Pathophysiology of Pelvic Organ Prolapse

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Pelvic organ prolapse is a prevalent and disabling condition with suboptimal treatment. Multiple mechanisms have been hypothesized as contributors to the development of prolapse, but none fully explain the origin and natural history of this process. Epidemiologic studies indicate that vaginal birth and aging are two major risk factors for the development of pelvic organ prolapse. Other factors include increased abdominal pressure, increased body mass index, and connective tissue disorders. Hence, there is little doubt that pelvic organ prolapse is multifactorial in etiology and may involve more than one pathology to exhibit full anatomic loss of support. Furthermore, loss of support that evolves decades after vaginal delivery may involve an initial insult compounded by loss of support associated with aging. Currently, it is believed that a complex of pathologies are involved in failure of pelvic organ support. These pathologies include those related to genetics, loss of pelvic floor striated muscle support, and loss of connective attachments of the vaginal wall to striated muscles and structures of the pelvis.

In this article, we review the potential mechanisms for loss of pelvic organ support in women and new insights into the role of elastic fibers in the pathophysiology of pelvic organ prolapse.

FAILURE OF PELVIC ORGAN SUPPORT: POTENTIAL MECHANISMS

The pelvic floor comprises several different tissue types that act in concert to provide support and maintain normal physiologic function of the rectum, vagina, urethra, and bladder. All tissue types of the pelvic floor are important for normal support of the...
pelvic organs, and failure of one or more of the tissue support systems may be involved in the pathophysiology of pelvic organ prolapse.

**Levator Ani**

The levator ani is a set of striated muscles comprising three regions. The iliococcygeal portion forms a flat horizontal shelf spanning from one pelvic sidewall to the other. The pubococcygeus muscle arises from the pubic bone on either side, is attached to the walls of the perforating pelvic organs and perineal body, and inserts on the coccyx. The pubococcygeus thereby is important in suspending the vaginal wall to the pelvis. The third portion of the levator ani, the puborectalis, forms a sling around and behind the rectum and extends to the external anal sphincter. Connective tissue covers superior and inferior fascia of the levator muscles. In the healthy state, baseline resting contractile activity of the levator ani muscles elevates the pelvic floor, and compresses the vagina, urethra, and rectum toward the pubic bone, narrowing the genital hiatus and preventing prolapse of the pelvic organs.

It is widely believed that the levator ani muscles sustain either direct or denervation injury during childbirth and that these injuries are involved in the pathogenesis of pelvic organ prolapse. It is hypothesized that nerve injury (due to stretch or compression or both) during the second stage of labor results in partial denervation of the levator ani and, as the denervated muscle loses tone, the genital hiatus opens, thereby leading to prolapse of the pelvic viscera.2–5

Experimental evidence for the relationship between denervation-induced injury of the levator ani and pelvic organ prolapse has been difficult to obtain. For example, although pudendal nerve neuropathy has been associated with pelvic organ prolapse,6 the levator ani muscles are not innervated by the pudendal nerve, but rather by neurons originating from sacral nerve roots S3-S5, which traverse the superior surface of the pelvic floor.7–10 Thus, pudendal nerve injury may not be related to denervation of levator ani muscles. Investigators who have directly assessed levator ani muscles disagree regarding neuromuscular damage in women with pelvic organ prolapse. Some studies demonstrate histomorphologic abnormalities in the levator ani from women with prolapse and stress incontinence.6,11 Other studies fail to find histologic evidence of levator ani denervation.12,13 In addition, full-thickness biopsies obtained from three locations of the levator ani failed to find evidence of atrophy, small-angled fibers, or type grouping in specimens from parous versus nulliparous cadavers,14 suggesting that pregnancy and parturition have little or no effect on histomorphology of levator ani muscles. The lack of histologic features of denervation in levator ani muscles from women with prolapse agrees with our own findings using microarray analysis and histomorphology of pubococcygeus muscles from premenopausal women with pelvic organ prolapse compared with age-matched controls.15 Furthermore, gross disruption of levator ani muscles and its innervation was not observed in squirrel monkeys with or without defects in pelvic organ support.16 Myogenic changes occurred more frequently in the pubocaudalis compared with the iliocaudalis muscles of this animal model, and a significant association of myogenic alterations in the pubocaudalis was found with aging, but not with pelvic organ prolapse or parity. However, parity was associated with increased apoptosis of fibroblasts in paravaginal attachments.16

To determine whether experimental denervation of the levator ani contributes to development of pelvic organ prolapse in squirrel monkeys, Pierce and colleagues17 compared nulliparous squirrel monkeys without prolapse to those with bilateral levator neurectomy, to parous monkeys without prolapse, and to parous monkeys with prolapse. As expected, significant atrophy of levator ani occurred in denervated
animals. However, pelvic organ support was not affected by bilateral denervation of the levator ani. Taken together, experimental evidence does not support a role for denervation-induced injury in the pathophysiology of pelvic organ prolapse. However, loss of skeletal muscle volume and function occurs in virtually all striated muscles during aging. Results obtained from young and old women with pelvic organ prolapse and young and old baboons without prolapse (Marinis and Word, unpublished data, 2003.) indicate that the levator ani undergoes substantial morphologic and biochemical changes during aging, suggesting that loss of levator tone with age may contribute to failure of pelvic organ support in older women, possibly with preexisting defects in connective tissue support. As the striated muscles lose tone, ligamentous and connective tissue support of the pelvic organs must sustain more forces conferred by abdominal pressure. As the connective tissues bear these loads for long periods of time, they stretch and may eventually fail, resulting in clinically recognized prolapse.

INTERACTIONS BETWEEN LEVATOR ANI AND CONNECTIVE TISSUES OF THE PELVIC FLOOR

Ligaments and connective tissues surrounding the pelvic organs support and stabilize the organs in their position above the levator ani. Several connective tissue types are involved in this support system as discussed below.

Bony Pelvis

It has been suggested that women with prolapse have a wider pelvic diameter than women without prolapse and, in one study, pelvic organ prolapse was associated with a shorter obstetric conjugate. These findings may represent different types of bony pelvises in women of different racial background. Black women are more likely to have an anthropoid pelvis with a narrow transverse inlet and wide obstetric conjugate. Also, in some, but not all, studies, black women have been characterized as having decreased risk of pelvic organ prolapse. Recently, however, using MRI, postpartum bony and soft tissue pelvic dimensions were measured in 246 postpartum primiparous women with or without pelvic organ prolapse. Although a deeper sacral hollow was significantly associated with fecal incontinence and a wider intertuberous diameter, and pelvic arch was associated with urinary incontinence, there were no significant differences in pelvimetry measurements between women with and without prolapse. The long-term impact of pelvic dimensions on the development of pelvic organ prolapse in parous women is not known.

Arcus Tendineous Fascia Pelvis and Arcus Tendineus Levator Ani

Two prominent lateral connective tissue structures play an important role in muscular and connective tissue support of the pelvic organs. Arising as condensations of the parietal fascia of the obturator internus and levator ani muscle, these aggregations of connective tissue comprise dense regular connective tissue, similar to that of tendons, with fibrous collagen more organized than the visceral connective tissue surrounding the pelvic organs (Fig. 1). The arcus tendoneus levator ani provides anchorage for the origin of the iliococcygeus and pubococcygeus muscle inserting at the pubic rami and then crossing over the obturator internus to insert posteriorly at the ischial spine. In contrast, the arcus tendineous fascia pelvis is a condensation of the parietal fascia of the visceral connective tissue that envelops the anterior and
posterior vaginal wall (see Fig. 1). It provides the lateral anchor sites for the anterior and posterior vagina. For many years, it was believed that tendon and the fascia of its corresponding muscle were all one continuous structure. Electron microscopy studies, however, demonstrate that muscle endomysium (fascia) is a separate entity, with its own connective tissues, and is not identical to tendon tissue radiating from the muscle. The arcus tendineous fascia pelvis is, therefore, separate from the fascia of the levator ani and is well developed anteriorly, presenting as a white line emanating from fasciae covering the pubococcygeus and iliococcygeus muscles.

The arcus tendineous fascia pelvis is therefore poised to withstand descent of the anterior vaginal wall and proximal urethra. The ability to prevent descent of the proximal urethra during periods of increased abdominal pressure is crucial in maintenance of urinary continence. Overstretching or tearing of the arcus tendineous fascia pelvis during vaginal delivery is postulated to contribute to pelvic organ prolapse.

**Uterosacral Ligaments**

There are two main differences between tendons and ligaments. While ligaments and tendons both have bundles of parallel collagen fibers, the fibers in ligaments are arranged in multidirectional layers, whereas the tendon’s fibers remain in strictly parallel strands. Ligaments are composed mainly of bundles of white fibrous tissue closely interlaced with one another. Although ligaments are pliant and flexible to allow freedom of movement, they are nevertheless strong, tough, and not able to extend. Elastin between each layer of the ligament allows some movement between collagen and smooth muscle layers for flexibility and change of motion.

The uterosacral ligaments are believed to contribute to pelvic support by suspending and stabilizing the uterus, cervix, and upper vagina to the dorsal body wall. Connective tissues of uterosacral ligaments in women with and without pelvic organ prolapse have been compared.\textsuperscript{24–33} The ligament is comprised of about 20% smooth muscle. Using immunohistochemistry, Gabriel and colleagues\textsuperscript{25} found that smooth muscle and collagen type I content was similar in uterosacral ligaments from postmenopausal women with or without pelvic organ prolapse. Collagen III expression,
however, was significantly increased in ligaments from women with prolapse. In contrast, using a similar technique, Takacs and colleagues found that the fractional area of smooth muscle was decreased and that apoptotic cells were increased in ligaments from women with prolapse. Reisenauer and colleagues found that the distribution of smooth muscle in the uterosacral ligament was abnormal in women with pelvic organ prolapse compared with age-, parity-, and menopause-matched controls. Consistent with the findings of Takacs and colleagues, smooth muscle cell nuclei were smaller.

Brizzolara and colleagues conducted a comprehensive study designed to study gene expression profiles in ligamentous support tissue. In this study, 34 microarrays interrogating 32,878 genes were conducted from 17 women with or without pelvic organ prolapse. Investigators found 249 differentially expressed genes between the two groups and these genes most commonly belonged to immunity and defense pathways. Interleukin-6, thrombospondin 1 and prostaglandin-endoperoxide synthase 2 (COX-2) were increased significantly in ligaments from women with prolapse. Using light microscopy, investigators found no inflammatory infiltrates in the tissue. These studies suggest that the transcriptional program of cells involved in ligamentous support of the pelvic organs is altered in women with prolapse. These alterations most likely lead to altered matrix production, cell shape, and mechanical properties, and aberrant inflammatory and healing processes. The role of stretch and mechanical strain on the ligaments in producing these changes is not known.

**Vaginal Wall**

Abnormalities in the vaginal wall or in the attachments of the vaginal wall to the pelvic floor muscles may be involved in the pathogenesis of pelvic organ prolapse. The supportive connective tissue of the pelvic floor is a continuous interdependent sheet that envelops the vagina and suspends it to the levator ani muscles of the pelvic floor via the arcus tendineous fascia pelvis (see Fig. 1). The connective tissue of the vaginal wall comprises the lamina propria, the vaginal muscularis, and the vaginal adventitia (see Fig. 1). Connective tissue of the vaginal wall (formerly referred to as endopelvic fascia) coalesces laterally to the arcus tendineous fascia pelvis and superior fascia of the levator ani. In the lower third of the vagina, the vaginal wall is attached directly to surrounding structures—the perineal membrane, and the perineal body. This suspensory system, together with the uterosacral ligaments, prevents the vagina and uterus from descent when the genital hiatus is open. Loss of connective tissue “resilience” is believed to contribute to pelvic organ prolapse during aging.

**EVIDENCE THAT THE VAGINAL WALL IS ABNORMAL IN WOMEN WITH PELVIC ORGAN PROLAPSE**

**Collagen**

It has been suggested that abnormal synthesis or degradation of collagen and elastin fibers of the vaginal wall contributes to the pathophysiology of prolapse. The extracellular matrix of connective tissues comprises predominantly fibrillar collagens and elastic fibers embedded in a nonfibrillar ground substance of proteoglycans, glycosaminoglycans, and hyaluronan. Collagen synthesis in pelvic floor connective tissues in women with pelvic organ prolapse has been reviewed comprehensively. Using hydroxyproline assays (an index of cross-linked collagen) and analyses of collagen alpha chains, Jackson and colleagues found decreases in total collagen content in vaginal epithelium from women with prolapse compared with premenopausal controls. The ratios of collagen I to collagen III were similar between the two groups.
These findings agree with those of Soderberg and colleagues, who, using the same assay, found total collagen content of parauthral ligaments to be decreased in young women with prolapse. Histologic methods, such as immunofluorescent techniques with antibodies to collagen subtypes, are not as quantitative as hydroxyproline assays. These techniques have the advantage, however, of localizing collagen subtypes in the connective tissues and can thereby distinguish between vascular and nonvascular matrix in the epithelium, lamina propria, and muscularis of connective tissues. Using immunohistochemical techniques, Kokcu and colleagues and Moalli and colleagues found increased total collagen in vaginal apical biopsies in premenopausal women with prolapse. The discrepancies in findings regarding collagen content in connective tissues of women with prolapse likely stem from differences in technical assessments of collagen content. Newly formed collagen is more extractable and contains more pentosidine cross-links. It is difficult to differentiate between mature and immature collagen using immunohistology. Thus, increased collagen content by immunofluorescence likely represents both mature and newly formed immature collagen, a finding consistent with that of Jackson and colleagues and Soderberg and colleagues, who showed that formed collagen increases in the vaginal wall during aging. Suzme and colleagues found that hydroxyproline content was decreased in the uterosacral ligament of women with pelvic organ prolapse despite histopathological evidence of increased collagen density. Collagen synthesis is increased in fibroblasts from women with prolapse compared with controls and our studies indicate collagen type I and type III mRNA are increased in vaginal muscularis from women with prolapse compared with age-matched controls (Boreham and Word, data not published). Taken together, the data suggest that collagen synthesis is increased in the vaginal wall of women with prolapse, but that the newly formed immature collagen is more susceptible to endogenous proteases and therefore is unlikely to contribute to mature cross-linked collagen that confers strength and durability to connective tissues.

Histomorphology

The normal vaginal wall comprises mucosa (epithelium and lamina propria), a fibroelastic muscularis layer, and an adventitial layer composed of loose areolar tissue, abundant elastic fibers, and neurovascular bundles. Smooth muscle cells of the anterior vaginal wall obtained from the apex of the vagina were identified by immunohistochemistry with antibodies to smooth muscle α-actin. In normal vaginal wall, smooth muscle of the muscularis is well organized in discrete fascicles constituting 45% of total cross-sectional area (Fig. 2A). In women with prolapse, smooth muscle bundles are smaller and disorganized. Smooth muscle content in this location of the vagina is decreased significantly (~22%), compared with the normal well-suspended vagina (Fig. 2B). In addition, nerve bundles are large and numerous in the deep muscularis of asymptomatic controls, but are smaller and fewer in number in women with pelvic organ prolapse. Although smooth muscle cell density is decreased in the vaginal apex of women with pelvic organ prolapse, there is no evidence that vaginal wall thickness is altered, suggesting that decreased smooth muscle cell volume may be replaced by other cell types or by the extracellular matrix. Furthermore, there is considerable variability in the amount of smooth muscle in this location of the vagina, even in control subjects. Using full-thickness sagittal sections of vaginal wall from fresh female cadavers, we found substantial variability in vaginal wall thickness and smooth muscle cell density along the length of the vaginal wall. In specimens obtained from women undergoing colpocleisis or vaginectomy, smooth muscle cells may be hypertrophied, particularly in midvagina or at the level of maximal bulge.
Contractile Protein Expression

Caldesmon and smooth muscle myosin heavy chain (SM-MHC) are two proteins that serve as molecular markers for smooth muscle cell differentiation. We studied expression, location, and isoform distribution of these proteins in the normal anterior vaginal wall and found caldesmon was predominantly expressed as the high molecular weight isoform (h-caldesmon) and was localized in smooth muscle cells of the vaginal muscularis and vasculature. The predominant isoforms of myosin heavy chain in the normal vagina were SM1 and SM2. Content of caldesmon and SM-MHC were increased in smooth muscle cells of the anterior vaginal wall in women with pelvic organ prolapse. This increase in contractile protein expression was not due to hyperplasia or hypertrophy of smooth muscle cells. Thus, the disproportionate increase in caldesmon expression relative to SM-MHC indicates an abnormal phenotype of smooth muscle cells in the vaginal muscularis of women with pelvic organ prolapse.

Ultrastructural Morphology

Preliminary studies have been conducted to define the ultrastructural morphology of the vaginal muscularis. In agreement with light microscopy studies, smooth muscle cells of asymptomatic controls were organized in fascicles. The cells are closely aligned and contain numerous intermediate junctions indicative of cell-to-cell communication (Fig. 3). In contrast, in vaginal muscularis from women with pelvic organ prolapse, smooth muscle cells were dispersed in a sea of collagen surrounding the muscle bundles and interspersed within the bundle, effectively separating the myocytes from direct cell-to-cell communication (see Fig. 3). In addition, smooth muscle cells were characterized by (1) a perinuclear halo of dilated, abundant sarcoplasmic reticulum, indicating increased protein synthesis; (2) an indented nucleus; (3) a thick basal lamina; and (4) irregularities of the sarcolemma with numerous caveolae (Fig. 4). The cells exhibit classic morphologic features of myofibroblasts, cells characteristically involved in numerous pathologic processes associated with wound healing and inflammation. Intracellular amorphous inclusions were observed specifically in smooth muscle cells in women with prolapse, suggesting a unique type of degeneration in these cells. The inclusions were remarkably common in seven of eight women with prolapse, but absent or rare in normal controls (six of six). Specifically, smooth muscle cells (not fibroblasts or any other cell type) contained large cytoplasmic inclusions specific to the vaginal muscularis.
vacuoles filled with proteinaceous material (see Fig. 4). It has been proposed that the vacuoles may be filled with either tropoelastin or procollagen (immature collagen or elastin) synthesized and secreted by activated myofibroblasts in response to cellular insult. Macrophages were also observed throughout the deep muscularis in women with pelvic organ prolapse. These cells provide a rich source of metalloproteases and elastases.

Protease Activation in Connective Tissues of the Pelvic Floor

Uninhibited protease activity has been identified as a key underlying cause of matrix degradation during the onset and progression of certain types of degenerative
diseases. Neutrophil elastase has been shown to disrupt the integrity of the microvascular barrier, and direct infusion of elastase has been shown to contribute to the development of emphysema and aortic aneurysms. Up-regulation of matrix metalloproteinase (MMP) 9 (MMP-9) and pro-MMP-2 has been reported in the vaginal wall of women with prolapse.

Proteases take part in a multitude of physiologic processes and their action varies from the very broad and indiscriminate (eg, proteases in digestion) to the exceptionally specific cleaving single peptide bonds in a single target protein. Proteolytic enzymes are implicated in regulating a wide range of fundamental biologic processes, such as blood coagulation, cell-cycle progression, development, wound healing, and apoptosis. In the human genome, more than 560 genes are annotated as proteases. An analysis of PubMed, however, reveals no experimental data on the gene products from approximately 27% of these protease genes (ie, >150 genes), and a further 28% of human protease genes have undergone only preliminary characterization. Much effort is needed to explore the physiologic role of genes encoding in a protease because the complexity of the tasks involved for different proteases varies widely. Such approaches as proteomics and genomics will enable investigators to identify proteins responsible for changes in the extracellular matrix of pelvic floor connective tissues that confer loss of support of the pelvic organs. Furthermore, identification of these cellular processes will provide novel targets for therapeutic intervention either for the prevention of pelvic organ prolapse in women at risk for this problem, or for adjunctive therapy in reconstructive surgery.

MMPs represent the most thoroughly studied family of proteases. MMPs, mainly MMP-2, MMP-9, and MMP-12, also process elastolytic activity. MMP-2 and MMP-9 were first identified as 72-kD gelatinase (gelatinase-A) and 92-kD gelatinase (gelatinase-B), respectively, because of their ability to cleave gelatin. MMPs are zinc-dependent endopeptidases that degrade pericellular extracellular matrix proteins, such as collagens, gelatins, and elastin. All three enzymes contain an N-terminal propeptide domain, a catalytic domain, and a hemopexin-like C-terminal domain. They are secreted as inactive proforms, called zymogens, and undergo proteolytic cleavage or conformational change to become active enzymes. Activation of pro-MMP-2 occurs at the cell surface through formation of trimolecular complexes with membrane-type 1–MMP and tissue inhibitors of MMP-2. Activation of MMP-9 requires other proteinases, such as MMP-2, MMP-3, MMP-13, or serine proteinases, such as trypsin and plasmin. Serine and cysteine proteases have also been identified as major elastolytic proteases. Among cysteine proteases, cathepsins S and K have been considered the most potent elastolytic activities with cathepsin K exhibiting a slightly higher activity than cathepsin S.

MMP-9, a particularly important member of the MMP family, has been associated with degradation of the extracellular matrix (both collagen and elastin) in normal and pathologic conditions. After it is released from the cell, MMP-9 can be found in the extracellular space, but it is also associated with the cell surface through complexes with CD44 or with a chain of type IV collagen. Furthermore, internalization and catabolism of MMP-9 may result from its interaction with the low-density lipoprotein receptor–related protein. Most studies have focused on transcriptional regulation of MMP-9, but posttranscriptional mechanisms have also been described, including regulation of translational efficiency. The complexity of MMP activation is compounded by the presence of MMP endogenous tissue inhibitors and, more recently, it has been shown that in vitro active MMPs may be spontaneously inactivated by degradation into smaller fragments by autocatalysis. For example, estrogen and progesterone have been shown to increase...
proteolysis of MMP-13 and thereby inhibit MMP-13 activity in fibroblasts of the arcus tendineous fascia pelvis.

In summary, these results provide compelling evidence that the vaginal muscularis is abnormal in women with prolapse compared with age- and parity-matched controls. Fibromuscular tissue from the prolapsed vagina is characterized by loss of smooth muscle at the vaginal apex, myofibroblast activation, abnormal smooth muscle phenotype, and increased protease activity. It is not known whether these changes are a result of the mechanical forces imposed on the prolapsed tissues, or if these changes in the fibromuscular wall of the vagina play a role in the pathogenesis of prolapse. While epidemiologic studies indicate that vaginal birth and aging are two major risk factors for developing pelvic organ prolapse, the specific effects of pregnancy, parturition, and aging on pelvic floor support mechanisms have not been identified. Studies conducted with vaginal tissues from women with or without pelvic organ prolapse cannot provide information regarding a primary role of these changes in the pathogenesis of the disorder. Factors due to prolonged stretching, mechanical stress, and hypoxia within the vaginal wall may produce secondary effects.

LESSONS LEARNED FROM MOUSE MODELS OF PELVIC ORGAN PROLAPSE

As discussed in the introduction to this article, epidemiologic studies indicate that vaginal birth and aging are two major risk factors for developing pelvic organ prolapse. Nevertheless, the specific effects of pregnancy, parturition, and aging on pelvic floor support mechanisms have not been identified. Progress in this area has been hampered by the lack of readily available animal models to study the disease. Recent findings in mice with null mutations in genes that encode proteins involved in elastic fiber assembly and synthesis suggest that elastic fiber homeostatic networks are important in the pathogenesis of pelvic organ prolapse. In this section, we will briefly review the mouse models and relevant information in women.

LOXL1 Knockout Mice

Recent findings in mice with null mutations in the gene encoding lysyl oxidase–like 1 (LOXL1) suggest that lysyl oxidase–like 1 (LOXL1) is crucial for pelvic organ support. LOXL1 knockout mice are viable and appear grossly normal except for elastic fiber defects in the skin, lung, and postpartum uterus. Interestingly, mice lacking LOXL develop pelvic organ prolapse 1 to 2 days after giving birth. The elastin cross-link was markedly decreased in the postpartum uterus but not in the virgin uterus in LOXL1 mice. This finding suggested that the failure of the postpartum uterine wall to form fibers might be the reason for pelvic organ prolapse in LOXL-null mice.

However, a precise mechanism of elastic fiber remodeling and homeostasis in the pelvic organ associated with pregnancy and parturition is not known. Liu and colleagues published an extension of studies reported previously in LOXL1-null mice. They included detailed descriptions of genitourinary pathology and abnormal elastic fibers in the urethra, abnormal bladder function, LOXL1 gene expression in the cervix, and LOXL1 protein in the uterus of young and old mice. By 25 weeks of age, 50% of parous LOXL1-deficient mice developed genitourinary prolapse. It should be emphasized that prolapse also occurs in virginal animals as a function of age.

Fibulin-5 Knockout Mice

Fibulin-5 (Fbln5) knockout mice exhibit disrupted elastic fiber networks in organs rich in elastin. These mice survive into adulthood but develop severe
“elastinopathies,” including loose skin, vascular abnormalities, and emphysema.\textsuperscript{29,30,55} \textit{Fbln5}-null mice also develop pelvic organ prolapse and were reported to be among the first animal models with this condition.\textsuperscript{69} Fibulin-5 was first proposed to link elastic fibers to cell surface integrin via its arginyl-glycyl-aspartic acid (RGD) motif, thus providing a bridge between elastic fibers and surrounding cells. MMP-9 is up-regulated in the vaginal wall before the onset of prolapse.\textsuperscript{70} Although similarities exist in \textit{LOXL1}/C0 and \textit{Fbln5}/C0 mice, the postpartum bulge in \textit{LOXL1} knockout mice contains a huge, distended bladder. In \textit{Fbln5} knockout mice, the bladder appears normal. Furthermore, 91\% of \textit{Fbln5}-null female mice develop pelvic organ prolapse by 6 months of age, whereas only 50\% of virginal \textit{LOXL1}/C0 mice develop prolapse by 7 months. Other lysyl oxidases in the vaginal wall supportive tissues may ameliorate the development of prolapse in the absence of parturition-induced degradation of existing elastic fibers. Fibulin-5, on the other hand, may be specific for elastic fiber assembly; or it may have effects on proteins that affect other biologic processes in addition to elastic fiber assembly.

\textbf{Fibulin-3 Knockout Mice}

\textit{Efemp1} encodes fibulin-3, an extracellular matrix protein important in the maintenance of abdominal fascia.\textsuperscript{71} Recently, we sought to evaluate the role of fibulin-3 in pelvic organ support.\textsuperscript{72} Pelvic organ support was impaired significantly in female \textit{Efemp1} knockout mice (\textit{Fbln3}−/−), and overt vaginal, perineal, and rectal prolapse occurred in 26.9\%. Severity of prolapse increased with age but not parity. Interestingly, fibulin-f was up-regulated in vaginal tissues from \textit{Fbln3}−/− mice with and without prolapse. Despite increased expression of fibulin-5 in the vaginal wall, failure of pelvic organ support occurred in \textit{Fbln3}−/− animals, suggesting that factors related to aging led to prolapse. Elastic fiber abnormalities in vaginal tissues from young \textit{Fbln3}−/− mice progressed to relatively more severe disruption of elastic fibers with age, and vaginal MMP-9 activity was increased significantly in \textit{Fbln3}−/− animals with prolapse.

\textbf{ELASTIC FIBER: LINKING CELLS WITH THEIR MATRIX}

Cells within tissues specifically contact other cells. They also contact a complex network of secreted proteins and carbohydrates, the extracellular matrix. Animals contain many different types of extracellular matrices, each specialized for a different function. For example, tendons exhibit great strength, the extracellular matrix in the kidney is designed for filtration, and the uterus expands dramatically during pregnancy. Many tissues, including the uterus, cervix, and vagina, need to be both strong and extensible to function. This is accomplished by a network of elastic fibers in the extracellular matrix, which allows the tissue to stretch and recoil without damage. Elastic fibers are five times more extensible than a rubber band of the same cross-sectional area.\textsuperscript{73}

Two distinct entities comprise mature elastic fibers: (1) an abundant amorphous component made up of the protein elastin and (2) microfibrils, proteins that surround elastin (Fig. 5). Precursor elastin is secreted from the cell as a soluble monomer called \textit{tropoelastin}. In the extracellular space, lysine residues within tropoelastin are specifically modified to form covalent cross-links between tropoelastin chains. This cross-linked polymer has a high degree of reversible distensibility, including the ability to deform to large extensions with small forces. Cross-linking is initiated by copper-requiring extracellular enzymes, the lysyl oxidases. Microfibrils consist of several proteins, including fibrillin and microfibril-associated glycoprotein. These proteins
appear before tropoelastin secretion and form a scaffold upon which elastin is deposited before it is displaced to the periphery of the growing fiber (see Fig. 5).74

Defects in elastic fiber structure result in a myriad of pathologic conditions. Pelvic organ prolapse is just one. Cutis laxa, for example, is a connective tissue disorder resulting from markedly reduced dermal elastin content. With this disorder, skin becomes inelastic and hangs loosely in folds.75 Pelvic organ prolapse is common in women with cutis laxa. Damage or degradation of elastic fibers leads to emphysema, a degenerative disease of the lungs in which the air sacs lose their elasticity. Mutations in the fibrillin gene have been shown to cause Marfan syndrome, a common genetic disorder with clinical manifestations, including pelvic organ prolapse76 and aortic dilatation and dissection.75

**ELASTIC FIBERS AND AGING**

Elastin is produced early in life. Production of elastin reaches peak levels in the third trimester of fetal life and steadily decreases during early postnatal development. In undisturbed tissues, elastic fibers may last over the entire human lifespan. In a transgenic mouse line bearing a reporter linked to the elastin promoter, activity of the elastin promoter increases during postnatal development, reaching a peak at 3 months of age in skin, then decreases.77 In many respects, age-related modifications in elastic fibers (extensively described in all organs and tissues) may be largely interpreted as progressive degradation of a protein polymer produced early in life. In humans, the elastic meshwork grows largely undistorted during postnatal growth, in which fibers seem to enlarge proportionate with tissue growth. Later, in adults and in elderly subjects, elastic fibers gradually become tortuous, frayed, and porous.78 However, experiments

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**Fig. 5.** Elastic fiber assembly and the role of fibulin-5. Tropoelastin is secreted from fibroblasts and smooth muscle cells as a monomer. Fibulin-5 may tether elastin to the cell by interacting with integrins (orange) on the cell surface. Cell surface localization would target elastin to the microfibril scaffold (green) possibly by interacting with fibulin-2 located in the microfibril. In this way, fibulin-5 may regulate elastic fiber assembly by the microfibril machinery. Thereafter, assembled and coacervated tropoelastin is cross-linked by copper-dependent lysyl oxidases in the extracellular matrix to form mature elastic fibers surrounded by microfibrils (arrows). Elastic fibers are extraordinarily stable but may be broken down by activated MMPs or other proteases to form elastin degradative products (EDPs). EDPs are matrikines that amplify proteases to further degrade elastic fibers, tropoelastin, and collagen. Cu**, copper ions; Fbln-5, fibulin-5.
suggest that elastic fiber turnover in the female reproductive tract, unlike elastic fiber turnover in other adult organs, is continuous and accelerates after childbirth. This unique adaptation to synthesize and assemble new elastic fibers allows the vagina to expand during pregnancy and recover from childbirth.

FIBULIN-5 AND ELASTOGENESIS

Other proteins, including the emerging family of fibulin proteins, contact elastic fibers in vivo and are thought to promote the formation and stabilization of the fiber. The term fibulin is derived from the Latin word for clasp or buckle. Fibulins comprise five family members, each with overlapping, but distinct, patterns of expression. They are particularly prominent in tissues rich in elastic fibers, such as lung, blood vessels, bladder neck, and uterus. Recent studies identified fibulin-5 (also known as DANCE [developing arteries and neural crest epidermal growth factor–like protein] or EVEC [embryonic vascular epidermal growth factor–like repeat–containing protein]) as a protein that links elastic fibers to cells and regulates fiber assembly and organization. Fibulin-5 co-localizes with and binds to elastin on the surface of elastic fibers and to cells. Fbln5 binds to cells through interactions with integrin cell surface receptors. Fibulin-5 is essential for formation of new elastic fibers, but apparently not for the maintenance of already existing fibrils. The elastin fiber renewal process that occurs in the female reproductive tract after parturition is unique among adult tissues. The idea that fibulin-5 acts as a bridge between elastin and the cell surface adds an extra level of complexity to the existing model of elastic fiber assembly. Close collaboration between cells and elastin is needed for fiber formation to occur, and molecules that facilitate this have been identified. For example, elastin receptors have been shown to associate with tropoelastin and serve as chaperones to aid in its intracellular transport and extracellular assembly. Fibulin-5 also binds to LOXL1 and may coordinate the actions of these molecules by directing elastic fiber assembly at the cell surface.

ELASTIC FIBERS IN THE VAGINAL WALL OF WOMEN WITH PELVIC ORGAN PROLAPSE

In 1975, el-Kholi and Mina, using Verhoeffe’s Van Geison stain, studied elastic fibers from the vaginas of 48 women of different age groups with and without vaginal prolapse. Elastic fibers were minimal and showed marked fragmentation in women with pelvic organ prolapse compared with those in age- and parity-matched controls. A more recent investigation failed to find significant abnormalities in vaginal wall elastic fiber staining. According to investigators, the photomicrographs in this report do not support that conclusion. Nevertheless, it is not known whether the reported changes in elastic fibers are a result of the mechanical forces imposed on the prolapsed tissues, or if these changes in the fibromuscular wall of the vagina play a role in the pathogenesis of prolapse. Yamamoto and colleagues compared elastin mRNA expression and elastin synthesis in cultured fibroblasts derived from cardinal ligaments of elderly patients with pelvic organ prolapse with those derived from age-matched control patients. Marked decreases in elastin gene transcripts and elastin synthesis were found in quiescent fibroblasts cultured from women with pelvic organ prolapse. Klutke and colleagues found that desmosine content (an index of cross-linked elastin fibers) was similar in uterosacral ligaments from women with or without prolapse. Desmosine content, however, was significantly decreased in women with complete procidentia. Interestingly, although expression of LOX and LOXL1 mRNA was decreased, fibulin-5 mRNA was increased in uterosacral ligaments from women with prolapse compared with controls. These results differed from those of Jung and colleagues in which fibulin-5 mRNA levels were decreased and
LOXL1 mRNA levels increased in uterosacral ligaments from women with prolapse. Discrepancies in these two studies may stem from differences in the study populations since Jung’s investigation was limited to postmenopausal women. Both studies, however, indicate that expression of genes involved in elastic fiber assembly is altered in pelvic floor connective tissues from women with pelvic organ prolapse.

These findings together with the known association between inherited elastinopathies and pelvic organ prolapse suggests that defective elastic fiber assembly is a primary event in the pathophysiology of pelvic organ prolapse. Pelvic organ prolapse is common in women with inherited defects in elastic fiber synthesis or assembly. In addition to primary elastinopathies that have been directly linked to alterations in the elastin gene, a number of secondary elastinopathies have been described, caused by functional imbalance of other structural and auxiliary factors regulating elastic fiber deposition (Marfan disease, GM-1-gangliosidosis, Morquio B, Hurler disease, Costello syndrome, Ehlers Danlos syndrome, pseudo-xanthoma elasticum). Several investigators have suggested that alterations in collagen synthesis and collagen types are causally related to connective tissue disorders, such as inguinal hernia, pelvic organ prolapse, stress urinary incontinence, and benign joint hypermobility syndrome. In this respect, several aspects of collagen and elastic fiber homeostatic pathways are interrelated. Cellular pathways of elastic fiber and collagen synthesis and degradation converge in some areas. For example, the copper-dependent enzyme lysyl oxidase is crucial for cross-linking of elastin and collagen. Several MMPs (ie, MMP-12 and MMP-9) degrade both elastin and collagen. The relative importance of each pathway in the pathophysiology of pelvic organ prolapse is unknown.

SUMMARY

The pelvic floor is a complex dynamic system that supports the vagina and pelvic viscera. In women, failure of pelvic organ support is common. A wealth of literature has established that vaginal parity and aging are important risk factors for pelvic organ prolapse in women. The specific mechanisms by which vaginal delivery and aging lead to failure of pelvic organ support are unknown. For decades, pelvic surgeons have recognized that women with prolapse often exhibit abnormal connective tissues in the pelvic floor. In recent years, new information is accumulating to define the cellular and molecular mechanisms that confer abnormal structural and functional support to the pelvic organs.

By definition, remodeling of connective tissue involves both synthesis and degradation of the extracellular matrix. Remodeling of connective tissue of the pelvic floor in pelvic organ prolapse likely represents aberrations in both synthesis and degradation of matrix components. Early studies by Woessner and Brewer indicate that matrix synthesis and degradation of both collagen and elastin are regulated dramatically in the uterus during pregnancy and postpartum involution. Connective tissues of the pelvic floor also undergo matrix remodeling during pregnancy, parturition, and the puerperium. Recent investigations, together with the phenotype of LOXL1, Fbln-3, and Fbln-5 knockout mice, have led to the idea that pelvic organ prolapse, at least in some women, may represent an elastinopathy brought about by different conditions, including aging and incomplete remodeling of the vaginal wall after parturition. Disturbances in the balance between synthesis/assembly and degradation of matrix components of connective tissues of the pelvic floor may result in slow, but progressive, loss of pelvic organ support. Understanding the basic mechanisms of pelvic organ support will lead to the development of therapeutic strategies to prevent or
abrogate pelvic organ prolapse and its associated morbidities, such as urinary and fecal incontinence.

REFERENCES


