoxA1* siRNA through the nipple to mice with breast tumors led to a decrease in tumor formation, and silencing *HoxA1* within the mammary ducts in vivo led to a loss of hormonal expression and suppressed cell proliferation [102]. Strikingly, this phenomenon does not occur when the gene is injected directly into the tumor, suggesting that the dynamic interaction is locally and spatially mediated [102]. In summary, the studies highlighted here strongly demonstrate the role of DR in the mammary gland and that this interaction is responsible for maintaining development and function of the tissue. Also, when the DR-signaling exchange is altered, it can disrupt these homeostatic mechanisms and lead to a progression toward malignancy

DYNAMIC RECIPROCITY IN FOLLICULOGENESIS

There is increasing evidence that the ovarian ECM plays a critical role in follicle development. Primordial (dormant) follicles are localized to the collagen-rich ovarian cortex, which offers a rigid physical environment that supports follicular architecture and increases survival [103]. On the other hand, the rigidity of the cortical ECM limits expansion of the follicle and consequently oocyte maturation, maintaining the follicle in its quiescent state [104]. Throughout a woman's reproductive lifespan, a subset of follicles is recruited each cycle and enters the growing follicle pool. As a follicle migrates to the medulla of the ovary, it encounters a softer, more pliant ECM. This permits the follicle to expand and resume its development. Thus, changes in the stiffness of the ovarian ECM from cortex to medulla directly affect follicular cell behavior (Fig. 1B). The importance of ECM stiffness in folliculogenesis has been shown using in vitro models that recreate the complex ovarian microenvironment by using interpenetrating networks of fibrin and alginate with dynamic, cell-responsive mechanical properties [105]. Whereas older alginate-only hydrogels, which are nondegradable, became too rigid to support follicle growth as the follicle expanded (essentially sequestering the follicle in a cortex-like environment), the fibrin component in fibrin and alginate hydrogels degrades over time, softening the matrix and mimicking, in a temporal fashion, the spatial migration of a follicle from the stiff ovarian cortex to the soft medulla [106].

At the same time, these gels offer an excellent example of DR between matrix and cell. Follicular cells themselves produce their own ECM components, which are incorporated into the alginate scaffold. Additionally, the process of fibrin degradation is driven by soluble factors released by granulosa and thecal stromal cells, notably plasminogen activator [107, 108] and connective tissue growth factor (CTGF) [109]. Furthermore, physical fragmentation of ovaries from juvenile mice promoted follicle growth and led to the formation of mature oocytes through disruption of the Hippo-signaling pathway [110]. Remarkably, women with primary ovarian insufficiency who underwent ovarian fragmentation, Akt stimulation treatment, and autologous transplantation of the remnant tissue generated mature oocytes following in vitro fertilization methods [110]. In one patient, a live birth was achieved. It may be that fragmentation relieves the inhibition of the stiff matrix forces, allowing the residual follicles to

develop. In agreement with this model, anovulatory women with polycystic ovary syndrome have increased number of follicles held quiescent in the ovarian cortex, which is stiffer and contains more collagen compared to normal ovaries [111].

There is also evidence that follicular fluid in follicles is accumulated by the osmotic forces of hyaluronan and versican, which are glycoproteins produced by granulosa cells [112]. Granulosa cells cultured in a 3D environment of collagen type I with leukemia-inhibiting factor were successfully transplanted back into the ovaries of immunodeficient mice and preferentially localized within antral follicles [113]. Thus, the growing understanding of the importance of DR in follicular development has already begun to be translated into advances in tissue bioengineering, with important implications for the field of fertility preservation.

DYNAMIC RECIPROCITY, CYTOKINES, AND OVULATION

Extensive matrix remodeling is paramount for the oocyte to proceed through folliculogenesis, ovulation, and development of a highly vascularized corpus luteum. Ovulation, the expulsion of an egg from a mature follicle, is triggered by the luteinizing hormone (LH) surge, which in turn stimulates morphological changes in the follicle that ultimately result in rupture. Follicular rupture is caused in part by decreased tensile strength at the follicular apex due to degradation of collagen fibers and in part by changes in intrafollicular pressure that facilitate rupture of the weakened follicular wall [114, 115].

The LH surge induces follicular cells to synthesize and secrete proteolytic enzymes, including MMPs, plasminogen activators and plasmin, and ADAMTS [116–120]. Under the influence of these mediators, the ECMs of the tunica albuginea and theca externa, as well as the basement membrane separating the granulosa and thecal cell layers, become fragmented and disorganized as collagen disintegrates. In mice, the process is similar with elevated levels of ECM components (laminin, collagen, perlecan, nidogens) in the basal lamina of developing follicles and corpora lutea, with collagen type IV being the predominant form at all stages of development [121]. Expression of matrix proteins, including HAPLN1, is driven by the LH surge, and deficiencies in key matrix proteins in the cumulus-oocyte complex reduce ovulation rates [122–126]. Mice lacking ADAMTS1 fail to cleave versican, an ECM proteoglycan, and demonstrate reduced rates of ovulation and fertilization as a result of impaired tissue remodeling [127]. Additionally, degradation of ECM by secreted plasmins and MMPs liberates ECM-bound proteins, including TNF-α and TIMP-3 [128]. The cytokine TNF-α resides at the interface of the cell and the ECM and may promote collagen fibril breakdown as well as apoptosis of ovarian superficial epithelial cells in the apical region of the preovulatory follicle [129]. Tissue inhibitors of metalloproteinase (TIMPs) counter the remodeling actions of MMPs and form a delicate balance of remodeling and maintenance [119]. This is a prime example of DR, whereby the cells orchestrate the disintegration of the matrix and in doing so, release cytokines and inhibitors that feed back to further modify cell behavior.

The final signal in ovulation may be endothelin-2 (EDN-2), which mediates smooth muscle cell (SMC) contraction. EDN-2 is transiently expressed in granulosa cells immediately prior to ovulation and is able to reach the SMC layer by diffusing across the thecal layer following carefully timed degradation of the thecal ECM [130, 131]. 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. The complex, back-and-forth choreography thus played out between soluble mediators, mechanical forces, matrix proteins, and shifting fluids is at the core of DR.

DYNAMIC RECIPROCITY IN UTERINE FIBROID GROWTH AND DEVELOPMENT

The hallmark of uterine fibroids is excessive ECM production and cell proliferation. The fibrotic matrix creates a stiff extracellular environment exerting mechanical force on surrounding cells. Transduction of this force ultimately results in a variety of cell responses, including cytoskeletal rearrangement, cell contraction, growth, and gene expression changes, including genes involved in ECM composition, all affecting how the fibroid cells interact with the extracellular environment. Therefore, as we begin to unravel the complexities of fibroid development and function, it has become clear that DR may underlie its growth and development.

The alteration of the ECM is altered compared to the surrounding myometrium is well described [22, 132]. Microarray analysis demonstrated that fibroids have elevated levels of genes involved in ECM formation, including collagen, proteoglycans, and elastin that are associated with growth [133, 134]. Furthermore, there is downregulation of other key ECM proteins, such as dermatopontin [135]. Electron microscopy analysis demonstrated that collagen fibrils are increased, loosely packed, and arranged in a nonparallel manner, not appreciated in nearby myometrium (Fig. 2A) [136]. The excessive matrix deposition and disorganization creates the fibrotic nature of this tissue, leading to an environment of increased mechanical force. TGFβ3, a known promoter of ECM production, is increased in fibroid tissue and also contributes to formation of its abnormal environment [137]. In addition, key proteins known to interact with TGFβ3, including dermatopontin and thrombospondin, have altered expression in fibroids compared to myometrium that may lead to increased TGFβ3 activity [135, 138].

Expression patterns of integrins are critical to understanding how the cell responds to its altered environment. Integrin β1 is overexpressed in fibroid cells and plays a role in determining cell shape and proliferation [139, 140]. Decreased β1 activity is demonstrated to alter cytoskeletal integrity, inhibit cell spreading, and decrease growth. Also, disrupting β1 signaling leads to decreased activity of downstream proteins RhoA and ERK, demonstrating that integrin signals through the Rho family GTP-signaling proteins [140]. Integrin α11 is also elevated in fibroid cells and may play a role in myofibroblast differentiation [141]. Fibroid-derived myofibroblasts contribute to production of the excessive ECM microenvironment and stimulate leiomyoma cell proliferation [135, 142, 143].

Fibroid cells recognize their stiff microenvironment through the process of MT; however, compared to myometrial cell, fibroid cells have a defective perception of mechanical stress and are unable to respond to mechanical cues [144, 145]. The Rho-signaling cascade plays a role in the MT response. Levels of active RhoA and AKAP13, which activates RhoA, are increased in fibroid cells compared to myometrial cells. Inhibition of AKAP13 through siRNA inactivation while simultaneously treatment with lysophosphatidic acid, a known promoter of RhoA, was shown to lead to decreased levels of RhoA compared to myometrial cells [146]. Inhibition with Fasudil of Rho kinase (ROCK), a downstream target of RhoA, led to relaxed contraction of fibroid cells in 3D collagen gels

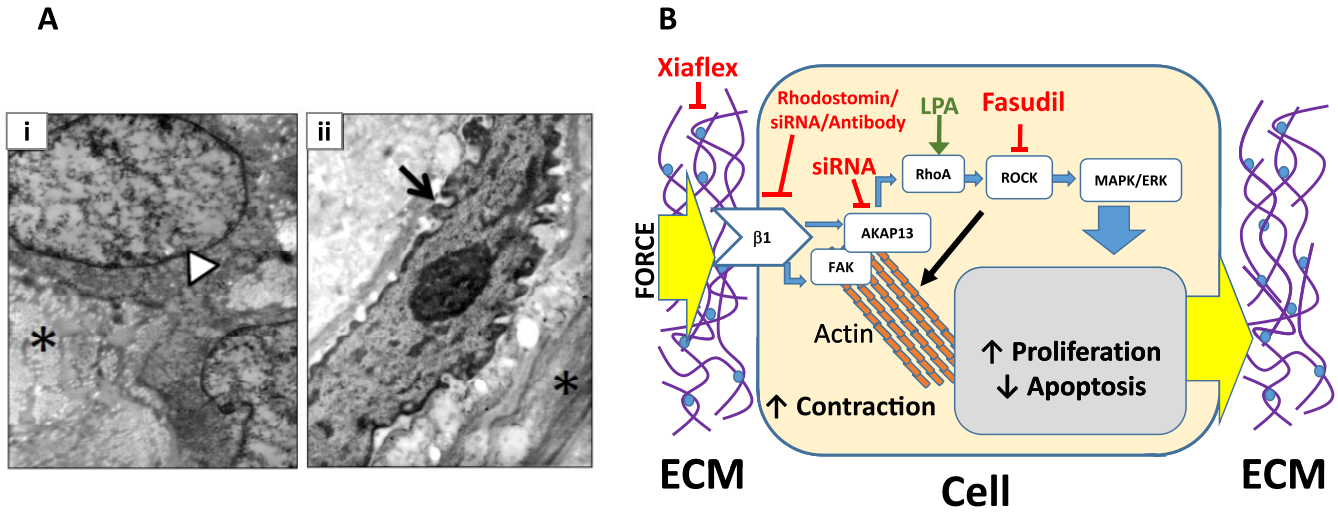

FIG. 2. **A**) Electron microscopy images of myometrial and fibroid tissue. **i**) Normal myometrial SMC with a large, smooth nucleus (white arrow head), surrounded by tightly packed, organized collagen fibrils (asterisk) seen in cross-section. Original magnification ×11 500 (adjusted to magnification ×15 500 for image). **ii**) Image of a myofibroblast from a uterine fibroid. Note the angular and notched nucleus (black arrow) and condensed chromatin within the nucleus. Extracellular matrix features disordered collagen fibrils in the fibroid tissue (asterisk). Magnification ×15 500. **B**) A schematic representation of DR in fibroid cells. Mechanical stress is sensed by the fibroid cell that internalizes the signal and changes how the cell interacts with the ECM, altering the composition and organization of the external microenvironment. Integrin β1 signals the stiffness of the ECM, leading to activation of downstream-signaling events. FAK initiates actin polymerization resulting in cell contraction. AKAP13 activates RhoA, which in turn interacts with ROCK and activates the MAPK/ERK-signaling cascade, resulting in changes in cell proliferation, decreased apoptosis, and upregulation of genes involved in ECM composition and remodeling. Agents that perturb/stimulate the pathway are also listed (see text for explanation).

[147]. In addition, fibroid cells plated on a stiff collagen substrate resembling the fibroid microenvironment demonstrated increased phosphorylation of FAK, decreased levels of p21, and activation of the MAPK-signaling pathway, leading to increased proliferation and altered cytoskeletal organization [148]. Disruption of the dense collagen matrix of fibroid cells with Xiaflex, a collagenase, results in relaxation of the cell and adoption of a phenotype similar to myometrial cells [149]. Furthermore, Fasudil treatment led to a decrease in ECM gene transcripts known to contribute to the fibroid fibrotic environment [147]. Thus, mechanical stress is sensed by the fibroid cell and leads to downstream activation of mechanical-signaling pathways that ultimately affect cell shape and contractility, promote growth, and direct changes in the ECM environment itself (Fig. 2B). This is a clear demonstration of the bidirectional interaction between the fibroid cell and its microenvironment that defines DR.

In addition to mechanical stress, fibroid cells are subjected to osmotic stress and have increased fluid content relative to the myometrium [150, 151]. The Rho-GEF Brx (AKAP13), previously described above in mechanical signaling, plays a key role in transducing the osmotic response through the transcription factor NFAT5, including upregulating osmolarity response genes [152]. Fibroid cells have increased NFAT5 expression compared to nearby myometrium and demonstrate increased expression of hyperosmolarity genes when exposed to osmotic stress [153]. The cellular osmotic response results in fluid exchange between the cell and ECM, thus affecting both cell shape and ECM composition, demonstrating another example of DR [154].

CONCLUSION

This minireview of DR and its impact on the physiologic functioning of female reproductive organs highlights the dynamic state between the cell and its surrounding ECM, leading to cyclic changes in tissue development that characterize the reproductive tract. The concept of DR allows us to avoid the popular emphasis of one molecule or one gene to explain these processes and utilizes a flexible approach that takes into account the signaling interactions that lead to changes in cell shape, tissue architecture, and the microenvironment. While DR in the mammary gland has been the most extensively explored, we provide direct evidence that similar dynamic interactions occur in other parts of the female reproductive tract as well. Our aim in writing this review was to emphasize the critical role of DR in reproduction and stimulate interest toward investigation of this concept in reproductive biology. Studies reviewed here demonstrate the exciting potential of this research to translate into the clinical realm, including fertility preservation in women with primary ovarian insufficiency or definitive management of uterine fibroids without relying on surgical intervention. Therefore, in addition to explaining normal physiological function, exploring DR may shed new light into the pathologic processes that occur in these tissues and provide an inspiring opportunity for novel therapeutic intervention. This pursuit may have powerful implications in the field of reproductive health.

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