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Adenomyosis pathogenesis: insights from next-generation sequencing

Serdar E. Bulun [1,*], Sule Yildiz [1], Mazhar Adli [1], and Jian-Jun Wei [1,2]

[1]Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA [2]Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA

*Correspondence address. Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, 250 E Superior, Room 3-2306, Chicago, IL 60611, USA. E-mail: s-bulun@northwestern.edu
https://orcid.org/0000-0003-0850-8637

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BACKGROUND: Adenomyosis, characterized by the presence of islands of endometrial tissue surrounded by hypertrophic smooth muscle cells within the myometrium, is one of the most challenging uterine disorders in terms of diagnosis and management. Adenomyosis presents with pelvic pain, excessive uterine bleeding, anemia and infertility. The relative contributions of abnormal endometrial tissue and myometrial smooth muscle cells to the development and growth of adenomyosis are not well understood. Moreover, there is continuing debate on the origins of adenomyosis; two competing theories describe the invagination of basal endometrium into the myometrium or the metaplastic differentiation of remnant endometrial stem/progenitor cells within the myometrium.

OBJECTIVE AND RATIONALE: A recent series of next-generation sequencing (NGS) studies have provided the best scientific evidence thus far regarding the cellular origins of adenomyosis and the contributions of new signaling pathways to its pathogenesis, survival, and growth. These seminal studies on endometrium, adenomyosis and endometriosis demonstrate or support the following key points. (i) Mutations of *KRAS* map to both intracavitary endometrial tissue and proximally located adenomyotic samples, supporting the invagination theory of pathogenesis. Driver mutations found in smooth muscle cells of uterine fibroids are absent in adenomyosis. (ii) *KRAS* and other less frequent mutations are limited to endometrial-type epithelial cells. They are also observed in endometriosis, indicating that the disease process in adenomyosis is similar to that in endometriosis and distinct from that of uterine fibroids. (iii) Activating mutations of *KRAS* stimulate specific pathways to increase cell survival and proliferation and are associated with progesterone resistance in adenomyosis. Together, these findings suggest that distinct cell populations in eutopic endometrial tissue play key roles in the etiology of adenomyosis.

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Dependence on ovarian steroids and ovulatory cycles for disease severity is a unique feature of adenomyosis. In this context, common patterns of aberrant gene expression have been reported both in adenomyosis and endometriosis. These include pathways that favor increased estrogen biosynthesis, decreased estradiol metabolism, a unique estrogen receptor beta (ESR2)-driven inflammatory process, and progesterone resistance due to decreased progesterone receptor expression. Since adenomyosis exhibits a uniquely estrogen-driven inflammatory process and progesterone resistance, we discuss the interactions between these molecular characteristics and signaling pathways induced by the newly discovered *KRAS* mutations.

SEARCH METHODS: We conducted a comprehensive search using PubMed for human and animal studies published until 2020 in the following areas: adenomyosis, endometriosis, endometrium, NGS, whole-exome sequencing, whole-genome sequencing, RNA sequencing, targeted deep sequencing, epigenetics, driver mutation, *KRAS*, progesterone resistance, estrogen action and steroid production.

OUTCOMES: Targeted deep sequencing analyses of epithelial cells in adenomyosis and adjacent basalis endometrial glands demonstrated recurring *KRAS* mutations in both cell types. This finding suggests that adenomyosis originates from basalis endometrium. Epithelial cells of the endometrium, adjacent adenomyosis and co-occurring endometriosis also share identical *KRAS* mutations. These findings suggest both adenomyosis and endometriosis are oligoclonal tissues that arise from endometrial cell populations carrying a specific driver mutation that most commonly affects the *KRAS* gene.

WIDER IMPLICATIONS: Adenomyosis usually follows an event such as pregnancy that has disrupted the integrity of the endometrial–myometrial junction followed by repetitious menstrual episodes that increase the likelihood of the entrapment of the basalis endometrium within the myometrium. Glandular epithelial cells carrying *KRAS* mutations and located within the deep crypts of basalis endometrium may become entrapped and invade myometrial tissue to give rise to adenomyosis. Evidence suggests that *KRAS* mutations may be responsible, in part, for previously observed phenomena such as prolonged cell survival and progesterone resistance in adenomyosis.

Key words: adenomyosis / endometrium / endometriosis / next-generation sequencing / NGS / driver mutation / KRAS / ESR1 / PGR / progesterone resistance

Introduction

The presence of endometrial glands in the myometrium was first described in 1860 by Rokitansky, who used the term 'cystosarcoma adenoides uterinum' (Rotkitansky, 1860). Thereafter, Von Recklinghausen and Cullen described similar pathologic entities, using the terms adenomyomata, cystoadenomyomata, adenomyoma and diffuse adenomyom (Von Recklinghausen, 1896; Cullen, 1908). It was Frankl in 1925 who first designated the disorder as 'adenomyosis uteri' (Frankl, 1925). The definition of adenomyosis introduced by Bird *et al.* in 1972, 'the benign invasion of endometrium into the myometrium, producing a diffusely enlarged uterus which microscopically exhibits ectopic, non-neoplastic, endometrial glands and stroma surrounded by the hypertrophic and hyperplastic myometrium', is still used today (Bird *et al.*, 1972; Ferenczy, 1998; Benagiano and Brosens, 2006).

Adenomyosis, uterine leiomyomas and endometriosis are extremely common and frequently co-exist. Adenomyotic uteri often contain uterine leiomyomas (Ferenczy, 1998). Moreover, sonographically, it may be challenging to distinguish between an adenomyoma and a leiomyoma. Although adenomyosis has been considered a variant of endometriosis, i.e. endometriosis interna, we acknowledge that there are both clinical and pathologic similarities and distinctions between the two entities (Emge, 1956; Israel and Woutersz, 1959). Despite certain distinctions, adenomyosis seems to be strongly associated with endometriosis. For example, one study showed that women with endometriosis also exhibit an irregular endometrial–myometrial junction and a higher rate of adenomyosis (Larsen *et al.*, 2011). Additionally, severe endometriosis is associated with adenomyosis with a deeper myometrial invasion (Larsen *et al.*, 2011). The lack of definitive molecular data, have made it challenging to understand the associations between these uterine disorders. A recent series of publications on next-generation sequencing (NGS)-based analyses of these pathologies, signal a paradigm shift in our ability to clarify the underlying disease mechanisms and the possible associations between them (Li *et al.*, 2014; Anglesio *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019; Moore *et al.*, 2020).

Most pathologists and clinicians postulate that adenomyosis develops when the normal junction between the basalis endometrium and the myometrium is disrupted (Fig. 1) (Uduwela *et al.*, 2000). This disruption may facilitate the invasion of the myometrium by endometrial glands, resulting in ectopic intramyometrial glands that induce hypertrophy in adjacent myometrial smooth muscle cells. What triggers the initial disruption of this junction and the entire disease process underlying adenomyosis is unclear (Vercellini *et al.*, 2006). During pregnancy, invading trophoblasts disturb the endometrial–myometrial junction. In contrast to endometriosis, the frequency of adenomyosis was found to be higher in parous women compared with nulliparas (Parazzini *et al.*, 1997; Vercellini *et al.*, 2006). Similarly, women with a history of one or more spontaneous abortions were also at increased risk (Parazzini *et al.*, 1997; Vercellini *et al.*, 2006). This may be explicable by a higher risk for endometrial–myometrial junction breakdown induced by pregnancy. The endometrial–myometrial junction may also be disrupted by repeated episodes of menstruation and associated myometrial contractions (Parazzini *et al.*, 1997; Vercellini *et al.*, 2006). In fact, the risk is higher in women reporting heavy periods (Parazzini *et al.*, 1997; Vercellini *et al.*, 2006). This view resonates with the involvement of tissue injury and repair as a mechanism for adenomyosis (Leyendecker *et al.*, 2009).

The breakdown of the normal endometrial–myometrial boundary may be followed by the invagination of the endometrial basalis into the myometrium (Fig. 1). Islands of adenomyosis may be scattered throughout the myometrium, giving origin to the diffuse form of the disease; less frequently, these islands occur in a localized form, termed adenomyoma (Ferenczy 1998). The ectopic mucosa resembles basal endometrium, and a direct connection between the basalis portion of

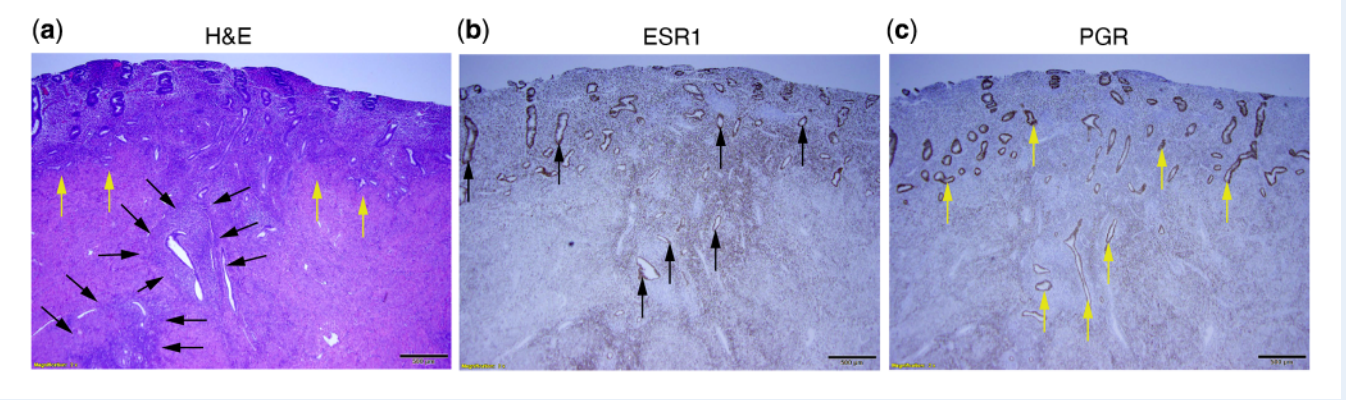

Figure 1. Adenomyosis. (**a**) Hematoxylin and eosin (H&E)-stained specimen showing abnormal endometrial–myometrial junction in the center of the field, where basal endometrium extends deep into the myometrium (black arrowheads). This area is flanked on both sides by normal appearing myometrial-endometrial junction (yellow arrowheads). (**b** and **c**) Glandular epithelial cells show immunoreactive nuclear estrogen receptor-α (ESR1, black arrows) and progesterone receptor (PGR, yellow arrows) indicated by brown staining in both endometrium and adenomyosis. Immunoreactive ESR1 and PGR are also observed in the nuclei of stromal cells. Immunoreactive ESR1 and PGR appear less intense in adenomyosis compared with endometrial tissue indicating fewer steroid receptors.

the endometrium and adenomyotic foci has been shown consistently (Fig. 1) (Ferenczy 1998). Interestingly, the posterior myometrium is usually affected to a greater extent than the other portions of the uterine wall (Ferenczy 1998; Bazot *et al.*, 2001; Chapron *et al.*, 2020).

The endometrium and myometrium undergo drastic cyclic steroid-dependent changes involving vasoconstriction, necrosis, shedding, myometrial contractions and rapid angiogenesis and regeneration. The extension and intramyometrial spreading of adenomyotic tissue seem to be associated with the anti-apoptotic, angiogenic and proliferative properties of the basalis endometrium in the presence of a hyper-estrogenic state (Ferenczy, 1998). Eutopic endometrial tissue of women with adenomyosis synthesizes estrogen locally and exhibits progesterone resistance and abnormal cytokine production. These properties enhance both ability of the endometrium to infiltrate the junctional zone myometrium and the growth of adenomyotic tissue (Benagiano and Brosens, 2012). Clinically and pathologically, adenomyosis is hormone-sensitive and contains steroid receptors; aromatase inhibitors and gonadotropin-releasing hormone (GnRH) analogs suppress the extent and symptoms of adenomyosis (Fig. 1) (Bergeron *et al.*, 2006; Kimura *et al.*, 2007). In contrast, exogenous progestogenic agents are less effective for the treatment of adenomyosis, possibly due to altered progesterone receptor (PGR) expression and activity (Fig. 1) (Inoue *et al.*, 2019).

Next-generation sequencing and uterine disorders

The term NGS is an umbrella term used to describe many existing and emerging high-throughput approaches to DNA or RNA sequencing. Such approaches utilize massively parallel processing of clonally amplified millions of DNA templates in a flow cell, in contrast to the time-honored Sanger sequencing that electrophoretically separates nucleic acid molecules according to their sizes in an individual sequencing experiment. NGS technologies form the backbone of nearly all contemporary genomic approaches, including whole-exome sequencing (WES, sequencing of mostly protein-coding regions termed exons of some 21 000 human genes) and whole-genome sequencing (WGS; sequencing of the entire coding regions of all genes plus large sequences that separate the genes) as well as various other genome-scale or targeted deep sequencing methods. Since NGS became widely available starting in the early 2000s, it has been applied to a number of tumors or diseased tissues, including uterine disorders such as adenomyosis.

Here, we review the key findings from WES and WGS analyses of adenomyosis and related tissues. Additionally, one NGS-based microbiome analysis of the female lower genital tract in adenomyosis patients showed that throughout the reproductive tract, there was depletion or enrichment of many bacteria, some of which overlapped with bacteria associated with anemia, which was consistent with clinical links between the two conditions (Chen *et al.*, 2017). At this time, it is not clear whether this association has a causative role in adenomyosis. We also could not find any genome-wide association study of adenomyosis.

Although this review focuses on adenomyosis, the epithelial mutations in eutopic endometrial glands are intricately related to the origins of both adenomyosis and endometriosis; thus, it is also relevant that we discuss NGS studies of endometriosis (Table 1) (Li *et al.*, 2014; Anglesio *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019). By the same token, the mutations found in uterine leiomyoma smooth muscle cells are very different in their frequency and type compared with the epithelial cells of endometrium or adenomyosis; therefore, we also discuss these mutations briefly to emphasize the distinct origins of uterine leiomyomas and adenomyosis (Mäkinen *et al.*, 2011; Mehine *et al.*, 2013; Inoue *et al.*, 2019).

Since 2011, a number of seminal WES or WGS studies of uterine disorders have been published. These studies, which primarily aimed to uncover somatic mutations in extremely common benign uterine disorders, have significantly improved our understanding of the mechanisms of disease underlying uterine leiomyomas, endometriosis and adenomyosis (Table 2) (Mäkinen *et al.*, 2011; Mehine *et al.*, 2013;

Table I Key next-generation sequencing studies of endometrium, endometriosis and adenomyosis.

Author, year and journal	Methodology and sample size (patients)	Tissues compared	Key findings
Li *et al.*, 2014, *Human Mol Genet*	Laser-capture microdissection (LCM) of epithelial cells Whole-exome sequencing (n = 21) Targeted sequencing	Ovarian endometriotic lesions and eutopic endometrium of endometriosis patients vs normal endometrium of healthy women	Somatic mutations were found in both eutopic endometrial and endometriotic epithelial cells.
Anglesio *et al.*, 2017, *N Engl J Med*	LCM of epithelial and stromal cells Whole-exome sequencing (n = 24) Targeted sequencing (n = 3) Droplet digital PCR (n = 14)	Deep infiltrating endometriotic (extra-ovarian) lesions vs matched eutopic endometrium	Driver mutations involving genes such as *KRAS, ARID1A, PIK3CA* and *PPP2R1A* were found in deep infiltrating endometriotic epithelial cells. No mutations were found in endometriotic stroma.
Suda *et al.*, 2018, *Cell Rep*	LCM of epithelial cells Whole-exome sequencing (n = 24) Targeted sequencing (n = 74)	Ovarian endometriotic lesions vs normal endometrium	*KRAS, PIK3CA, FBXW7, PPP2R1A*, and *PIK3R1* were recurrently mutated in the majority of both endometriotic and disease-free eutopic endometrial epithelial cell clones in deep invaginating glandular crypts.
Inoue *et al.*, 2019, *Nat Commun*	LCM of epithelial cells Whole-exome sequencing (n = 51) Targeted sequencing (n = 19)	Adenomyosis vs endometriosis, matched eutopic endometrium, leiomyoma, normal myometrium and normal endometrium	*KRAS* mutations were significantly enhanced in eutopic endometrial epithelial cell clones in deep invaginating glandular crypts and adjacent adenomyotic tissue compared with the adenomyosis-free group.
Moore *et al.*, 2020, *Nature*	LCM of epithelial cells Whole-genome sequencing (n = 28)	Histologically normal endometrium	Recurrent *PIK3CA* and *KRAS* mutations were found in histologically normal endometrial epithelial cells.

Table II Key recurrent mutations in gynecologic disorders.

Mutations	EE	Adenomyosis	Endometriosis	Leiomyoma
KRAS	+	+	+	
PIK3CA	+	+	+	
PPP2R1A	+	+	+	
ARID1A			+	
MED12				+

EE, eutopic (normally located) endometrium.

Li *et al.*, 2014; Anglesio *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019). A number of investigators had suggested that the clinically normal-appearing eutopic endometrium might serve as the precursor tissue for endometriosis and adenomyosis (Sampson, 1927; Noble *et al.*, 1996; Tseng *et al.*, 1996; Bulun, 2009; Carrarelli *et al.*, 2017). The recently published NGS studies not only verified this previously postulated role of eutopic endometrium in endometriosis or adenomyosis, but they also provided a compelling link between eutopic endometrial cells and ovarian cancer (Noble *et al.*, 1996; Tseng *et al.*, 1996; Li *et al.*, 2014; Carrarelli *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019; Moore *et al.*, 2020). WES of whole tissues was the most common initial methodology, which identified remarkably large numbers of single-nucleotide variations and other genetic alterations, some of which turned out to be mutations that disrupt or alter protein structures (Li *et al.*, 2014; Anglesio *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019). WGS in some of these studies provided a more complete picture of genomic alterations in leiomyomas and eutopic endometrium (Mehine *et al.*, 2013; Moore *et al.*, 2020).

These initial analyses of whole tissues were followed by targeted deep sequencing of whole tissues or laser-capture micro-dissected cellular components suspected of bearing these recurrent mutations. Notably, nearly all recurrent mutations were detected in micro-dissected epithelial cells (Li *et al.*, 2014; Anglesio *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019; Moore *et al.*, 2020). The genetic alterations identified in these cells accounted for all of the mutations found in whole tissues of eutopic endometrium, endometriosis and adenomyosis, whereas the abundantly present stromal cells were mutation-free (Li *et al.*, 2014; Anglesio *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019; Moore *et al.*, 2020). In the majority of uterine leiomyomas, the smooth muscle cell, which comprises the majority of the tumor cells, was found to contain an identical mutation because of the overwhelmingly high recurrent nature of mutations in a single gene (*MED12*) in the entire tumor; this observation supported the hypothesis that a leiomyoma arises from the clonal expansion of a mutated progenitor myometrial smooth muscle cell (Table 2) (Mäkinen *et al.*, 2011; Bulun, 2013; Mehine *et al.*, 2013). Conversely, endometriosis and adenomyosis seemed to originate from the ectopic proliferation and expansion of multiple mutated epithelial cell clones that also contain an attached stromal cell population (Suda *et al.*, 2018; Inoue *et al.*, 2019; Moore *et al.*, 2020). In clinically normal eutopic endometrial glands, multiple epithelial cell clones with distinct driver mutations often originated early in life and subsequently progressively colonized the endometrium's epithelial lining in a mosaic-like fashion (Table 2) (Suda *et al.*,

2018; Inoue et al., 2019; Moore et al., 2020). In the endometrium, endometriosis, adenomyosis and leiomyoma, the most prominently detected genetic alterations in *PIK3CA*, *KRAS*, *PPP2R1A* and *MED12* were predicted to be activating-type mutations, whereas ARID1A mutations lead to a loss of function (Table 2) (Mehine et al., 2013; Anglesio et al., 2017; Suda et al., 2018; Inoue et al., 2019; Moore et al., 2020).

Driver mutations in endometrial glandular epithelial cells

Moore et al. (2020) assessed whether endometrial glands comprise clonal cell populations and examined the variant allele fractions of somatic mutations. Distributions of variant allele fractions in 91% (234 out of 257) of micro-dissected endometrial glands indicated that each gland consists predominantly of a cell population descended from a single epithelial progenitor stem cell. Intriguingly, normal human endometrial glands were clonal cell populations with total mutation burdens that increased at about 29 base substitutions per year, a rate many-fold lower than that in endometrial cancers (Moore et al., 2020).

A total of 209 driver mutations were found in the great majority of normal eutopic endometrial glands from 89% (25 out of 28) of women. *PIK3CA* was the most frequently mutated cancer gene (Table 2). Numerous cell clones with at least one (57%) or more driver mutations colonized much of the eutopic endometrial epithelium, in contrast to the colon, another glandular epithelium (Suda et al., 2018; Lee-Six et al., 2019), where approximately 1% of normal crypts in middle-aged individuals carry a driver mutation (Suda et al., 2018; Lee-Six et al., 2019). This may be attributable to intrinsic differences between the endometrium and colon in structure and physiology. In the endometrium, the cyclical process of tissue breakdown, shedding and remodeling iteratively opens up a denuded terrain for pioneering clones of endometrial epithelial cells with driver mutations to colonize in preference to wild-type cells (Moore et al., 2020).

In the fraction of histologically normal eutopic endometrial glands carrying a driver gene mutation, the mean number of mutations per gland and the number of different driver mutations in each individual were positively correlated with the age of the individual. It was concluded that cell clones with driver mutations often originate during the first decades of life and subsequently progressively colonize the endometrium's epithelial lining (Moore et al., 2020).

In a similar study, Suda et al. (2018) isolated 109 single endometrial glands from the uteri of three subjects and explored the presence of somatic mutations by applying targeted-gene sequencing. This group concluded that individual endometrial glands within a normal uterus of the same individual carry distinct somatic mutations, which aligns with their sporadic and spontaneous somatic origins during menstrual glandular proliferation cycles (Suda et al., 2018). *PIK3CA* was found to be the most frequently mutated gene in the eutopic endometrial glands examined (Table 2). The authors identified less frequent mutations within the other cancer-associated genes, including *KRAS*. The mutant allele frequencies of the somatic mutations in endometrial glands indicated that clonal expansion had occurred within the gland, starting with cells located deep in the base of the invaginated gland. They found that approximately one-third of the endometrial glands carried a mutation in *PIK3CA*, although the mutation in each gland was distinct and involved different amino acid alterations (Table 2). Only a few endometrial glands had the same mutation. These findings of genetic variation between distinct glands underscore the heterogeneity of the uterine endometrial epithelium and may help explain the observed mosaic-like nature of its genome (Suda et al., 2018).

Link between endometrium and endometriosis

Using WES, Suda et al. (2018) detected 4192 somatic mutations in 13 ovarian endometriotic and 11 normal eutopic endometrial epithelium samples. A number of driver genes, including *KRAS*, *PIK3CA*, *FBXW7*, *PPP2R1A* and *PIK3R1*, were found to be recurrently mutated in the majority of both endometriotic and eutopic endometrial epithelium samples, although the epithelia were histologically benign and normal (Suda et al., 2018). These findings were validated by targeted sequencing in a larger cohort and were consistent with an earlier report by Anglesio et al. (2017), who mapped similar driver mutations in eutopic endometrium to extraovarian deep-infiltrating endometriosis (Table 2).

In endometriotic and eutopic endometrial epithelial cells, *KRAS* and *PIK3CA*, respectively, were found to be the most commonly mutated genes (Table 2). All mutations in the *KRAS* gene were located at amino acids 12, 13 or 61, and led to impaired guanosine triphosphate (GTP) hydrolysis by the KRAS GTPase-accelerating proteins (GAPs). This in turn led to constitutive activation of GTP-bound RAS and its downstream PI3K and extracellular signal-regulated kinase pathways (Scheffzek et al., 1997). The mutations in *PIK3CA* were found in several functional domains of the gene. Three mutations were at residue H1047 and other mutations were similar to mutations that had been previously identified in cancers (Forbes et al., 2017). Based on the finding that the majority of *PIK3CA* mutations in cancers show gain-of-function effects and growth advantages, the presence of these same mutations in endometriotic and eutopic endometrial epithelial cells are expected to have functional significance in disease pathogenesis (Gymnopoulos et al., 2007).

Driver mutations in adenomyosis

Inoue et al. (2019) recently demonstrated numerous somatic mutations in adenomyosis. Using WES, they detected 134 unique synonymous and non-synonymous single-nucleotide variations in 60.8% (31/51) of whole tissues of adenomyosis, supporting the possibility that adenomyosis is a clonal disorder with somatic mutations (Inoue et al., 2019). These adenomyosis single-nucleotide variations were present in low numbers and at low variant allele frequencies, comparable to those in co-occurring endometriosis but much lower than those in co-occurring leiomyoma or ovarian cancer.

Subsequent application of targeted deep sequencing demonstrated recurrent somatic pathogenic *KRAS* mutations in 37.1% (26/70) of adenomyosis cases, in which 25 samples had $KRAS^{G12}$ and one sample had $KRAS^{Q61}$ amino acid alterations. Somatic mutations encoding the

PIK3CA p. H1047 alteration were validated in two out of 70 patient lesions, as was a mutation encoding *PPP2R1A* p.P179 in a lesion in one out of 70 patients. Inoue *et al.* (2019) concluded that somatic *KRAS* mutation is a critical genomic alteration associated with adenomyosis. Samples of endometriosis obtained from some of the adenomyosis patients contained both *KRAS* and *PIK3CA* mutations (Inoue *et al.*, 2019) consistent with previously published mutational profiles of endometriosis (Anglesio *et al.*, 2017; Suda *et al.*, 2018). On the other hand, most leiomyomas from some of the adenomyotic uteri harbored *MED12* mutations, which was consistent with the previous WES studies on fibroids (Mehine *et al.*, 2013). Importantly, multi-regional sampling and targeted deep sequencing (Mehine *et al.*, 2013) showed that most mutations detected in adenomyosis and co-existing leiomyoma were mutually exclusive, implying the lack of a clonal relationship between these disorders (Inoue *et al.*, 2019). Collectively, these studies suggest that adenomyosis has a slightly different mutation profile to endometriosis and a distinct mutation profile compared with fibroids, even if all three disease samples originate from a single patient (Table 2) (Inoue *et al.*, 2019).

Compared with whole tissue samples, variant allele frequencies were markedly higher in laser-captured epithelial cell components, demonstrating that the low frequencies detected in the WES analyses of bulk frozen adenomyosis lesions were due to low epithelial cell content rather than poor expansion of mutated adenomyosis clones (Inoue *et al.*, 2019). Targeted deep sequencing of isolated epithelial cells indicated that somatic mutations in adenomyosis reside in this cell type, and that adenomyosis may therefore originate from the ectopic proliferation of mutated epithelial cell clones (Inoue *et al.*, 2019). This observation dovetails with the reports of Moore *et al.* (2020) and Suda *et al.* (2018), who found that the endometrial glands arise from clonal expansion of mostly mutated epithelial cells deep in the base of the tubular gland. It is tempting to hypothesize that adenomyosis primarily stems from the basalis portions of the endometrial glands that harbor a *KRAS* mutation and are trapped within the myometrium during traumatic processes at the endometrial-myometrial junction, such as recurrent menstruation or pregnancy (Fig. 2) (Parazzini *et al.*, 1997; Vercellini *et al.*, 2006).

Link between endometrium and adenomyosis

Inoue *et al.* (2019) investigated whether the genomic alterations in adenomyosis originate in histologically normal eutopic endometrium. They compared mutations detected in multi-regional adenomyosis samples with those in adjacent eutopic endometrial or myometrial tissues. Most mutations were detected only in adenomyotic lesions, but some mutations, including those in *KRAS* and *PIK3CA*, also appeared in eutopic endometrium. Thus, these mutations likely arose in eutopic endometrial cells before they invaded the myometrium, consistent with a previously proposed etiology of endometriosis (Suda *et al.*, 2018; Inoue *et al.*, 2019). Although adenomyosis frequently co-occurs with endometriosis, until recently, there was no molecular evidence to support a common cellular origin. However, Inoue *et al.* (2019) found identical *KRAS* mutations in co-existing adenomyotic and endometriotic lesions in multiple patients. These results are in line with the frequent co-occurrence of endometriosis anatomical subtypes, such as ovarian endometriomas ($P = 0.001$) and deep-infiltrating endometriosis ($P < 0.05$), in a cohort of *KRAS*-mutated adenomyosis patients (Inoue *et al.*, 2019). The NGS data reported by at least four different laboratories collectively support the mechanism by which *KRAS*-mutated clones arising in eutopic endometrium acquire enhanced invasiveness and proliferative capacity that enable them to grow ectopically, driving adenomyosis as well as ovarian endometriomas and deep-infiltrating endometriosis (Anglesio *et al.*, 2017; Suda *et al.*, 2018; Inoue *et al.*, 2019; Moore *et al.*, 2020).

Frequently, *KRAS* and *PIK3CA* mutations are found in histologically normal eutopic endometrium from women without endometriosis or adenomyosis (Anglesio *et al.*, 2017; Suda *et al.*, 2018; Moore *et al.*, 2020). These mutations are also found in eutopic endometrium adjacent to adenomyotic lesions, prompting Inoue *et al.* (2019) to investigate whether the frequency of *KRAS*, *PIK3CA* and *PPP2R1A* mutations was altered in eutopic endometrium adjacent to an adenomyotic lesion. They compared microdissected samples of endometrial tissue from women with or without adenomyosis or endometriosis (Inoue *et al.*, 2019). Among dissected eutopic endometrial samples, *KRAS* mutations were commonly observed in the adenomyosis (55.6%) and endometriosis (50%) groups, but less frequently in the disease-free group (29.1%). *PIK3CA* mutations in eutopic endometrial tissue were also observed but to a lesser extent in adenomyosis (11.1%), endometriosis (35.7%) and disease-free (25%) groups (Inoue *et al.*, 2019). These observations are consistent with other publications reporting recurrent *KRAS* and *PIK3CA* mutations in endometrium that appears to be histologically normal (Suda *et al.*, 2018; Moore *et al.*, 2020). Remarkably, the variant allele frequencies of mutations encoding oncogenic *KRAS* p.G12/G13 alterations (but no other mutations) were significantly enhanced in eutopic endometrial samples from the adenomyosis group compared with the disease-free group, suggesting that *KRAS*-mutated clones had expanded in the eutopic endometrium of individuals with adenomyosis (Inoue *et al.*, 2019). These genomic analyses of eutopic endometrium suggest that an increase in expanded *KRAS*-mutated clones in this histologically normal tissue may be an early step in the molecular pathogenesis of adenomyosis (Fig. 2) (Inoue *et al.*, 2019).

Summary of NGS studies of adenomyosis

Mutated epithelial clones localized in the eutopic endometrial glands seem to play important roles in the pathophysiology of adenomyosis. A number of recurrent driver mutations were found in these endometrial glandular epithelial cells, including mutations affecting *PIK3CA* and *KRAS*. Although PIK3CA is the most commonly mutated gene in eutopic endometrial cells, mutations found in adenomyosis epithelium almost exclusively affected the *KRAS* gene. *KRAS* was also the most recurrently mutated gene in epithelial cells of endometriotic lesions. Collectively, these NGS data suggest that *KRAS*-mutated epithelial clones play an important and common role in the etiologies of adenomyosis and endometriosis.

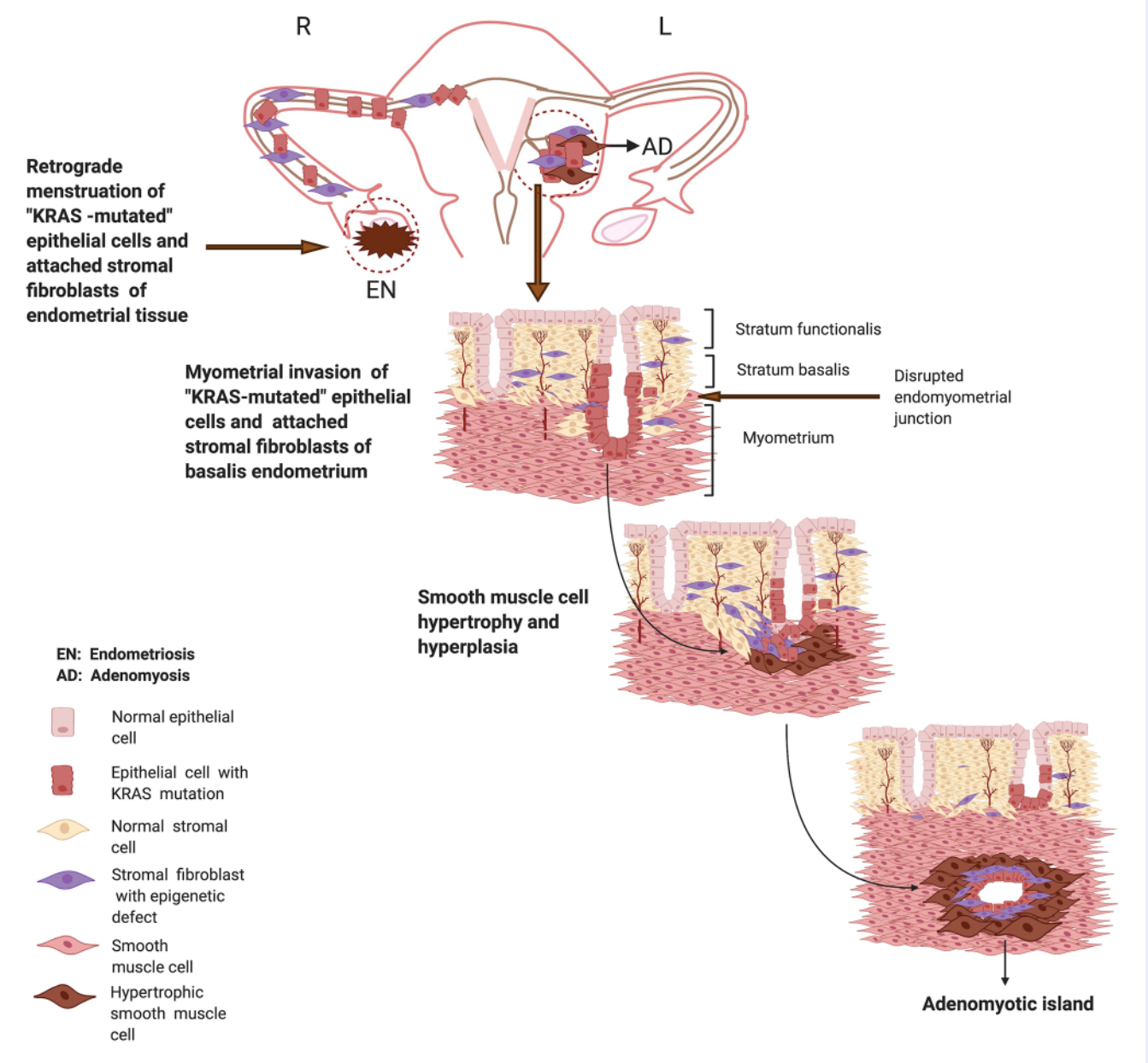

Figure 2. Pathophysiology of adenomyosis supported by combined histological and mutational analyses of adenomyotic and adjacent endometrial tissues. Mapping of identical driver mutations that primarily affect the *KRAS* gene in the epithelial cells of basalis endometrium and adjacent adenomyosis strongly suggests that distinct cellular clones in deeply invaginating crypts are trapped in myometrial tissue. Activating *KRAS* mutations in these clones conferred survival and growth advantages, leading to their expansion to eventually become clinically recognizable adenomyosis.

Abnormalities in gene expression in adenomyosis

Until the recent discovery of *KRAS* mutations in epithelial cells, the reported molecular abnormalities in adenomyosis were mostly of epigenetic nature or involved abnormal expression of downstream genes as a consequence of an unknown mutation (Inoue *et al.*, 2019). In other words, compared with eutopic endometrium, there were significant differences in the levels of mRNA or protein expression of a number of genes in adenomyosis, but no mutations involving these genes were reported (Vannuccini *et al.*, 2017). These differences in gene expression most often relied on mRNA analysis of whole tissues. This was occasionally coupled with studies of protein expression or changes in DNA methylation associated with the gene of interest. The cellular localization of the abnormalities in adenomyosis was heavily dependent on immunohistochemistry and often not verified by other methods because primary culture of the cellular components of adenomyosis has not been achieved. Most of the identified gene

expression abnormalities centered on excessive estrogen formation and progesterone resistance and involved steroid receptors and other transcription factors (Vannuccini *et al.*, 2017). Intriguingly, similar abnormalities were reported in the transcriptome of eutopic endometrial tissue of women with adenomyosis (Xiang *et al.*, 2019).

Estrogen excess

Estrogen plays a key role in the etiology of adenomyosis. Treatments that diminish ovarian production of estradiol, such as GnRH analogs and aromatase inhibitors, suppress adenomyosis whereas tamoxifen, which acts as an estradiol agonist in the uterus, aggravates adenomyosis (Ugwumadu *et al.*, 1993; Cohen *et al.*, 1995; Bergeron *et al.*, 2006; Kimura *et al.*, 2007). The enzyme encoded by the aromatase (*CYP19A1*) gene is responsible for estrogen production from its precursors in human tissues (Bulun *et al.*, 2005). The presence of aromatase activity and gene expression in adenomyotic tissue was reported even before they were revealed in endometriotic tissue (Yamamoto *et al.*, 1993; Noble *et al.*, 1996; Fang *et al.*, 2002; Pavone and Bulun, 2012).

Aromatase overexpression in endometriotic stromal cells is coupled with an epithelial deficiency of the enzyme HSD17B2 that inactivates estradiol by converting it into estrone in adenomyosis (Vierikko *et al.*, 1985; Casey *et al.*, 1994; Zeitoun *et al.*, 1998). It is likely that HSD17B2 may also be deficient in adenomyotic epithelial cells, as endometriosis and adenomyosis share multiple molecular features. The combination of aromatase excess and HSD17B2 deficiency may give rise to excessive levels of estradiol in adenomyosis (Bulun, 2009). Moreover, the expression of genes encoding the enzymes that convert arachidonic acid to prostaglandins, including cyclooxygenases, have been reported in adenomyosis (Maia *et al.*, 2006; Vannuccini *et al.*, 2017). Excessive formation of prostaglandins, especially PGE_2, may stimulate aromatase expression and cause pain associated with menses in adenomyosis (Bulun, 2009; Vannuccini *et al.*, 2017).

Steroid receptor expression and progesterone resistance

In the endometrial glands and stroma and myometrial tissue from adenomyotic uteri, the expression of the estrogen receptor-1 (ESR1) gene was lower overall compared to normal controls during the mid-secretory phase (Mehasseb *et al.*, 2011). In contrast, ESR2 expression was higher in functionalis endometrial glands and basalis stroma and myometrium from adenomyotic uteri (Mehasseb *et al.*, 2011). Expression of the PGR-A gene was similar to that of PGR-B, with reduced expression in the basalis endometrial stroma and inner and outer myometrium in the adenomyotic uteri (Mehasseb *et al.*, 2011). Interestingly, in adenomyotic foci, the pattern of ESR2, PGR-A and PGR-B expression was found to be similar to that in endometrial basalis tissue (Mehasseb *et al.*, 2011). This steroid receptor expression is also similar to that in endometriotic stromal cells, where ESR2 is upregulated and ESR1 is downregulated via opposite DNA methylation patterns (Xue *et al.*, 2007; Dyson *et al.*, 2014). The inverted ratio of ESR1 to ESR2 may play a role in decreased PGR expression in addition to hypermethylation of this gene (Bulun *et al.*, 2019). Additionally, PGR-B was reported to be suppressed by DNA methylation in adenomyotic stromal cells (Jichan *et al.*, 2010). In summary, adenomyotic tissue seems to exhibit progesterone resistance and aberrant estrogen action regulated primarily via ESR2. Since ESR2 and excessive production of prostaglandins induces inflammation in endometriotic stromal cells, a similar mechanism may affect adenomyosis (Bulun, 2009; Bulun *et al.*, 2019).

Immune system

An activated inflammasome seems to be a common pathway in both adenomyosis and endometriosis. This postulated mechanism is supported by increased expression of inflammatory cytokines such as tumor necrosis factor, IL1B, IL6 and CCL2 (MCP-1) in adenomyosis or in eutopic endometrium from adenomyosis patients (Sotnikova *et al.*, 2002; Zhihong *et al.*, 2016; Carrarelli *et al.*, 2017; Xiang *et al.*, 2019). On the other hand, local or circulating levels of other cytokines such as IL10, IL23, IL25, IL31, IL33 and IL37 are decreased in adenomyosis patients (Jiang *et al.*, 2018; Bourdon *et al.*, 2019). This observation was interpreted as the presence of a perturbed immune balance or immune-tolerant process in patients with adenomyosis (Bourdon *et al.*, 2019). Moreover, a number of laboratories showed decreased expression of IL10, LIF and its receptor LIFR in eutopic endometrial tissue from adenomyotic uteri, which was associated with diminished STAT3 activation and associated signaling (Yen *et al.*, 2017; Wang *et al.*, 2018). This was suggested as a mechanism for implantation failure or early pregnancy loss in patients with adenomyosis (Yen *et al.*, 2017; Wang *et al.*, 2018).

A number of abnormalities involving key immune cells in the peripheral blood or uterine tissue of adenomyosis patients have also been reported. The balance between regulatory T cells and T-helper 17 cells was found to be perturbed in the peripheral circulation and uteri of patients with both diffuse and focal adenomyosis; this immune cell abnormality correlated with the severity of dysmenorrhea (Lin *et al.*, 2012). In another study, the expression of killer cell inhibitory receptors on natural killer cells was shown to be decreased in eutopic endometrium in women with adenomyosis (Yang *et al.*, 2004). Together, these reports of widespread immune cell abnormalities are highly suggestive of a key role of the immune system in the pathophysiology of adenomyosis.

Link between KRAS mutations and abnormalities in gene expression

The three human *RAS* genes (*KRAS*, *NRAS* and *HRAS*) are the most frequently mutated genes in human cancers, with mutations appearing in 90% of pancreatic cancers, 35% of lung cancers and 45% of colon cancers (McCormick, 2015; Porru *et al.*, 2018). The recent discovery of widespread epithelial mutations in adenomyosis, more than half of which involve the *KRAS* gene, brought a paradigm-shifting perspective to differential gene expression (Inoue *et al.*, 2019). *RAS* genes encode proteins that are components of the mitogen-activated protein kinase signaling pathway, which is activated by a ligand binding to a receptor tyrosine kinase (RTK) such as the epidermal growth factor receptor (Fig. 3) (McCormick, 2015; Porru *et al.*, 2018). KRAS, as well as the other RAS proteins, exist in a non-active guanosine diphosphate (GDP)-bound state or active state (GTP-bound) (McCormick, 2015; Porru *et al.*, 2018). The transition between these two states is responsible for signal transduction from the cell surface RTK to the inside of

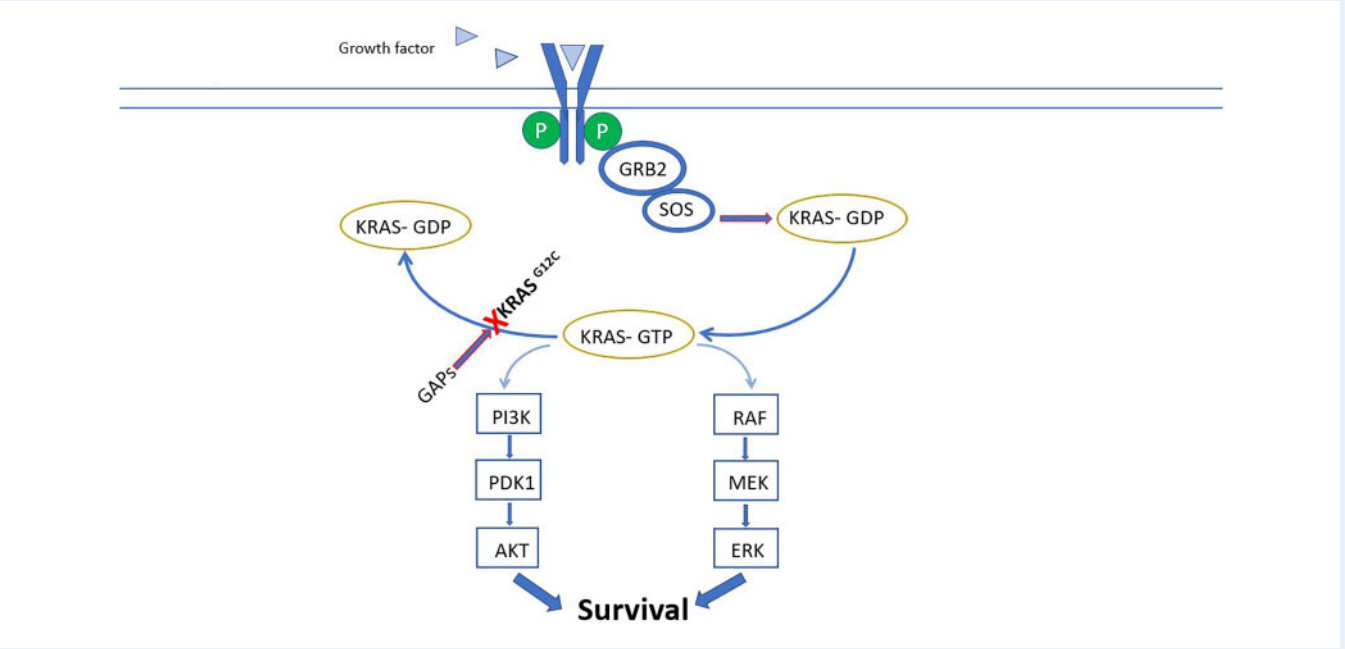

Figure 3. Autonomously activated KRAS/MAPK signaling pathway as a result of the *KRAS* mutation G12C. AKT, protein kinase B; ERK, extracellular signal-regulated kinase; GAPs, GTPase-activating proteins; GDP, guanosine diphosphate; GRB2, growth factor receptor-bound protein 2; MEK, mitogen-activated protein kinase-kinase; PDK1, pyruvate dehydrogenase lipoamide kinase isozyme 1; PI3K, phosphoinositide 3-kinase; RAF, RAF kinase; RTK, receptor tyrosine kinase; SOS, son of sevenless.

the cell, which is physiologically crucial for cell growth and differentiation (McCormick, 2015; Porru *et al.*, 2018). Upon binding of a ligand to the RTK, guanine exchange factors known as the son of sevenless-1/2 promote the activation of RAS proteins by stimulating a GDP for GTP exchange (Fig. 3). KRAS also has intrinsic GTPase activity, which means that the protein can hydrolyze a bound GTP molecule into GDP, but it is a relatively poor catalyst on its own. Thus, it requires binding of GTPase-activating proteins (GAPs), which accelerate KRAS-mediated GTP hydrolysis to GDP and return *KRAS* to an inactive state. The glycine to valine mutation at residue 12 (G12) renders the GTPase domain of KRAS insensitive to inactivation by GAP, leading to persistent accumulation of the active, GTP-bound protein and autonomous activation of multiple downstream effectors (Fig. 3) (McCormick, 2015; Porru *et al.*, 2018). Notably, 25 out of 26 *KRAS* mutations found in adenomyosis involved the G12 residue (Inoue *et al.*, 2019).

Constitutively activated mutant KRAS may induce a number of downstream pathways including PI3K-PDK1-AKT and RAF-MEK1/2-ERK1/2 (McCormick, 2015; Porru *et al.*, 2018). Both pathways favor cell proliferation and survival (Fig. 3). So far, efforts to therapeutically target these pathways in cancers with *KRAS* mutations have been generally disappointing because of their complexity. However, more promising progress has been made in targeting the KRAS protein directly (McCormick, 2015; Porru *et al.*, 2018). The potential use of these future therapeutic strategies in adenomyosis will depend on their effectiveness and side effect profiles.

As summarized previously, some 373 to 1024 genes were found to be differentially regulated in eutopic endometrial tissues of patients with or without adenomyosis (Herndon *et al.*, 2016; Xiang *et al.*, 2019). Mutated *KRAS* has been reported to influence gene expression via altering downstream signaling pathways (McCormick, 2015). Moreover, Inoue *et al.* (2019) provided evidence that mutated *KRAS* induces hypermethylation of the *PGR* gene and decreases PGR-A and PGR-B expression levels in endometrial epithelial cells, giving rise to progesterone resistance. The *KRAS* mutations in adenomyosis, however, are limited to epithelial cells and primarily detected in the glandular epithelium of basalis endometrium and adenomyotic tissue. In contrast, the great majority of adenomyotic tissue or basalis endometrium is comprised of endometrial stromal cells that possibly account for differential gene regulation and cellular function.

In addition to the considerations raised earlier, many questions remain unanswered. To what extent do the constitutively active *KRAS*-mutated epithelial cells account for the observed differential gene/protein expression or biologic function (e.g. proliferation, apoptosis, differentiation) in adenomyosis and associated eutopic endometrium? How do mutated epithelial cells affect the adjacent stromal cells in a paracrine manner with respect to epigenetic changes and consequent gene/protein expression? What are the abnormal secretory products of mutated epithelial cells? Do these mutations influence embryo implantation and fertility? Why is it that malignancy rarely arises from adenomyotic foci with epithelial mutations? Since the uterus is the most estrogen/progesterone-sensitive tissue, how do epithelial mutations interact with steroid hormone action? Do genotoxic products of estrogen metabolism, such as quinones, render epithelial cells prone to mutagenesis?

Overall summary: epithelial *KRAS* mutations and pathophysiology of adenomyosis

The recently uncovered epithelial mutations that mostly affect the *KRAS* gene and are seen in both the glandular endometrium and adjacent adenomyosis provide high-quality evidence that adenomyotic islands arise from the basalis layer (Fig. 2). This has long been suspected and hypothesized. The recent mapping of *KRAS* mutations in the glandular epithelial cells of the basalis epithelium to adenomyotic epithelium provide further supporting evidence for this concept (Fig. 2). Questions remain as to why and how adenomyosis occurs. The majority of reproductive-age women experience repeated menstruation episodes, which involves shedding of the functionalis layer and its expulsion aided by uterine contractions and simultaneous activation of regenerative processes involving the tissue stem cells in the basalis endometrium. One can envision that the menstruation process itself may increase the risk for the entrapment of fragments of the basalis layer within the myometrium.

Although the majority of women have repetitious menstrual episodes, a fraction of them develop adenomyosis; we can speculate on the potential factors involved. (i) Anatomically, a previous pregnancy may disrupt the endometrium-myometrium junction and increase the risk of endometrial entrapment in myometrial tissue during the following menstrual cycles. (ii) The most common mutation in the endometrium of clinically unremarkable women involve *PIK3CA* mutations that are rare in adenomyotic tissue, whereas adenomyosis and adjacent endometrium almost exclusively display *KRAS* mutations. This suggests that clonal glandular epithelial fragments with a *KRAS* mutation are more prone to survive once entrapped in the myometrium (Fig. 2). A *KRAS* mutation possibly enhances the expansion or invasion of these clones. (iii) The majority of the endometrial tissue in adenomyotic implants is comprised of endometrial stromal cells. Pre-existing epigenetic defects leading to aromatase excess, progesterone resistance and a hyperactive inflammasome in stromal cell populations in the basalis layer may increase the risk for adenomyosis. The risk factors for these genetic and epigenetic abnormalities may be acquired during embryonic or perinatal life and may even be transmitted to the next generation.

Although the sample sizes in these recent NGS studies were relatively small, and replication and validation are needed, their conclusions were very consistent (Tables I and 2). There is a compelling association between endometrial epithelial *KRAS* mutations and the development of adenomyosis and endometriosis. Intriguingly, the majority of these mutations are limited to an alteration of a specific amino acid (G12), which inhibits the GTPase activity of KRAS and renders it constitutively active (Fig. 3). We expect that in the immediate future, studies will focus on understanding the intracrine and paracrine effects of autonomously active KRAS and its downstream signaling pathways in epithelial cells, the neighboring stromal cells and other cell types. We anticipate that identifying targetable molecules may help limit the further spread of adenomyosis within the myometrium and suppress inflammatory processes in this tissue. This in turn may improve the rates of embryo implantation and decrease pregnancy losses associated with adenomyosis. These future targeted treatments may also reduce excessive uterine bleeding and obviate hysterectomy, a radical treatment modality for adenomyosis.

Data availability

The data underlying this article are available in the article.

Authors' roles

J.-J.W.: contribution to pathology-related portions. M.A.: contribution to next-generation sequencing portions. S.Y.: contribution to figures and references. S.E.B.: main writer of manuscript.

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Conflict of interest

All authors of this paper confirm that they do not have a conflict of interest related to this work.

References

Anglesio MS, Papadopoulos N, Ayhan A, Nazeran TM, Noë M, Horlings HM, Lum A, Jones S, Senz J, Seckin T *et al.* Cancer-associated mutations in endometriosis without cancer. *N Engl J Med* 2017;**376**:1835–1848.

Bazot M, Cortez A, Darai E, Rouger J, Chopier J, Antoine JM, Uzan S. Ultrasonography compared with magnetic resonance imaging for the diagnosis of adenomyosis: correlation with histopathology. *Hum Reprod* 2001;**16**:2427–2433.

Benagiano G, Brosens I. History of adenomyosis. *Best Pract Res Clin Obstet Gynaecol* 2006;**20**:449–463.

Benagiano G, Brosens I. The endometrium in adenomyosis. *Womens Health (Lond)* 2012;**8**:301–312.

Bergeron C, Amant F, Ferenczy A. Pathology and physiopathology of adenomyosis. *Best Pract Res Clin Obstet Gynaecol* 2006;**20**:511–521.

Bird CC, McElin TW, Manalo-Estrella P. The elusive adenomyosis of the uterus–revisited. *Am J Obstet Gynecol* 1972;**112**:583–593.

Bourdon M, Santulli P, Chouzenoux S, Maignien C, Bailly K, Andrieu M, Millischer AE, Doridot L, Marcellin L, Batteux F *et al.* The disease phenotype of adenomyosis-affected women correlates with specific serum cytokine profiles. *Reprod Sci* 2019;**26**:198–206.

Bulun SE. Endometriosis. *N Engl J Med* 2009;**360**:268–279.

Bulun SE. Uterine fibroids. *N Engl J Med* 2013;**369**:1344–1355.

Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, Martin R, Utsunomiya H, Thung S, Gurates B *et al.* Regulation of aromatase

expression in estrogen-responsive breast and uterine disease: from bench to treatment. *Pharmacol Rev* 2005;**57**:359–383.

Bulun SE, Yilmaz BD, Sison C, Miyazaki K, Bernardi L, Liu S, Kohlmeier A, Yin P, Milad M, Wei J. Endometriosis. *Endocr Rev* 2019;**40**:1048–1079.

Carrarelli P, Yen CF, Funghi L, Arcuri F, Tosti C, Bifulco G, Luddi A, Lee CL, Petraglia F. Expression of inflammatory and neurogenic mediators in adenomyosis. *Reprod Sci* 2017;**24**:369–375.

Casey ML, MacDonald PC, Andersson S. 17 beta-Hydroxysteroid dehydrogenase type 2: chromosomal assignment and progestin regulation of gene expression in human endometrium. *J Clin Invest* 1994;**94**:2135–2141.

Chapron C, Vannuccini S, Santulli P, Abrão MS, Carmona F, Fraser IS, Gordts S, Guo SW, Just PA, Noël JC *et al*. Diagnosing adenomyosis: an integrated clinical and imaging approach. *Hum Reprod Update* 2020;**26**:392–411.

Chen C, Song X, Wei W, Zhong H, Dai J, Lan Z, Li F, Yu X, Feng Q, Wang Z *et al*. The microbiota continuum along the female reproductive tract and its relation to uterine-related diseases. *Nat Commun* 2017;**8**:875.

Cohen I, Beyth Y, Tepper R, Figer A, Shapira J, Cordoba M, Yigael D, Altaras MM. Adenomyosis in postmenopausal breast cancer patients treated with tamoxifen: a new entity? *Gynecol Oncol* 1995; **58**:86–91.

Cullen TS. *Adenomyoma of the Uterus*. Philadelphia: W.B.Sanders, 1908.

Dyson MT, Roqueiro D, Monsivais D, Ercan CM, Pavone ME, Brooks DC, Kakinuma T, Ono M, Jafari N, Dai Y *et al*. Genome-wide DNA methylation analysis predicts an epigenetic switch for GATA factor expression in endometriosis. *PLoS Genet* 2014;**10**: e1004158.

Emge LA. Problems in the diagnosis of adenomyosis uteri; with special reference to dysfunctional bleeding. *West J Surg Obstet Gynecol* 1956;**64**:291–304; discussion, 304–305.

Fang Z, Yang S, Gurates B, Tamura M, Simpson E, Evans D, Bulun SE. Genetic or enzymatic disruption of aromatase inhibits the growth of ectopic uterine tissue. *J Clin Endocrinol Metab* 2002;**87**: 3460–3466.

Ferenczy A. Pathophysiology of adenomyosis. *Hum Reprod Update* 1998;**4**:312–322.

Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, Cole CG, Ward S, Dawson E, Ponting L *et al*. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res* 2017;**45**: D777–D783.

Frankl O. Adenomyosis uteri. *Am J Obstet Gynecol* 1925;**10**:680–684.

Gymnopoulos M, Elsliger MA, Vogt PK. Rare cancer-specific mutations in PIK3CA show gain of function. *Proc Natl Acad Sci USA* 2007;**104**:5569–5574.

Herndon CN, Aghajanova L, Balayan S, Erikson D, Barragan F, Goldfien G, Vo KC, Hawkins S, Giudice LC. Global transcriptome abnormalities of the eutopic endometrium from women with adenomyosis. *Reprod Sci* 2016;**23**:1289–1303.

Inoue S, Hirota Y, Ueno T, Fukui Y, Yoshida E, Hayashi T, Kojima S, Takeyama R, Hashimoto T, Kiyono T *et al*. Uterine adenomyosis is an oligoclonal disorder associated with KRAS mutations. *Nat Commun* 2019;**10**:5785.

Israel SL, Woutersz TB. Adenomyosis; a neglected diagnosis. *Obstet Gynecol* 1959;**14**:168–173.

Jiang JF, Xiao SS, Xue M. Decreased expression of interleukin-37 in the ectopic and eutopic endometria of patients with adenomyosis. *Gynecol Endocrinol* 2018;**34**:83–86.

Jichan N, Xishi L, Guo SW. Promoter hypermethylation of progesterone receptor isoform B (PR-B) in adenomyosis and its rectification by a histone deacetylase inhibitor and a demethylation agent. *Reprod Sci* 2010;**17**:995–1005.

Kimura F, Takahashi K, Takebayashi K, Fujiwara M, Kita N, Noda Y, Harada N. Concomitant treatment of severe uterine adenomyosis in a premenopausal woman with an aromatase inhibitor and a gonadotropin-releasing hormone agonist. *Fertil Steril* 2007;**87**: 1468.e9–12.

Larsen SB, Lundorf E, Forman A, Dueholm M. Adenomyosis and junctional zone changes in patients with endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2011;**157**:206–211.

Lee-Six H, Olafsson S, Ellis P, Osborne RJ, Sanders MA, Moore L, Georgakopoulos N, Torrente F, Noorani A, Goddard M *et al*. The landscape of somatic mutation in normal colorectal epithelial cells. *Nature* 2019;**574**:532–537.

Leyendecker G, Wildt L, Mall G. The pathophysiology of endometriosis and adenomyosis: tissue injury and repair. *Arch Gynecol Obstet* 2009;**280**:529–538.

Li X, Zhang Y, Zhao L, Wang L, Wu Z, Mei Q, Nie J, Li X, Li Y, Fu X *et al*. Whole-exome sequencing of endometriosis identifies frequent alterations in genes involved in cell adhesion and chromatin-remodeling complexes. *Hum Mol Genet* 2014;**23**:6008–6021.

Lin FJ, Jiang GR, Shan JP, Zhu C, Zou J, Wu XR. Imbalance of regulatory T cells to Th17 cells in IgA nephropathy. *Scand J Clin Lab Invest* 2012;**72**:221–229.

Maia H Jr, Casoy J, Correia T, Freitas L, Pimentel K, Athayde C, Coutinho E. Effect of the menstrual cycle and oral contraceptives on aromatase and cyclooxygenase-2 expression in adenomyosis. *Gynecol Endocrinol* 2006;**22**:547–551.

Mäkinen N, Mehine M, Tolvanen J, Kaasinen E, Li Y, Lehtonen HJ, Gentile M, Yan J, Enge M, Taipale M *et al*. MED12, the mediator complex subunit 12 gene, is mutated at high frequency in uterine leiomyomas. *Science* 2011;**334**:252–255.

McCormick F. KRAS as a therapeutic target. *Clin Cancer Res* 2015; **21**:1797–1801.

Mehasseb MK, Panchal R, Taylor AH, Brown L, Bell SC, Habiba M. Estrogen and progesterone receptor isoform distribution through the menstrual cycle in uteri with and without adenomyosis. *Fertil Steril* 2011;**95**:2228–2235, 2235. e2221.

Mehine M, Kaasinen E, Mäkinen N, Katainen R, Kämpjärvi K, Pitkänen E, Heinonen HR, Bützow R, Kilpivaara O, Kuosmanen A *et al*. Characterization of uterine leiomyomas by whole-genome sequencing. *N Engl J Med* 2013;**369**:43–53.

Moore L, Leongamornlert D, Coorens THH, Sanders MA, Ellis P, Dentro SC, Dawson KJ, Butler T, Rahbari R, Mitchell TJ *et al*. The mutational landscape of normal human endometrial epithelium. *Nature* 2020;**580**:640–646.

Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. *J Clin Endocrinol Metab* 1996;**81**:174–179.

Parazzini F, Vercellini P, Panazza S, Chatenoud L, Oldani S, Crosignani PG. Risk factors for adenomyosis. *Hum Reprod* 1997;**12**:1275–1279.

Pavone ME, Bulun SE. Aromatase inhibitors for the treatment of endometriosis. *Fertil Steril* 2012;**98**:1370–1379.

Porru M, Pompili L, Caruso C, Biroccio A, Leonetti C. Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities. *J Exp Clin Cancer Res* 2018;**37**:57.

Rotkitansky K. Ueber uterusdruesen-neubildung. *Z Gesellschaft Aerzte Wien* 1860;**16**:577–581.

Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tiffue into the peritoneal cavity. *Am J Obstet Gynecol* 1927;**14**:422–469.

Scheffzek K, Ahmadian MR, Kabsch W, Wiesmüller L, Lautwein A, Schmitz F, Wittinghofer A. The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science* 1997;**277**:333–338.

Sotnikova N, Antsiferova I, Malyshkina A. Cytokine network of eutopic and ectopic endometrium in women with adenomyosis. *Am J Reprod Immunol* 2002;**47**:251–255.

Suda K, Nakaoka H, Yoshihara K, Ishiguro T, Tamura R, Mori Y, Yamawaki K, Adachi S, Takahashi T, Kase H *et al*. Clonal expansion and diversification of cancer-associated mutations in endometriosis and normal endometrium. *Cell Rep* 2018;**24**:1777–1789.

Tseng JF, Ryan IP, Milam TD, Murai JT, Schriock ED, Landers DV, Taylor RN. Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. *J Clin Endocrinol Metab* 1996;**81**:1118–1122.

Uduwela AS, Perera MA, Aiqing L, Fraser IS. Endometrial-myometrial interface: relationship to adenomyosis and changes in pregnancy. *Obstet Gynecol Surv* 2000;**55**:390–400.

Ugwumadu AH, Bower D, Ho PK. Tamoxifen induced adenomyosis and adenomyomatous endometrial polyp. *Br J Obstet Gynaecol* 1993;**100**:386–388.

Vannuccini S, Tosti C, Carmona F, Huang SJ, Chapron C, Guo SW, Petraglia F. Pathogenesis of adenomyosis: an update on molecular mechanisms. *Reprod Biomed Online* 2017;**35**:592–601.

Vercellini P, Viganò P, Somigliana E, Daguati R, Abbiati A, Fedele L. Adenomyosis: epidemiological factors. *Best Pract Res Clin Obstet Gynaecol* 2006;**20**:465–477.

Vierikko P, Kauppila A, Rönnberg L, Vihko R. Steroidal regulation of endometriosis tissue: lack of induction of 17 beta-hydroxysteroid dehydrogenase activity by progesterone, medroxyprogesterone acetate, or danazol. *Fertil Steril* 1985;**43**:218–224.

Von Recklinghausen F. *Die Adenomyomata und Cystadenomata der Uterus und Tubenwandung: Ihre Abkunft von Resten des Wolff'schen Koerpers*. Berlin: August Hirschwald Verlag, 1896.

Wang J, Huang C, Jiang R, Du Y, Zhou J, Jiang Y, Yan Q, Xing J, Hou X, Zhou J *et al*. Decreased endometrial IL-10 impairs endometrial receptivity by downregulating HOXA10 expression in women with adenomyosis. *Biomed Res Int* 2018;**2018**:1–9.

Xiang Y, Sun Y, Yang B, Yang Y, Zhang Y, Yu T, Huang H, Zhang J, Xu H. Transcriptome sequencing of adenomyosis eutopic endometrium: a new insight into its pathophysiology. *J Cell Mol Med* 2019;**23**:8381–8391.

Xue Q, Lin Z, Cheng YH, Huang CC, Marsh E, Yin P, Milad MP, Confino E, Reierstad S, Innes J *et al*. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. *Biol Reprod* 2007;**77**:681–687.

Yamamoto T, Noguchi T, Tamura T, Kitawaki J, Okada H. Evidence for estrogen synthesis in adenomyotic tissues. *Am J Obstet Gynecol* 1993;**169**:734–738.

Yang JH, Chen MJ, Chen HF, Lee TH, Ho HN, Yang YS. Decreased expression of killer cell inhibitory receptors on natural killer cells in eutopic endometrium in women with adenomyosis. *Hum Reprod* 2004;**19**:1974–1978.

Yen CF, Liao SK, Huang SJ, Tabak S, Arcuri F, Lee CL, Arici A, Petraglia F, Wang HS, Kayisli UA. Decreased endometrial expression of leukemia inhibitory factor receptor disrupts the STAT3 signaling in adenomyosis during the implantation window. *Reprod Sci* 2017;**24**:1176–1186.

Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, Johns A, Meng L, Putman M, Carr B *et al*. Deficient 17beta-hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17beta-estradiol. *J Clin Endocrinol Metab* 1998;**83**:4474–4480.

Zhihong N, Yun F, Pinggui Z, Sulian Z, Zhang A. Cytokine profiling in the eutopic endometrium of adenomyosis during the implantation window after ovarian stimulation. *Reprod Sci* 2016;**23**:124–133.