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Cancer-associated mutations in endometriosis: shedding light on the pathogenesis and pathophysiology

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BACKGROUND: Endometriosis is a benign gynaecological disease. Thus, it came as a complete surprise when it was reported recently that the majority of deep endometriosis lesions harbour somatic mutations and a sizeable portion of them contain known cancer-associated mutations (CAMs). Four more studies have since been published, all demonstrating the existence of CAMs in different subtypes of endometriosis. While the field is still evolving, the confirmation of CAMs has raised many questions that were previously overlooked.

OBJECTIVE AND RATIONALE: A comprehensive overview of CAMs in endometriosis has been produced. In addition, with the recently emerged understanding of the natural history of endometriotic lesions as well as CAMs in normal and apparently healthy tissues, this review attempts to address the following questions: Why has there been such a wild discrepancy in reported mutation frequencies? Why does ectopic endometrium have a higher mutation rate than that of eutopic endometrium? Would the presence of CAMs in endometriotic lesions increase the risk of cancer to the bearers? Why do endometriotic epithelial cells have much higher mutation frequencies than their stromal counterpart? What clinical implications, if any, do the CAMs have for the bearers? Do these CAMs tell us anything about the pathogenesis and/or pathophysiology of endometriosis?

SEARCH METHODS: The PubMed database was searched, from its inception to September 2019, for all papers in English using the term 'endometriosis and CAM', 'endometriosis and cancer-driver mutation', 'somatic mutations', 'fibrosis', 'fibrosis and epigenetic', 'CAMs and tumorigenesis', 'somatic mutation and normal tissues', 'oestrogen receptor and fibrosis', 'oxidative stress and fibrosis', 'ARID1A mutation', and 'Kirsten rat sarcoma mutation and therapeutics'. All retrieved papers were read and, when relevant, incorporated into the review results.

OUTCOMES: Seven papers that identified CAMs in endometriosis using various sequencing methods were retrieved, and their results were somewhat different. Yet, it is apparent that those using microdissection techniques and more accurate sequencing methods found more CAMs, echoing recent discoveries that apparently healthy tissues also harbour CAMs as a result of the replicative aging process. Hence endometriotic lesions, irrespective of subtype, if left intact, would generate CAMs as part of replicative aging, oxidative stress and perhaps other factors yet to be identified and, in some rare cases, develop cancer. The published data still are unable to paint a clear picture on pathogenesis of endometriosis. However, since endometriotic epithelial cells have a higher turnover than their stromal counterpart due to cyclic bleeding, and since the endometriotic stromal component can be formed by refresh influx of mesenchymal cells through epithelial–mesenchymal transition, endothelial–mesenchymal transition, mesothelial–mesenchymal transition and other processes as well as recruitment of bone-marrow-derived stem cells and outflow due to smooth muscle metaplasia, endometriotic epithelial cells have much higher mutation frequencies than their stromal counterpart. The epithelial and stromal cellular components develop in a dependent and co-evolving manner. Genes involved in CAMs are likely to be active players in lesional fibrogenesis, and hyperestrogenism and oxidative stress are likely drivers of both CAMs and fibrogenesis. Finally, endometriotic lesions harbouring CAMs would conceivably be more refractory to medical treatment, due, in no small part, to their high fibrotic content and reduced vascularity and cellularity.

WIDER IMPLICATIONS: The accumulating data on CAMs in endometriosis have shed new light on the pathogenesis and pathophysiology of endometriosis. They also suggest new challenges in management. The distinct yet co-evolving developmental trajectories of endometriotic stroma and epithelium underscore the importance of the lesional microenvironment and ever-changing cellular identity. Mutational profiling of normal endometrium from women of different ages and reproductive history is needed in order to gain a deeper understanding of the pathogenesis. Moreover, one area that has conspicuously received scant attention is the epigenetic landscape of ectopic, eutopic and normal endometrium.

Key words: bone-marrow-derived stem cells / cancer-associated mutation / developmental trajectory / endometriosis / endothelial–mesenchymal transition / epithelial–mesenchymal transition / fibrogenesis / mesothelial–mesenchymal transition / pathogenesis / pathophysiology

Introduction

Endometriosis is a benign gynaecological disease characterized by the ectopic deposition of endometrial-like tissues outside of the uterine cavity. It has three major subtypes, namely ovarian endometrioma (OE), deep endometriosis (DE) and superficial peritoneal endometriosis (PE) (Nisolle and Donnez, 1997). Featuring elevated local oestrogen production and inflammation, endometriosis is one of the major contributors to dysmenorrhea, infertility and chronic pelvic pain, impacting negatively on the quality of life in afflicted women (Vercellini *et al.*, 2014). Although OE is reported to be linked with increased risk of ovarian cancer (OVCA) of certain histotypes (Kurman and Shih Ie, 2010; Pearce *et al.*, 2012; Saavalainen *et al.*, 2018), the magnitude of elevated risk is fairly moderate and the resultant absolute risk of OVCA is still low (Pearce *et al.*, 2012; Guo, 2015; Saavalainen *et al.*, 2018). For extraovarian endometriosis, the risk of developing into cancer is near zero (Saavalainen *et al.*, 2018; Bulun *et al.*, 2019). Thus, it came as a complete surprise when Anglesio *et al.* reported in 2017 that the majority (79%) of DE lesions harbor somatic mutations and a sizeable portion of them (26%) contain known cancer-driver mutations on genes coding for AT-rich interactive domain-containing protein 1A (ARID1A), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit α (PIK3CA), Kirsten rat sarcoma (KRAS), or protein phosphatase 2 scaffold subunit Aα (PPP2R1A) in the epithelial, but not the stromal, component (Anglesio *et al.*, 2017). Although the authors were careful and did not say that the cancer-driver mutations in DE are synonymous with increased risk of developing cancer, the connotation of 'cancer-associated mutations' (CAMs) is nonetheless unnerving and somewhat unsettling or even alarming.

Since the report by Anglesio *et al.* (2017) four more studies have been published. While the study by Suda *et al.* reported

that 100% of OE lesions harboured somatic mutations and the majority of them also carried CAMs (Suda *et al.*, 2018), another study reported only 3% of OE had CAMs (Zou *et al.*, 2018). Lac *et al.* reported 10% of iatrogenic endometriosis (IE) and 22% of DE carried CAMs (Lac and Huntsman, 2018). Noe *et al.* reported that all six non-superficial endometriosis carried somatic mutations, and that the mutations were significantly enriched in epithelial but not stromal components of all lesions, suggesting that epithelium is clonal and its development is independent of stroma (Noe *et al.*, 2018).

Collectively, these studies shed new light on the pathogenesis and pathophysiology of endometriosis. As the circle of our knowledge expands, however, more questions can be raised.

Why is there such a large discrepancy in reported mutation frequencies? How can we reconcile such a discrepancy? Why does ectopic endometrium have a higher mutation rate than that of eutopic endometrium? Would the occurrence of CAMs in endometriotic lesions render an increased risk of cancer to their carriers? How often do CAMs occur? Do all patients with endometriosis, deep or otherwise, have CAMs in lesions sooner or later? What clinical implications, if any, do the CAMs have for the bearers? When a patient with endometriosis is found to have CAMs, should she be concerned or worried? OE is now well documented as being linked with OVCA, but why does extraovarian endometriosis seldom lead to cancer? Do these CAMs tell us anything about the pathogenesis and/or pathophysiology of endometriosis? Are there, if any, limitations in these studies? Finally, what kind of future research is needed so that we can build upon our knowledge and further unveil some long-standing mysteries and conundrums in endometriosis?

In this paper, I will provide a comprehensive overview on the current status of somatic mutations, especially CAMs, in endometriosis, identify possible causes of the CAMs, explain why there is discrepancy among studies, describe what these reported CAMs tell us about the pathogenesis and/or pathophysiology, and elaborate on the possible clinical implications of CAMs. Above all, I shall address the questions raised above and expose areas in need of further research so that we can learn more about the pathogenesis and/or pathophysiology of endometriosis.

Methods

PubMed was searched for all peer-reviewed original and review articles related to CAMs in endometriosis published in English from its inception to September 2019. The literature search was performed using main terms 'endometriosis and CAM', and 'endometriosis and cancer-driver mutation'. In addition, PubMed was also searched using the keywords and MeSH terms 'somatic mutations', 'fibrosis', 'fibrosis and epigenetic', 'CAMs and tumorigenesis', 'somatic mutation and normal tissues', 'oestrogen receptor and fibrosis', 'oxidative stress and fibrosis', 'ARID1A mutation', and 'KRAS mutation and therapeutics'. All retrieved papers were carefully assessed and, when relevant, incorporated into the review results. Their reference lists were also checked to identify any other study that could be relevant to this review. The eligibility of the retrieved studies was based mainly on abstract. The decision as whether or not the study was included in this review was made after careful assessment of its content.

Mutations and CAMs in endometriosis

Somatic mutations, defined as permanent and irreversible changes of the nucleotide sequence that are different from the host's germline, in endometriotic lesions are nothing new. Various forms of mutations, such as chromosomal aneuploidy (Shin *et al.*, 1997; Kosugi *et al.*, 1999), loss of heterozygosity (LOH) (Sato *et al.*, 2000) and copy number changes/genomic alterations (Gogusev *et al.*, 1999; Wu *et al.*, 2006a,b,c; Yang *et al.*, 2013) have been reported since the 1990s. Negative findings also have been reported (Rai *et al.*, 2010; Saare *et al.*, 2012).

Somatic mutations and cancer-driver mutations

Developed from a single fertilized egg, which is essentially just one single cell, a human being consists of 10^{13}–10^{14} cells in his/her body thanks to successive mitoses or cell divisions (Bianconi *et al.*, 2013). In its entirety, the 3.1 billion basepairs of human genome comprise the entire nucleic acid sequence encoded as DNA within the 23 chromosome pairs in the cell nuclei and a small amount of DNA sequence (0.054‰ of the genome) in the mitochondria.

In normal physiological conditions, cells in different organs/tissues in humans are constantly experiencing turnover. This renewal process maintains the cellular homeostasis and is vital to the health of an organism. Depending on the worn-out rate of the organs/tissues, some organs/tissues, such as intestinal epithelium, have a faster turnover, with new cells replenished within days. Other organs/tissues, such as the brain, have a much slower turnover and remain mostly in dormancy.

During development or in adulthood, mitosis entails DNA replication. While the replication mechanism has a very high fidelity, with the mutation rate in the order of 10^{-8}–10^{-7} per basepair per cell division (Nachman and Crowell, 2000; Araten *et al.*, 2005), mutation is still bound to occur given the enormous size of the genome and the number of replications. In just one cell division, the chance that *no* mutation occurred is $\sim 3.4 \times 10^{-14}$ assuming a mutation rate of 10^{-8} per basepair per division. This miniscule probability amounts to tossing a fair coin consecutively 45 times and getting the head up all the time.

Hence, mutagenesis is essentially stochastic in normal physiological conditions and inevitable. Because mutation occurs as a replication error, aging, which is intimately linked with an increasing number of cell divisions, is an important factor in causing genomic mutations (Rozhok and DeGregori, 2016). In fact, it has been shown that the total number of stem cell divisions, which varies greatly among different tissue types, is highly correlated with cancer risk. This explains why the incidence of certain types of cancer, such as colorectal cancer, is much higher than others, such as stomach cancer (Tomasetti and Vogelstein, 2015). Since human endometrium is a highly regenerative tissue undergoing monthly cycles of growth, differentiation and shedding during a woman's reproductive life, and since stem cells are involved in endometrial regeneration (Gargett *et al.*, 2016), spontaneous mutations are bound to occur in normal endometrium, and this may explain, at least in part, the mutations found in eutopic endometrium (Suda *et al.*, 2018).

In addition, when the organism/organs/tissues are exposed to an adverse environment, such as mutagenic chemicals/agents, UV light,

radiation, oxygen radicals, persistent inflammation and other deleterious factors, mutagenesis may be further accelerated (Cogliano *et al.*, 2011) leading eventually to DNA damage, inactivation of tumour suppressor genes and oncogene activation. Moreover, similar to the evolution of species, cells with different genetic variants are under selective pressure: those with a high explicative fitness would eventually outnumber cells with lower fitness. As a result, the existence of somatic mosaicism is fully documented in humans, and has been viewed as an aging phenotype (De, 2011; Risques and Kennedy, 2018).

Not all mutations are deleterious. In fact, the majority of mutations are harmless and have no impact on functionality or behaviours of the cell that bears the mutation and, as such, these mutations are accumulated passively (Martincorena *et al.*, 2017). But every now and then, an important gene is mutated, and the cells bearing the mutation have a competitive edge over other cells, such as higher proliferative or survival propensity, resulting in the gradual and progressive domination of the cells with that mutation. Such cells, called the mutant clones, may acquire and accumulate further mutations and are the origin of cancer cells (Fialkow, 1976; Nowell, 1976).

In contrast to 'passenger' mutations, which occur randomly and confer no fitness to their bearers (Muller *et al.*, 2012), cancer 'driver' mutations or CAMs are implicated in pathways that are critical in determining the proliferative, survival and metastatic propensity of tumour cells (Kato *et al.*, 2016). Thus, CAMs are thought to be rare in benign conditions such as endometriosis, are present mostly in pre-malignancy and are most frequent in metastatic cancer or those with a metastatic potential (Kato *et al.*, 2016).

Detection of somatic mutations

Somatic mutations are traditionally detected by many, mostly low-resolution, methods. For detecting copy number changes, cytogenetic approaches such as multi-colour fluorescence *in situ* hybridization (FISH), conventional or array-based comparative genomic hybridization (CGH) and LOH analysis are often used. For detecting gene mutations, genotyping and Sanger sequencing are often used. FISH, CGH, LOH, genotyping and Sanger sequencing are of low resolution and of higher error rate, and, as such, can only detect mutations in a few predetermined loci or in large chromosomal segments, and are restricted to detecting variant allele frequencies typically higher than 10% (Strom, 2016; Risques and Kennedy, 2018).

With the advent, and particularly the increasing affordability, of the next generation sequencing (NGS) technologies, the resolution of detection has increased dramatically and the error rate has been reduced substantially. The new error-correction NGS can further increase the resolution and reduce the error rate, making the detection of low-frequency mutations much easier and more accurate. Currently, error-corrected NGS technologies can detect mutations in the 0.001–0.1% range (Risques and Kennedy, 2018). Consequently, as the detection resolution increases, higher and higher somatic mutation rates have been reported in some benign diseases and even in tissues that are physiologically normal (Risques and Kennedy, 2018). This dramatic improvement in detection accuracy has fundamentally changed our views on somatic mutation burdens, mutational signatures, structural variants and the frequency of CAMs in apparently normal individuals. For example, older studies using NGS technology reported CAMs in about 10% of individuals older than 65 years, but studies using error-corrected NGS technologies indicate that the CAMs prevalence in adults is nearly 100% (Krimmel *et al.*, 2016; Young *et al.*, 2016).

In addition, since different cell types in the same tissue often have distinct developmental trajectories and the proportions of different cell types in the same tissue vary greatly in different people, the use of microdissection in harvesting the desired cell type greatly increases the signal-to-noise ratio and helps to detect the true mutations in a particular cell type. For example, before the use of microdissection, the clonality of endometriotic epithelial cells could not be unequivocally determined in about 18–40% of the cases due to cell contamination, but with microdissection the clonality can be determined in all cases (Wu *et al.*, 2003).

This can explain why studies that did not use microdissection often reported a much lower somatic mutation rate. For example, Vestergaard *et al.* reported that only 1 (4.3%) out of 23 patients with endometriosis was found to harbour mutations (Vestergaard *et al.*, 2011) (Table I). Similarly, Zou *et al.* reported that 3 (3.0%) of 101 OEs were found to have CAMs (Zou *et al.*, 2018). Consequently, while these data appear to be genuine, the fact that the endometriotic epithelium and stroma apparently have different mutational profiles (Noe *et al.*, 2018) renders their conclusions questionable.

Moreover, it also explains why studies using low-resolution detection methods reported many fewer mutations. For example, using PCR in combination with denaturing gradient gel electrophoresis (DGGE) on nine cancer-associated genes, Vestergaard *et al.* reported that only 1 (4.3%) out of 23 patients with endometriosis was found to harbour mutations (Vestergaard *et al.*, 2011) (Table I). In contrast, using the whole-exome sequencing method (much more accurate than DGGE), Li *et al.* found all 16 (100%) patients with OE harboured various somatic mutations (Li *et al.*, 2014).

Therefore, whether or not microdissection is used, the accuracy of the detection method (so that low-abundance mutations can be detected) and the scope of detection (detecting specific mutations or unbiased whole-exome or whole-genome sequencing) should largely determine the mutation frequency. The difference in detection methodology accounts for the discrepancy in reported mutation rates.

Somatic mutations in tumours and in normal tissues

Somatic mutations used to be thought to occur exclusively in pathological tissues such as cancer, pre-neoplastic tissues, or normal tissue adjacent to tumours. As mutation detection techniques become more accurate, affordable and higher resolution, it becomes evident that spontaneous somatic mutations or genomic alterations can, and do, occur in apparently normal tissues and benign conditions (Kato *et al.*, 2016; Risques and Kennedy, 2018). Notably, use of the microdissection technique and elimination of sequencing errors greatly increases the detection accuracy, permitting detection of low-frequency mutations in apparently normal or healthy tissues/people (Krimmel *et al.*, 2016; Dong *et al.*, 2017) that would otherwise be missed using the older sequencing methods. In addition, mutations are found to be increasingly more abundant in aging tissues, indicating that aging or the number of replications is a major driving force in generating mutations (Kinde *et al.*, 2011; Schmitt *et al.*, 2012; Hsieh *et al.*, 2013; Blokzijl *et al.*, 2016; Hoang *et al.*, 2016; Nair *et al.*, 2016; Mattox *et al.*, 2017; Martincorena *et al.*, 2018). Within the same individual, there is a substantial variation

Table I Summary results of studies reporting CA mutations in endometriosis.

Study	Type of endometriosis	Sample size	Patient age (years)	Detection method	Names of mutated genes	Percentage of patients who had mutations	Major findings	Microdissection (epithelium)
Vestergaard *et al.* (2011)	Not reported	$N = 23$	31.0 ± 5.2	9 CA genesPCR in combination with denaturing gradientgel electrophoresis (DGGE) Sensitivity: ~5%	BRAF, HRAS, NRAS, CTNNB1, CDK4, FGFR3, PIK3CA, TP53 and PTEN	1/23 = 4.3%		No. Mixed cell populations
Li *et al.* (2014)	OE	$N = 16$	32.4 ± 6.2	Whole-exome sequencing (Estimated to be able to detect 76% of existing mutation)		100%	C-T Mutations sig. higher Eu and Ec have distinctive mutation profiles	Yes
Anglesio *et al.* (2017)	DE	$N = 24$	36.7 ± 7.4	Exome-wide sequencing	various	19 (79.2%) 5 (20.8%) cancer-driver	Only in epithelium	Yes
Suda *et al.* (2018)	OE	$N = 13$	42.9 ± 11.0	whole-exome sequencing	Various	13 (100.0%) Driver mutations: 10 (76.9%)	Discordant mutational profiles between eutopic and ectopic endometrium is found	Yes
Lac *et al.* (2018)	Iatrogenic endometriosis and DE	$N = 40$ $N = 36$	36.5 ± 5.5 33.9 ± 7.0	Targeted sequencing	33 genes (exons and hot spots)	4 (10.0%) 8 (22.2%)		Yes
Zou *et al.* (2018)	OE	$N = 101$	32 (median)	PCR amplification of the potential mutational hotspot regions of KRAS, PPP2R1A, PIK3CA, BRAF, NRAS, HRAS, ERK1, ERK2 and PTEN genes	KRAS, PPP2R1A, PIK3CA, BRAF, NRAS, HRAS, ERK1, ERK2 and PTEN genes, as well as the entire coding region and corresponding intron/exon boundaries of the ARID1A gene. Then sequencing and compared against the DNA derived from PBMC.	3 (3.0%)	KRAS p.G12 V, PPP2R1A p.S256F, ARID1A	No
Noe *et al.* (2018)	Mixed	DE: $n = 5$ OE: $n = 1$	40.3 ± 8.8	Exome sequencing		6 (100%)	Significantly enriched mutations in epithelial but not in stromal components	Yes

CA: cancer associated; DE: deep endometriosis; Ec: ectopic endometrium; Eu: eutopic endometrium; OE: ovarian endometrioma; PBMC: peripheral blood mononuclear cell, ARID1A: AT-rich interactive domain-containing protein 1A; BRAF: B-Raf proto-oncogene, serine/threonine kinase; CDK4: cyclin-dependent kinase 4; CTNNB1: catenin $\beta 1$; ERK1: extracellular signal-regulated kinase 1; ERK2: extracellular signal-regulated kinase 2; FGFR3: fibroblast growth factor receptor 3; HRAS: HRas proto-oncogene, GTPase; KRAS: KRAS proto-oncogene, GTPase; NRAS: NRAS proto-oncogene, GTPase; PIK3CA: phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit α; PPP2R1A: protein phosphatase 2 scaffold subunit Aα; PTEN: phosphate and tension homology deleted on chromsome ten; TP53: tumour protein p53.

in genomic alterations among different tissues, with more alterations seen in tissues with a fast turnover and in genes involved in cell regulation (O'Huallachain *et al.*, 2012). Moreover, these age- or replication-related mutations are often CAMs (Martincorena *et al.*, 2015; Krimmel *et al.*, 2016; Martincorena *et al.*, 2018). That is, somatic mutation and even CAMs can, and do, occur in apparently healthy or normal tissues.

Noteworthy is that massive mutations in physiologically normal tissues have been detected when deep sequencing methods are used (Martincorena *et al.*, 2015; Martincorena *et al.*, 2018). Healthy cells in the oesophageal epithelium, for example, carry at least several hundred mutations per cell in people in their 20s or over 2000 mutations in older people (Martincorena *et al.*, 2018), suggesting that replicative mutations accumulate with age. In addition, in tissues exposed to the sun, large numbers of mutant clones under positive selection are found, and the signatures of the mutations are consistent with the DNA damage induced by UV rays (Martincorena *et al.*, 2015), highlighting the impact of environmental exposure on somatic mutations. Many of these mutations are CAMs, which confer selective advantages. Surprisingly, the prevalence of NOTCH1 (a cancer-driver gene) mutations in normal oesophagus is found to be several times higher than in oesophageal cancers (Martincorena *et al.*, 2018). While these clones appear to be a result of normal aging, it is likely that they may acquire more genetic advantages over time and eventually transform into malignancy (Martincorena *et al.*, 2018). Indeed, a fitness benefit of merely 0.4% over the time course of 20 years might be enough to lead to malignant transformation (Bozic *et al.*, 2010). Hence, the presence of CAMs is not necessarily synonymous with cancer or tumour development.

Of course, the mutational processes underlying normal aging also are operative in tumorigenesis in a given organ or tissue (Kinde *et al.*, 2011; Schmitt *et al.*, 2012; Hoang *et al.*, 2016; Mattox *et al.*, 2017; Risques and Kennedy, 2018). So much so that half or more of somatic mutations in tumours are estimated to have arisen before initiation of a tumour (Tomasetti *et al.*, 2013), and that replication-induced mutations have been proposed to account for up to two-thirds of the mutations in human cancers even after adjustment for an environmental and hereditary propensity for malignancy (Tomasetti *et al.*, 2017).

Extensive sequencing of adult stem cells of various organs with different cancer incidences has shown that these organs gradually accumulate mutations at very similar rates, but their mutation profiles vary from tissue to tissue (Blokzijl *et al.*, 2016). Hundreds to thousands of mutations are present in tumour cells and are shared by most or all tumour cells, but the mutation burden correlates with patient's age (Welch *et al.*, 2012; Milholland *et al.*, 2015). Consistently, the cancer incidence seems to be correlated with the number of stem cell divisions across a wide variety of cancer types (Tomasetti and Vogelstein, 2015). In fact, these age-associated or replication-driven mutations appear to have particular mutational signatures (Alexandrov *et al.*, 2013; Alexandrov *et al.*, 2015). The acquisition of CAMs apparently confers an explicative advantage, resulting in the clonal expansion of the founder cell (Vogelstein *et al.*, 2013; Martincorena *et al.*, 2018).

Consistent with the reported massive mutations in various physiologically normal tissues (Martincorena *et al.*, 2015; Blokzijl *et al.*, 2016; Franco *et al.*, 2018; Lee-Six *et al.*, 2018; Martincorena *et al.*, 2018), a recent study employing targeted sequencing of hotspot regions in cancer-related genes in combination with immunohistochemistry analysis on 110 women who had undergone either hysterectomy or iatrogenic procedures reports that 51–64% of women carry CAMs in their endometrium without any evidence of malignancy or even subtle pathology, with KRAS (28.2%), PIK3CA (12.7%), and phosphate and tension homology deleted on chromsome ten (PTEN), PTEN (27.3%) being the most common (Lac *et al.*, 2019). Consistently, the results showed that the mutation rate is a linear function of age, with the likelihood of harbouring a mutation in endometrial tissue increased by 5% per year, independent of menstrual phase (Lac *et al.*, 2019).

Using microdissection and NGS whole-genome sequencing method, another study reports mutation burden, and signatures and CAMs on 215 histologically normal endometrial glands isolated from 18 women (Moore *et al.*, 2018). Remarkably, it finds that, in normal endometrial glands, there is an average of 1324 base substitutions and an average of 85 insertions/deletions (indels) per woman (Moore *et al.*, 2018). Again, the mutation rate correlated linearly with age, accumulating ~28 base substitutions per gland per year during adult life and an extra 20 substitutions with each increasing unit of BMI (Moore *et al.*, 2018). Many of these mutations occur early in life, and different mutational processes appear to be operative (Moore *et al.*, 2018). Nearly 95% of women evaluated are found to harbour various CAMs in their endometrial glands, including KRAS, PIK3CA, phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1), Rho GTPase activating protein 35 (ARHGAP35), PPP2R1A and F-box and WD repeat domain containing 7 (FBXW7), which have been reported to be present in ectopic endometrium (Anglesio *et al.*, 2017, Suda *et al.*, 2018) as well as in endometriosis-associated ovarian cancer (EAOC) (Kuo *et al.*, 2009).

CAMs in endometriosis: what do they tell us?

Given the above discussion, it seems evident that we can disregard, without loss of much information, those studies reporting mutations in endometriosis that did not use microdissection to harvest cells of the desired type and/or used low-accuracy detection methods. After this screening, only five studies were considered to be trustworthy.

Using whole-exome sequencing of endometriotic epithelial cells, Li *et al.* reported all 16 patients with OE harbour various somatic mutations and identified frequent alterations in genes involved in cell adhesion and chromatin-remodelling complexes (Li *et al.*, 2014). This is the first study to show that all OE lesions seem to have somatic mutations (Table I). In addition, their pathway analyses using genes found to be recurrently mutated identified chromatin remodelling as one of the enriched functional groupings. In particular, their data suggest that mutated genes encode a histone methyltransferase involved in histone H3 lysine 4 (H3K4) modification, echoing previous reports of aberrant H3K4 methylation in endometriosis (Xiaomeng *et al.*, 2013; Monteiro *et al.*, 2014; Sun *et al.*, 2016). Their results thus lend support for the notion that endometriosis can be characterized with epigenetic aberrations (Guo, 2009a,b).

The study by Anglesio *et al.* showed that in women with DE a sizeable portion of lesions contain known CAMs in the epithelial component only (Anglesio *et al.*, 2017) (Table I). Since DE is rarely reported to be associated with malignancy, this study presents results that are quite unnerving. However, the clinical significance remains largely unclear.

The same group recently published another study on mutations in IE as well as DE (Lac *et al.*, 2018). Here, IE refers to endometriotic lesions resulting from the surgical scars of previous obstetric or gynaecological procedures. Using microdissection and a hypersensitive cancer hotspot

sequencing panel, they found that 10% of IE and 22% of DE lesions harbour CAMs. In addition, 18% of IE and 14% of DE lesions exhibited, by immunoreactivity, loss of PTEN, a tumour suppressive gene, in the epithelial component (Lac *et al.*, 2018). Combining sequencing data and immunohistochemistry results, they reported an overall rate of CAMs in 28% of IE and 36% DE lesions (Lac *et al.*, 2018) (Table I), confirming their previous study (Anglesio *et al.*, 2017). In addition, it demonstrates the similarity of the two types of endometriosis (harbouring considerable CAMs) and their differences (somewhat different mutation profiles) (Lac *et al.*, 2018). That is, CAMs are not exclusively confined to DE but can actually be seen in other subtypes of endometriosis as well.

Noe *et al.* recently reported that, among 19 mutations sequenced in six patients with OE or DE, all were significantly enriched in epithelial cells but not in stromal cells, suggesting that the evolution of non-superficial endometriosis is not straightforward: epithelium is clonal and its development is independent of stroma (Noe *et al.*, 2018). Using droplet digital PCR analysis of microdissected epithelium- and stroma-enriched endometriosis tissues, they report that the 19 somatic passenger mutations analyzed were predominantly found in the epithelial compartment, in contrast to very few mutations in the stromal one (Noe *et al.*, 2018) (Table I). These findings are consistent with the previous report that the endometriotic epithelial cells are monoclonal (Wu *et al.*, 2003), whereas stromal cells may be continuously regenerated or recruited during lesional progression and development. This led to the conclusion that the evolution of endometriosis is complex, in that epithelium is clonal and its development is independent of stroma (Noe *et al.*, 2018). In the authors' own words, the results 'do not support the views that endometriosis originate from a single stem/progenitor cell, which differentiate to both epithelial and stromal cells, or the epithelial cells differentiate into stromal cells through epithelial–mesenchymal transition at the site of endometriosis' (Noe *et al.*, 2018). Lac and Huntsman (2018) further elaborated this point, proposing that endometriotic epithelium and stroma may have distinct developmental trajectories. In particular, they argue that, given the data, the most likely scenario is that progenitor cells undergo clonal expansion to give rise to epithelial cells, whereas stromal cells come into being without clonal expansion (Lac and Huntsman, 2018).

By using a combination of microdissection, independent discovery and validating samples, whole-exome and target-gene sequencing, multiregional sequencing of several sites (multiple lesions as well as eutopic endometrium) from the same individuals, and single endometrial gland sequencing, the study by Suda *et al.* is by far the most informative one to date on CAMs in endometriosis, especially for OE. They reported that epithelial cells within OE lesions exhibit extensive CAMs and clonal expansion, and that the genomic architecture of epithelial cells in uterine endometrium is heterogeneous (Table I). While overall the OE lesions and normal endometrium had a similar number of somatic mutations per Mb sequenced, the mutational profiles in ectopic and eutopic endometrium from the same patient were discordant (Suda *et al.*, 2018). In addition, single endometrial glands carry distinct CAMs, even though the tissues appeared to be histologically benign and normal (Suda *et al.*, 2018). In general, the distributions of mutant allele frequency (MAF) in endometriotic epithelium were higher than those in uterine endometrial epithelium, and some endometriotic epithelium harboured arm-level allelic imbalances that are consistent with LOH in regions harbouring CAMs. One of their interesting findings is that lesions that were in close physical proximity appeared to have similar mutations, yet lesions located on the right and left ovaries displayed entirely different mutations, suggesting different developmental origins (Suda *et al.*, 2018). Individual endometrial glands within the normal uterus of the same individual carried distinct somatic mutations. In many ways, the study by Suda *et al.* (2018) has provided a greater understanding of the spatiotemporal evolution of OE and a much-needed glimpse at the unique mutational profiles of endometriotic epithelium and uterine endometrial epithelium, and also demonstrated a clear explicative advantage of acquiring these CAMs.

Based on these findings, Suda *et al.* offered a plausible explanation for the pathogenesis of OE, which originates from eutopic endometrium that already carries CAMs that confer selective advantages once regurgitated into the peritoneal cavity through retrograde menstruation, resulting in clonal expansion and ultimately causing symptoms (Suda *et al.*, 2018). In other words, endometriosis originates from a defective endometrium that is harbouring CAMs.

Shedding light on pathogenesis and pathophysiology

Pathogenesis

In endometrium, each menstrual cycle is analogous to classic tissue injury and repair, which include inflammation, its resolution, angiogenesis, tissue formation and remodelling or re-epithelialization (Maybin and Critchley, 2015). Each gland in the endometrium appears to be regenerated from a committed endometrial stem cell (Tanaka *et al.*, 2003). Similar to eutopic endometrium, the ectopic endometrium sheds glandular epithelial cells during menstruation, but considerably less so in the endometriotic stromal cells, which also house recruited progenitor cells and transdifferentiated cells (see below).

The study by Suda *et al.* has demonstrated beautifully and convincingly the power of sequencing in establishing the phylogenetic relationship between two clones of cells. Based on their results, Suda *et al.* proposed that the endometrium of women with OE contains, prior to the formation of OE lesions, endometrial glands with pre-existing CAMs that may have selective advantages, which subsequently acquire more CAMs after successfully implanting onto the ectopic sites, which then go through clonal expansions (Figure S7 in (Suda *et al.*, 2018)).

However, caution should be exercised here. First, the finding of CAMs in endometrial glands is based on target-gene sequencing of 109 single endometrial glands from the uteri of three women, aged 38, 47 and 49 years old, respectively (Suda *et al.*, 2018). Small sample size aside, the three patients were older than the mode of the age at first surgery in women with OE (Liu *et al.*, 2008). OE is frequently diagnosed before 38 years of age and in some cases in adolescent girls (Saridogan, 2017) even though a diagnostic delay is well documented in endometriosis (Hadfield *et al.*, 1996; Arruda *et al.*, 2003). Given the somewhat ubiquitous age-related somatic mutations in healthy organs/tissues (Risques and Kennedy, 2018), and since endometrium is a highly regenerative tissue that displays monoclonality in each endometrial gland yet the entire endometrium exhibits a mosaic pattern of clonal distribution (Tanaka *et al.*, 2003; Wu and Guo, 2008), it is conceivable that normal endometrium, especially from older women, may harbour somatic mutations or even some CAMs. In fact, it has

been shown recently that the most common CAMs in apparently normal endometrium are KRAS and PIK3CA (Lac *et al.*, 2019). The whole-genome sequencing study by Moore *et al.* also shows that many CAMs detected in the glands of endometrium, such as KRAS, PIK3CA, PIK3R1, ARHGAP35, FBXW7, fibroblast growth factor receptor 2 (FGFR2), PP2R1A, PTEN, zinc finger homeobox 3 (ZFHX3), and AT-rich interaction domain 5B (ARID5B), are all present in histologically normal endometrium from women without endometriosis or uterine fibroids (Moore *et al.*, 2018). Thus, the proposal, based on the *post hoc* evidence of CAMs in eutopic endometrium from women with endometriosis, that women with endometriosis or OE have pre-existing CAMs before the genesis of endometriosis is simply premature, especially in the absence of any data suggestive of a phylogenetic relationship between endometriotic lesions and the eutopic endometrium.

Second, while the suspicion that endometriosis may originate from defective endometrium has long been raised (Vinatier *et al.*, 2000), we need to understand that most, if not all, data on the endometrial aberrations are collected from patients who have already been surgically and histologically diagnosed with endometriosis. In fact, data from several well designed animal studies of endometriosis consistently and unequivocally indicate that, once endometriosis is induced artificially, the eutopic endometrium then acquires various molecular and histological aberrations (Kim *et al.*, 2007; Lee *et al.*, 2009; Sherwin *et al.*, 2010; Naqvi *et al.*, 2016; Kim *et al.*, 2019). More remarkably, the extent of endometrial aberrations appears to depend on the proximity of endometriotic lesions to the uterus (Naqvi *et al.*, 2016). These data strongly suggest that the numerous endometrial aberrations in women with endometriosis may be more likely to be the consequence, rather than the cause, of endometriosis.

In fact, the data presented in the Suda *et al.* (2018) study actually support this view: one, the mutation profiles between ectopic endometrium and their eutopic counterpart are different (Fig. 1B; Tables S2 and S3 in (Suda *et al.*, 2018)); and, two, the number of high MAF mutations per Mb is higher in endometriotic epithelium than that in endometrial epithelium, even though KRAS and PIK3CA are the most frequently mutated genes in both ectopic and eutopic endometrium (Fig. 1B and Fig. S1B in (Suda *et al.*, 2018)). Should mutations in eutopic endometrium be responsible for the genesis and formation of ectopic endometrium, the endometriotic lesions can be considered as a *de facto* clonal expansion of its eutopic ancestry, and the MAF of the mutated genes in eutopic endometrium would be no lower than that of the ectopic endometrium. Of course, since endometrium is polyclonal (Tanaka *et al.*, 2003), and since only a few endometrial samples were harvested and sequenced in the study (Suda *et al.*, 2018), it is possible that the eutopic endometrial tissue samples sequenced may not be the clone that was descended from the one that caused endometriosis. Regardless, however, the data presented in Suda *et al.* (2018) are insufficient to conclude that endometrial CAMs predate the genesis of endometriosis.

Hence, based on the very recent data on the mutational burden and CAMs in histologically normal endometrium (Moore *et al.*, 2018; Lac *et al.*, 2019) and the evidence that eutopic endometrium apparently acquires molecular aberrations after the induction of endometriosis (Kim *et al.*, 2007; Lee *et al.*, 2009; Sherwin *et al.*, 2010; Naqvi *et al.*, 2016; Kim *et al.*, 2019), the notion that the endometrium of women with endometriosis contains, prior to the lesion formation, endometrial glands with pre-existing CAMs, which are responsible for the genesis of endometriosis is questionable at least, especially because these CAMs are detected *post hoc,* after endometriosis has been diagnosed.

From distinct developmental trajectories to partners in crime

One question left unanswered in Noe *et al.* (2018) is: if endometriotic stromal cells are under the same pressure of replication error as epithelial cells, why do they harbour much less mutations than the latter? Lac and Huntsman speculate that 'epithelial cells may be an integral process in the pathogenesis of endometriosis. Stromal cells, in contrast, may play a more-supportive role in endometriosis, and are likely to be continuously regenerated or recruited to the site of endometriotic lesions. It is possible that stromal cells may arise from the continuous induction of metaplasia of surrounding cells to become endometrium-like stroma' (Lac and Huntsman, 2018). They are correct in general, but perhaps a more complete account will help to shed light on the development of endometriosis, probably more on its pathophysiology than on its pathogenesis.

First, it turns out that, aside from the resident fibroblasts/stromal cells, the stromal component in endometriotic lesions consists of fibroblasts and myofibroblasts, which can be recruited or transdifferentiated from several sources. First, they can be differentiated from endometriotic epithelial cells through epithelial–mesenchymal transition (EMT), currently an area of active research with over 80 PubMed-indexed papers and counting (Matsuzaki and Darcha, 2012; Zhang *et al.*, 2016a,b). This may explain why the stromal component still shares some, but much fewer, mutations at some loci with the epithelial component, as observed in Noe *et al.* (2018). It could also explain why in some DE lesions the glandular epithelium is absent, yielding what is termed 'stromal endometriosis', that is, endometriotic lesions without glandular epithelium (Mai *et al.*, 1997; Clement, 2007), which is reported to be seen in 27–45% of cases of PE (Abrao *et al.*, 2003; Boyle and McCluggage, 2009; Kamergorodsky *et al.*, 2009), 0–13% of OE (Abrao *et al.*, 2003; Kamergorodsky *et al.*, 2009) and 12–15% of DE (Abrao *et al.*, 2003; Kamergorodsky *et al.*, 2009).

Second, the bone-marrow-derived stem cells (BMDSCs) can be recruited into the stroma. In fact, in a mouse model of endometriosis that received bone marrow transplantation, it is found that approximately 0.1% of stromal cells and 0.04% of epithelial cells in lesions are of donor origin (Du and Taylor, 2007). In other words, the stroma and epithelium recruited BMDSCs in the ratio of 2.5:1. In another study, most BMDSCs were found in endometriotic stroma in mice with induced endometriosis (Ersoy *et al.*, 2017). Thus, the lesional stromal component recruits more BMDSCs that naturally contain less somatic mutations than the epithelial component.

Third, endometriotic stromal cells also can be transdifferentiated from other cells, such as endothelial cells through endothelial–mesenchymal transition (EndoMT) or mesothelial cells through mesothelial–mesenchymal transition (MMT), as shown in a recently established mouse model of deep endometriosis (Yan *et al.*, 2017) (also Yan *et al.*, unpublished data). They might also be transdifferentiated from cells other than endothelial and mesothelial cells, such as pericytes, or fibrocytes and perhaps monocytes (Mack and Yanagita, 2015).

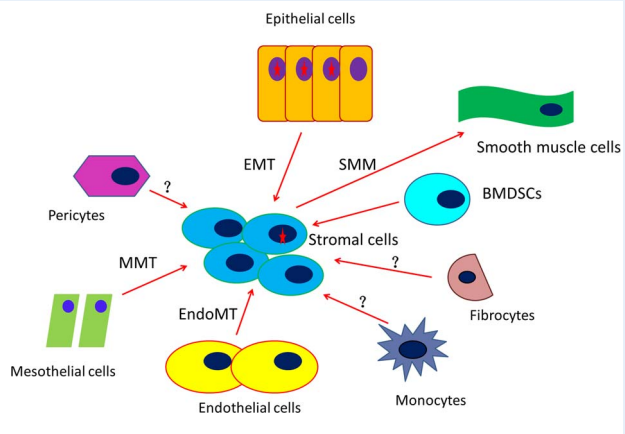

Figure 1 Schematic illustration that explains the distinct developmental trajectories of endometriotic stroma and epithelium. In essence, in contrast to endometriotic epithelial cells that experience turnover, the stromal component of endometriotic lesions recruits other cells, such as endothelial cells, mesothelial cells and epithelial cells, through the endothelial–mesenchymal transition (EndoMT), mesothelial–mesenchymal transition (MMT) and epithelial–mesenchymal transition (EMT). In addition, the stromal component may recruit bone marrow-derived stem cells (BMDSCs), pericytes, fibrocytes and monocytes. Moreover, outflow of stromal cells due to smooth muscle metaplasia (SMM) can cause the loss of older stromal cells. Cells with a red start indicate that these cells carry cancer-associated mutations (CAMs). The red arrows indicate the direction of transdifferentiation and/or recruitment. The question mark indicates the uncertainty towing to lack of evidence.

Lastly, in addition to the inflow of cells that are typically devoid of CAMs, there is also an outflow of cells from the stromal component due to smooth muscle metaplasia, i.e. stromal cells eventually transdifferentiated into smooth muscle cells. This would effectively move those older, originally stromal cells, out of the stromal component since these cells now become smooth muscle cells.

As myofibroblasts are the major effector cells of fibrogenesis, the ever-increasing stromal component would result in progressive fibrogenesis, which, in turn, leads to increased matrix stiffness but reduced vascularity and cellularity (Liu *et al.*, 2018a,b). The increased matrix stiffness has been shown to attenuate EMT (Matsuzaki *et al.*, 2017), which reduces the number of stromal cells differentiated from epithelial cells (which may or may not carry CAMs already). The reduced EMT effectively cuts the supply of CAM-carrying epithelial cells to the stromal component. This, coupled with supplementation of other cells that are devoid of CAMs to the component, may explain why there is a much lower mutation frequency in the stromal component (Noe *et al.*, 2018) as the component also recruits endothelial, mesothelial and other cells.

In view of the above, the stromal component of endometriotic lesions is formed from multiple sources: endometriotic epithelial cells, endothelial cells, mesothelial cells, BMDSCs and perhaps other cells as well. These cells may intrinsically have much lower mutation rates than that of endometriotic epithelial cells, giving rise to the results reported in Noe *et al.* (2018). A diagram depicting the scenario described here is shown in Figure 1.

However, the seemingly independent developmental trajectories should not be construed to mean that the two components go separate ways, leaving the other completely alone. On the contrary, it is well known that the function and morphogenesis of endometrial epithelial cells are regulated by paracrine effectors secreted by stromal cells (Arnold *et al.*, 2001).

One particularly important player involved in the paracrine effect is the exosomes secreted by endometriotic stromal and epithelial cells. For example, exosomes secreted by epithelial cells induce myofibroblast transformation in stromal cells and also neovascularization (Han *et al.*, 2017). Likewise, stroma-derived exosomes can also promote epithelial wound healing (Samaeekia *et al.*, 2018). Exosomes derived from endometriotic stromal cells are reported to enhance angiogenesis (Harp *et al.*, 2016).

Endometriotic epithelial cells, on the other hand, express many growth factors and chemokines that are responsible for the migration, proliferation and activation of fibroblasts. For example, transforming growth factor (TGF)-β1 is most prominently expressed in the epithelial component of lesions (Chegini *et al.*, 1994; Tamura *et al.*, 1999). The increased levels of TGF-β1, the archetypal profibrotic molecule, may induce fibroblast-to-myofibroblast transdifferentiation (FMT) in stromal cells (Zhang *et al.*, 2016a,b,c). Therefore, the passenger mutations exclusively enriched in endometriotic epithelial component, as found in Noe *et al.* (2018), do suggest distinct developmental trajectories of endometriotic epithelium and stroma, but the trajectories are by no means independent. They are actually dependent and co-evolve in the development of endometriosis.

But why do normal and eutopic endometrial epithelium have much lower mutation rates, as in ectopic endometrial epithelium, as shown in Suda *et al.* (2018)? There are several possibilities. First of all, BMDSCs have been shown to give rise to multiple endometrial cell types, including stromal, glandular and luminal epithelial cells (Taylor, 2004; Du and Taylor, 2007). These BMDSCs presumably harbour much less mutations when recruited to the endometrial epithelial component.

Second, in normal endometrium, the tissue regeneration in each menstrual cycle involves mesenchymal–epithelial transition (MET); i.e. stromal cells are transdifferentiated into epithelial cells and contribute to the 're-epithelialization' as part of the repair process (Huang *et al.*, 2012; Patterson *et al.*, 2013; Cousins *et al.*, 2014). Hence, the epithelial component in eutopic endometrium receives new recruits in each and every cycle. In contrast, the MET in ectopic endometrium appeared to be much diminished (Matsuzaki and Darcha, 2012). Since the endometrial stromal component may have recruited BMDSCs and thus have much lower mutations than their epithelial counterpart, as eluded to above, the transformed epithelial cells through MET in eutopic endometrium presumably should have less mutations than the original epithelial cells in ectopic endometrium, which are the descendants of successive cell divisions, essentially devoid of any replenishment from other sources.

Third, ectopic endometrium faces a much harsher microenvironment, comprising a DNA-damaging milieu. Fuelled by increased local oestrogen production and chronic inflammation, endometriotic lesions are known to have excess proliferative potential (Hapangama *et al.*, 2010). Yet chronic inflammation is intimately linked with increased oxidative stress, which is manifested by the increased production of reactive oxygen species (ROS) as well as reduced ROS detoxification (Alexandre *et al.*, 2006). Cellular proliferation also is closely correlated

with production of endogenous ROS through the activation of growth-related signalling pathways, including the mitogen-activated protein kinase ERK1/2 (McCubrey *et al.*, 2006). In cancer in particular, ROS production modulates tumour cell proliferation (Laurent *et al.*, 2005). Yet, ROS is one particularly destructive aspect of oxidative stress and can cause damage to cells and DNA (Evans and Cooke, 2004).

In view of the above discussion, it can be seen that the finding by Noe *et al.* (2018) highlights the distinct and co-evolving, but certainly not independent, trajectories of endometriotic epithelium and stroma, and underscores the multiple sources of fibroblasts/myofibroblasts in the stromal compartment. Since the myofibroblast is the major effector cell in fibrogenesis, this underscores fibrogenesis as a rather inevitable consequence of recruiting multiple, different cells into the stromal component of lesions.

There are at least two important implications for future research. First, while EMT is important in lesional development, its importance should not be over-emphasized. Second, the sources of myofibroblasts in endometriotic lesions deserve much more investigation, especially given the somewhat extensive research in EMT. In future research, lineage-tracing methods could be employed to ascertain the sources of myofibroblasts in the lesional stromal component.

Implications for treatment

Interestingly, few papers reporting CAMs, or somatic mutations for that matter, in endometriosis have discussed any possible clinical implications for treatment. Since mutations are permanent and irrepressible genomic alterations, unless the cells harbouring the mutations can be completely eliminated, the mutations in these cells are unlikely to be altered by pharmacological means and, as such, may exert their influence through aberrant gene and protein expression. This may be another reason for why medical treatment is challenging for endometriosis. This is particularly of concern when CAMs are present. Drugs that work when CAMs are absent may encounter resistance. For example, enhancer of zeste homolog 2 (EZH2) inhibitors that may work well for endometriosis (Zhang *et al.*, 2017) could experience resistance when lesions carry ARID1A mutations, as in OVCA (Wu *et al.*, 2018). As alluded to by Lac *et al.* (2018), targeting the RAS-pathway is difficult (Samatar and Poulikakos, 2014) and has potential toxicities related to phosphatidylinositol 3-kinase (PI3K)-AKT serine/threonine kinase (Akt) pathway inhibitors (Engelman, 2009).

In particular, while KRAS could potentially be a superb drug target for treating various cancers, its suppression by pharmacological means has faced serious challenges. The major reason for this is that the surface of KRAS protein lacks druggable pockets (Kessler *et al.*, 2019). Using a structure-based drug design, it was recently reported that a new small-molecule, BI-2852, a novel KRAS inhibitor that binds with nanomolar affinity to the small pocket on the KRAS surface that is previously thought to be undruggable, has been discovered (Kessler *et al.*, 2019). However, whether such a class of drugs can be used for treating endometriosis remains unclear. In addition, whether such drugs have an acceptable benefit/risk ratio is unknown. Nonetheless, with the advancement of novel drug design, the prospect that novel KRAS inhibitors will be discovered is bright.

The reported CAMs rate is 28% in IE (Lac *et al.*, 2018), over 40% in OE (Suda *et al.*, 2018) and 26% (Anglesio *et al.*, 2017) to 36% in extraovarian DE (Lac *et al.*, 2018), suggesting that, irrespective of subtype, a sizeable proportion of lesions harbour CAMs, on top of the fact that the majority (79%) of DE and all OE lesions harbour somatic mutations (Li *et al.*, 2014; Anglesio *et al.*, 2017; Suda *et al.*, 2018). Clearly, much more research is warranted to fully investigate the implications of CAMs.

Since recurrence is also a formidable challenge in the management of endometriosis (Guo, 2009a,b), one question is whether CAM-harbouring residual lesions not removed by surgery would have a higher risk of recurrence. This is a very practical issue given that the occult endometriosis is seemingly prevalent (Khan *et al.*, 2014).

As shown in Suda *et al.* (2018), lesions in one patient may harbour multiple CAMs and, as such, it is likely that there may be extensive intra-lesion mutational diversification, possibly resulting in diversification of DNA methylation and transcriptome states and differences in responses to drug treatment between even closely related cells of the same lesions or different foci, as in colorectal cancer (Roerink *et al.*, 2018). Should this be the case, it would pose another level of challenge in drug treatment of endometriosis harbouring CAMs.

Yet, perhaps one silver lining for CAM-harbouring endometriosis is that endometriotic epithelial cells harbouring these CAMs should express a series of proteins that are absent or present at lower levels in normal cells. These would lead to the presentation of an altered repertoire of MHC class I-associated peptides, so-called neoantigens, that are entirely absent from the normal human genome (Heemskerk *et al.*, 2013). In cancer, neoantigens have been postulated to be promising for tumour control (Hutchison and Pritchard, 2018). Since the quality of the T cell pool that is available for these neoantigens is not affected by central T cell tolerance (Gilboa, 1999), these antigens can be harnessed to activate the immune system to eradicate malignant cells or cells harbouring the CAMs that result in the production of the neoantigens (Pritchard, 2018). With the recent success of checkpoint blockade immunotherapy for various cancers, and the recent report that programmed death-1 (PD-1) and its ligand PD-1L are aberrantly expressed in endometriosis (Walankiewicz *et al.*, 2018; Wu *et al.*, 2019), it seems that immunotherapy targeting endometriosis-specific neoantigens may hold promise. This is particularly relevant given the painfully slow progress in development of novel therapeutics for endometriosis (Guo and Groothuis, 2018). Needless to say, further research is warranted to identify endometriosis-specific neoantigens for possible immunotherapy.

CAMs and fibrogenesis

In a recent review, this author found that the six cancer-driver genes, i.e. tumour protein p53 (TP53), ARID1A, PIK3CA, PTEN, KRAS and PPP2R1A, can participate in different aspects of fibrogenesis (Guo, 2018a). Importantly, the review suggests that cellular senescence may play a role in the lesional fibrogenesis (Guo, 2018a). It was argued that, given that CAMs can and do occur quite often in physiologically normal tissues, these CAMs in endometriosis are not necessarily synonymous with cancer or pre-cancer, but, rather, the result of immense pressure for lesional development and fibrogenesis (Guo, 2018a,b). This view appears to be accepted and supported by another recent review of the roles of these genes in hepatic, renal and pulmonary fibrosis (Kobayashi, 2019).

To better understand this, it helps to see the issue from the perspective that endometriotic lesions are wounds undergoing repeated

tissue injury and repair (ReTIAR), a notion that is summarized in Guo (2018a,b). One cardinal hallmark of endometriotic lesions is cyclic bleeding, just like the eutopic endometrium (Brosens, 1997). Because of bleeding, an indication for tissue injury, platelets and other immune cells are involved and indeed have recently been shown to play important roles in the development of endometriosis (Ding *et al.*, 2015; Guo *et al.*, 2016; Du *et al.*, 2017; Duan *et al.*, 2018). In particular, platelet-derived TGF-β1 drives smooth muscle metaplasia (SMM) and fibrosis through the induction of EMT and FMT in endometriotic lesions (Zhang *et al.*, 2016a,b,c). Platelets, as well as endometriotic stromal cells, secrete many bioactive factors, including thromboxane A_2, which may also act as a neutrophin, leading to hyperinnervation within or surrounding endometriotic lesions (Yan *et al.*, 2016). In fact, endometriotic cells may also secrete other neutrophins (Barcena de Arellano *et al.*, 2013), but this neurotrophic effect seems to be tilted in favour of sensory nerves at the expense of sympathetic nerve fibres (Arnold *et al.*, 2012; Scheerer *et al.*, 2017). While eutopic endometrium from women with endometriosis does not seem to have increased expression of neurotrophic factors (Barcena de Arellano *et al.*, 2012), it may promote neuroangiogenesis through exosome pathways (Sun *et al.*, 2019). Yet hyperinnervation or increased density of sensory nerve fibres in and around endometriotic lesions may further promote the development and fibrogenesis of endometriosis through release of neuropeptides such as substance P (Liu *et al.*, 2019; Yan *et al.*, 2019a,b). The higher expression of TrkB and p75, the two receptors for neutrophin brain-derived neurotrophic factor, in DE lesions than that of peritoneal endometriotic lesions (Dewanto *et al.*, 2016), concomitant with higher fibrotic content in the former lesions than the latter, also corroborates this notion. Moreover, platelets may also induce epigenetic changes, facilitating the gradual but progressive development of endometriosis, leading ultimately to tissue fibrosis (Zhang *et al.*, 2017). Both animal and human data lend support for the notion that endometriotic lesions are fundamentally wounds undergoing ReTIAR (Wu *et al.*, 2015; Zhang *et al.*, 2016a,b,c). The same processes also are operative in adenomyosis because of the commonality of cyclic bleeding (Liu *et al.*, 2016; Shen *et al.*, 2016).

Mutations in TP53, PTEN, ARID1A, PIK3CA, KRAS and PPP2R1A all appear to accelerate the development and fibrogenesis of endometriosis through regulating tissue repair, senescence, EMT, FMT and proliferation of fibroblasts/myofibroblasts (Guo, 2018a,b). On the other hand, as the natural history of endometriotic lesions appears to be gradual progression towards fibrosis (Guo, 2018a,b; Zhang *et al.*, 2016a,b,c), CAMs may be acquired and accumulated along the way. Hence, in future sequencing studies, evaluation of the extent of lesional fibrosis should be made since it seems to be evident that, everything else being equal, the older the lesion (which has experienced more episodes of cyclic bleeding), the more fibrotic content it contains, and the more CAMs or higher MAF it should have.

CAMs and malignant transformation

The link between OE and certain histotypes of OVCA is now well documented (Kurman and Shih Ie, 2010; Pearce *et al.*, 2012; Bulun *et al.*, 2019). However, the reports of cancers arising from extraovarian endometriosis are rare (Saavalainen *et al.*, 2018; Bulun *et al.*, 2019). According to the duality theory of OVCA (Kurman and Shih Ie, 2010), two possible origins of OVCA progenitor cells are tubal or uterine epithelium. Bulun *et al.* recently provide an excellent review on possible molecular mechanisms underlying the link between OE and OVCA (Bulun *et al.*, 2019); hence, in this review, only the essence of that review is sketched.

An overwhelming majority of OVCA cases are not heritable, and EAOC is mostly of the endometrioid and clear-cell histotype (Wiegand *et al.*, 2010; Bulun *et al.*, 2019). In these two histotypes of OVCA, CAMs such as ARID1A, B-Raf proto-oncogene, serine/threonine kinase (BRAF), PIK3CA, KRAS, PP2R1A, PTEN, CTNNB1, ARID1B, PIK3R1 and MLL3 have been reported (Wiegand *et al.*, 2010; Lu *et al.*, 2015; Murakami *et al.*, 2017). Remarkably, all these CAMs, with the only exception of CTNNB1 and BRAF, have been reported in OE and extraovarian endometriosis (Li *et al.*, 2014; Anglesio *et al.*, 2017; Lac *et al.*, 2018; Suda *et al.*, 2018; Zou *et al.*, 2018) (Table II). More remarkably, many of these CAMs have also been reported in histologically normal endometrium (Moore *et al.*, 2018; Lac *et al.*, 2019) (Table II).

As reviewed in Bulun *et al.* (2019), mutations at ARID1A, PIK3CA and PTEN have been proposed to be responsible for the malignant transformation of OE (Sato *et al.*, 2000; Wiegand *et al.*, 2010; Chandler *et al.*, 2015). EAOC and contiguous endometriosis frequently share the absence of ARID1A expression (Chene *et al.*, 2015). Experimental data provide evidence for the involvement of ARID1A, KRAS and PIK3CA mutation in endometriosis-induced malignant transformation. Activation of K-ras in donor endometrial tissues is found to facilitate lesion development in a mouse model of endometriosis (Cheng *et al.*, 2011). In mouse, local activation of K-ras through delivery of adenoviral Cre-induced ovarian or peritoneal endometriosis, as did conditional deletion of Pten (Dinulescu *et al.*, 2005). Yet the combined Pten deletion and K-ras activation in the ovary, however, resulted in metastatic endometrioid OVCA (Dinulescu *et al.*, 2005).

Of these CAMs, ARID1A is a particularly interesting one. It is frequently mutated in EAOC as well as in uterine endometrioid carcinomas (Jones *et al.*, 2010; Wiegand *et al.*, 2010; Wiegand *et al.*, 2011). One *in vitro* study reports that knocking down ARID1A in an endometriotic cell line is sufficient to initiate neoplastic transformation in conjunction with epigenetic reprogramming (Lakshminarasimhan *et al.*, 2017).

A recent study indicates that ARID1A normally preserves the endometrial epithelial cell identity by repressing genes responsible for mesenchymal cell fates (Wilson *et al.*, 2019). Combined ARID1A and PI3K mutations facilitate epithelial transdifferentiation and collective invasion (Wilson *et al.*, 2019). Another recent study reports that ARID1A inactivation causes aberrant telomere cohesion, which selectively eliminates gross chromosome aberrations during mitosis (Zhao *et al.*, 2019). This can explain why cancers associated with high frequency of ARID1A mutations often lack massive genomic instability, as seen in many cancers (Zhao *et al.*, 2019).

Yet in humans, endometriotic lesions harbouring any of the CAMs are not synonymous with cancer. This is first supported by the fact that some histologically normal endometrium may also carry these CAMs (Table II). According to the notion of ReTIAR, lesions undergo fibrogenesis as they progress and may acquire CAMs along the way. Consistent with this notion, when ARID1A is knocked down in an endometriotic epithelial cell line, it is found that the canonical pathways affected by differentially expressed genes are involved not only in cancer and the immune response but also in hepatic fibrosis

Table II CA mutations identified in ovarian endometrioma, extraovarian endometriosis, normal endometrium and endometriosis-associated ovarian cancer.

Tissue name	CA mutations	Other mutations	References
Ovarian endometrioma	ARID1A, PIK3CA, KRAS, PPP2R1A, ARID1B, PIK3R1, PTEN, MLL3, FBXW7 and ARHGAP35	TAF1, SPEG, TTN, ACRC, FAT1, FGFR2, HEATR1, FBN2,TAS2R31, PLXNB2, PTPN13,FRG1,KIAA1109, MUC6, ZFHX3, KMT2C and PLXNB2	(Suda *et al.*, 2018; Zou *et al.*, 2018)
Extraovarian endometriosis	ARID1A, PIK3CA, KRAS, PP2R1A,PTEN, CTNNB1, ERBB2, ARID1B, PIK3R1		(Anglesio *et al.*, 2017; Lac *et al.*, 2018)
Histologically normal endometrium	ARID1A, PIK3CA, TP53, KRAS, HRAS, PTEN, PIK3R1, PPP2R1A,ARHGAP35, FBXW7, ZFHX3, FOXA2, ERBB2, CHD4, NRAS, SPOP, FGFR2, AKT1, ERBB3 and BRAF	ARID5B, TAF1, SPEG, CHD4, ACRC, FAT1, FGFR2, FBN2,PLXNB2, CDKN1B, HGAP35, PTPN13,FRG1,KIAA1109, MUC6, ZFHX3, KMT2C, FOXA2, PLCG1, KDM3A, MLL5 and others	(Li *et al.*, 2014; Moore *et al.*, 2018; Suda *et al.*, 2018; Lac *et al.*, 2019)
Endometriosis-associated ovarian cancer	ARID1A, PIK3CA, KRAS, TP53, PTEN, CTNNB1, BRAF, ARID1B, PIK3R1 and MLL3		(Kuo *et al.*, 2009; Wiegand *et al.*, 2010; Lu *et al.*, 2015; Murakami *et al.*, 2017)

ACRC: acidic repeat containing; AKT1: AKT serine/threonine kinase 1; ARHGAP35: RhoGT Pase activating protein 35; ARID1B: AT-rich interaction domain 1B; ARID5B: AT-rich interaction domain 5B; CDKN1B: cyclin dependent kinase inhibitor 1B; CHD4: chromodomain helicase DNA binding protein 4; ERBB2: erb-b2 receptor tyrosine kinase 2; ERBB3: erb-b2 receptor tyrosine kinase 3; FAT1: FAT atypical cadherin 1; FBN2: fibrillin2; FBXW7: F-box and WD repeat domain containing 7; FGFR2: fibroblast growth factor receptor 2; FOXA2: forkhead box A2; FRG1: FSHD region gene 1; HEATR1: HEAT repeat containing 1; KDM3A: lysine demethylase 3A; MLL3: myeloid lymphoid or mixed-lineage leukaemia 3, also known as KMT2C; MLL5: myeloid lymphoid or mixed-lineage leukaemia 5; KMT2C: lysine methyltransferase 2C; MUC6: mucin 6; PIK3R1: phosphoinositide-3-kinase regulatory subunit 1; PLCG1: phospholipase Cgamma1; PLXNB2: plexinB2; PTPN13: protein tyrosine phosphatase non-receptor type 13; SPEG: striated muscle enriched protein kinase; SPOP: speckle type BTB/POZ protein; TAF1: TATA-box binding protein associated factor 1; TAS2R31: taste 2 receptor member 31; TTN: titin; ZFHX3: zinc finger homeobox 3.

(Lakshminarasimhan *et al.*, 2017). Consistently, monoallelic loss of ARID1A in the mouse endometrial epithelium is found to result in decreased expression of E-cadherin but increased expression of vimentin and collagens (Wilson *et al.*, 2019). That is, loss of ARID1A causes EMT and the over-production of extracellular matrix (ECM) products, which are two hallmarks of fibrotic disorders. These observations appear to lend support for the notion that ARID1A is involved in lesional fibrogenesis in endometriosis (Guo, 2018a,b).

Women with endometriosis frequently seek medical care because of pain or infertility, and this may happen before CAMs occur or while carrying these CAMs. Consequently, most endometriotic lesions do not exist long enough to acquire and accumulate further CAMs and transform to malignancy. If the lesion evades detection and removal long enough, it would eventually acquire and accumulate enough of the relevant repertoire of CAMs, and malignant transformation should ensue. Indeed, deep sequencing of multiple single cells from colorectal cancers indicates that most mutations that complete the malignant transformation are acquired during the final dominant clonal expansion of the cancer and result from mutational processes not seen in normal cells (Roerink *et al.*, 2018). The comparison of mutational profiles between normal endometrium and endometrial cancer also indicates that the latter exhibits much higher mutation loads than the former, and the latter additionally exhibits substantial structural variants and copy number changes (Zhang *et al.*, 2018) while the former essentially has none (Moore *et al.*, 2018).

It is well documented that cancer arises from cumulative CAMs—in fact three CAMs in lung and colorectal cancers (Tomasetti *et al.*, 2015; Vogelstein and Kinzler, 2015). Indeed, the majority of uterine endometrioid carcinomas have mutations in both ARID1A and PTEN (Liang *et al.*, 2012; Cancer Genome Atlas Research Network *et al.*, 2013). Conversely, ARID1A or PTEN mutation alone does not cause OVCA (Guan *et al.*, 2014). In particular, ARID1A inactivation/mutation alone is insufficient to initiate carcinogenesis, and it requires additional CAMs, such as a PIK3CA or PTEN, to complete the malignant transformation into clear cell carcinomas (Chandler *et al.*, 2015) or endometrioid carcinomas (Guan *et al.*, 2014). This also explains why endometrial epithelial cells carrying CAMs such as ARID1A, PTEN, KRAS and PIK3CA may still appear to be histologically normal (Moore *et al.*, 2018; Suda *et al.*, 2018; Lac *et al.*, 2019).

Hence, CAM-carrying lesions just need to acquire and accumulate relevant and enough CAMs in order to undergo malignant transformation, which may take years. Everything being equal, women with endometriosis-induced cancer should be older than those with endometriosis only, since malignant transformation takes extra time. On the other hand, women with endometriosis-induced cancer, in general, should be younger than those who developed cancer of the same type spontaneously. This is because, first, endometriotic lesions engender a microenvironment featuring hyperestrogenism, inflammation and oxidative stress that are individually and collectively mutagenic, generating a hotbed for DNA damage and thus CAMs. Second, women with endometriosis frequently manifest symptoms, such as pain, that prompt them to seek medical attention and, as such, their malignancy is likely to be diagnosed earlier.

Remarkably, these inferences appear to be borne out by published studies. Women with EAOC are reported to be significantly older than women with endometriosis but no OVCA (He *et al.*, 2017), suggesting that endometriosis-induced malignancy transformation does take time. The finding that most OVCA arising from endometriosis occurs 5 years

or longer after women had been diagnosed with OE (Saavalainen *et al.*, 2018) is consistent with this notion.

However, women with EAOC also tend to be significantly younger than those without EAOC and tend to have significantly more complaints of dysmenorrhea and menstrual disorders (Heaps *et al.*, 1990; Li *et al.*, 2019). This may explain why EAOC are mostly early-stage cancer (Li *et al.*, 2019). It may also explain why the incidence of OE-induced malignancy is very low (Kuo *et al.*, 2017), since various endometriosis-associated symptoms, such as pelvic pain and pelvic mass (Heaps *et al.*, 1990), would prompt these women to seek medical attention early.

However, one question remains: why do the majority of endometriosis-induced malignancies occur in ovaries instead of extraovarian sites (Heaps *et al.*, 1990; Stern *et al.*, 2001; Benoit *et al.*, 2006)? Indeed, data on extraovarian endometriosis-associated malignancy are extremely scanty (Benoit *et al.*, 2006; Barra *et al.*, 2018; Bulun *et al.*, 2019). Difference in tissue sensitivity or vulnerability aside, hyperestrogenism in ovarian tissues may be one of major culprits. Indeed, the oestrogen concentration in the ovary could be several orders of magnitude higher than other peripheral tissues (diZerega *et al.*, 1984; Bulun *et al.*, 2019). This elevated local concentration of oestrogens, coupled with increased expression of cytochromeP450family1subfamilyBmember1 (CYP1B1) (Piccinato *et al.*, 2016), which can act as a strong agonist of oestrogen receptors (ER) and also converts hydroxyestrogen to mutagenic quinones after being oxidized (Zhu and Conney, 1998), would turn the microenvironment of OE lesions into one conducive to gene mutations and CAMs. Interestingly, the expression levels of CYP1B1 in DE lesions is significantly lower than that of superficial lesions and is seemingly lower than that of OE lesions (Piccinato *et al.*, 2016), suggesting that the mutagenic potential at the ovarian sites may be higher than extraovarian sites. In addition, the increased expression of ERβ in endometriosis (Xue *et al.*, 2007; Han *et al.*, 2012) interacts with the inflammasome machinery, further stimulating inflammation and promoting survival of ectopic endometrium (Monsivais *et al.*, 2014; Han *et al.*, 2015; Bulun *et al.*, 2019). As a result, activated inflammatory cells become important sources of ROS and reactive nitrogen intermediates that cause DNA damage and genomic instability and thus CAMs (Canli *et al.*, 2017). Compared with OE, DE lesions have a lower expression of ERβ (Liu *et al.*, 2018), which might also suggest somewhat lower mutagenic potential at the extraovarian sites than at ovarian sites. Remarkably, compared with women with EAOC, women with extraovarian cancers arising in endometriosis are more likely to be post-menopausal and use HRT (Modesitt *et al.*, 2002), suggesting that endometriosis-induced tumorigenesis in extraovarian sites takes longer and may likely be fuelled by exogenous oestrogens.

In addition, there is a great variation in tissue-specific cancer risks due to the number of stem cell divisions (Tomasetti and Vogelstein, 2015). Hence, the location of the endometriotic lesions is also crucial. In particular, since women with DE tend to be older than those with OE only (Liu *et al.*, 2018), and since cell division rate decreases with age (Tomasetti *et al.*, 2019), DE lesions may have a lower cell division rate than OE lesions, resulting in lower occurrence of CAMs and consequently a reduced incidence of malignant transformation. Collectively, this may explain why OE is linked with certain histotypes of OVCA, but DE-induced malignancy is extremely rare.

Drivers of CAMs and fibrogenesis

One question that remains unaddressed is what, if any, are the common factors that drive CAMs and fibrogenesis? While the age of endometriotic lesions is a likely factor, there are at least two conspicuous culprits that can definitely drive CAMs as well as lesional fibrogenesis: oxidative stress and oestrogen.

Oxidative stress refers to the imbalance of ROS and antioxidants. Oxidative stress has been well documented to be involved in endometriosis (Donnez *et al.*, 2016). During the tissue repair process, innate immune cells are recruited and infiltrate the wounding site and, in the setting of endometriosis, endometriotic lesions. These cells, such as neutrophils, secrete proteolytic enzymes and proinflammatory cytokines, as well as large amount of ROS. In addition, inflamed tissues may also generate ROS through NADPH oxidase (Bedard and Krause, 2007). Indeed, NADPH oxidase 1 (NOX1) is overexpressed in endometriosis (Nassif *et al.*, 2016).

In addition, since ectopic endometrium, just like its eutopic counterpart, experiences cyclic bleeding this releases (from erythrocytes in and around lesions) cell-free haemoglobin (Hb) and its highly toxic by-products such as haem and iron. In particular, phagocytosis of senescent erythrocytes by macrophages results in the digestion of Hb and subsequent release of haem, which is converted by haem oxygenase into biliverdin, carbon monoxide and free iron (Maines, 1997). There is extensive evidence for iron overload in endometriosis (Defrere *et al.*, 2008), which induces iron-mediated damage, oxidative injury, inflammation and oxidative stress (Donnez *et al.*, 2016). For example, iron deposits in lesions (Moen and Halvorsen, 1992) and elevated iron levels in lesions (Takahashi *et al.*, 1996) have been reported. Consistently, the expression levels of 8-hydroxy-2′-deoxyguanosine (8-OhdG), a sensitive indicator of DNA damage because of oxidative stress (de Souza-Pinto *et al.*, 2001), are reported to be significantly elevated in normal ovarian cortex surrounding endometriotic cysts (Matsuzaki and Schubert, 2010). Not surprisingly, granulosa cells from patients with infertility and endometriosis exhibit a higher 8-OhdG index when compared with those from patients with other infertility causes (Seino *et al.*, 2002).

In endometriotic cells, the increased production of endogenous ROS, ERK activation and elevated proliferative capability are intimately linked (Ngo *et al.*, 2009). Not surprisingly, 8-OHdG has been reported in OE lesions (Kao *et al.*, 2005), in normal ovarian cortex surrounding OE lesions (Matsuzaki and Schubert, 2010) and in follicular and peritoneal fluids (Polak *et al.*, 2013; Da Broi *et al.*, 2016). Increased production of TP53 is also reported to be downregulated in OE, which is concomitant with increased expression of genes involved in autophagy and elevated protein expression of haem oxygenase-1, a sign of oxidative stress (Allavena *et al.*, 2015).

In addition to the increased production of ROS, the ability to eliminate ROS in endometriosis is reported to be substantially reduced. The redox-sensitive nuclear factor erythroid-derived 2-like 2 (NRF2), which controls the transcription of endogenous antioxidant enzymes and protects against inflammation-induced oxidative damage, is reported to be downregulated in endometriosis (Marcellin *et al.*, 2017). Its target gene, glutamate cysteine ligase, which is the first enzyme in the synthesis cascade of an important antioxidant, namely glutathione, also is found to be downregulated in endometriosis (Marcellin *et al.*, 2017). Consistently, endometriotic lesions induced in mice with NRF2

knocked down are found to be larger and exhibit more fibrosis than those in wild-type mice (Marcellin *et al.*, 2017).

Excessive oxidative stress results in redox imbalance, ineffective repair of DNA damage and generation of CAMs (Cooke *et al.*, 2003; McAdam *et al.*, 2016). On the other hand, NOX-dependent redox signalling can upregulate TGF-β/ suppressor of mothers against decapentaplegic (Smad) signalling in a feed-forward manner, accelerating fibrogenesis (Barcellos-Hoff and Dix, 1996; Jiang *et al.*, 2014). Thus, aging, oxidative stress and perhaps other factors yet to be identified, jointly drive CAMs and fibrogenesis. In addition CAMs also promote fibrogenesis, and the process of fibrogenesis as a whole may generate enormous selection pressure so that cells with CAMs have a higher fitness.

Increased local production of oestrogens is one important hallmark of endometriotic lesions (Bulun, 2009). Due to increased expression of CYP1B1 in endometriotic lesions (Piccinato *et al.*, 2016), the lesional microenvironment can be a hotbed for mutagenesis, thereby producing CAMs.

The increased oestrogen levels may also promote lesional fibrosis. ERα has been reported to promote pulmonary fibrosis through reduced expression of the microRNAs let-7a and let-7d, which can modulate AKT phosphorylation and TGF-β1 and SMAD7 expression (Elliot *et al.*, 2019). In addition, increased insulin-like growth factor (IGF)-1 levels in fibrotic tissues may stimulate ERα in an oestrogen-independent manner (Elliot *et al.*, 2019). Despite the report of lower ERα expression levels in (mostly OE) tissues and primary stromal cells (Brandenberger *et al.*, 1999; Fujimoto *et al.*, 1999) and high ERβ expression levels (Xue *et al.*, 2007), recent studies found that OE lesions have the lowest glandular ERα expression, but highest glandular ERβ expression as compared with fallopian, peritoneal and extrapelvic lesions (Colon-Caraballo *et al.*, 2018). ERα is expressed in the smooth muscle cell component in peritoneal (Barcena de Arellano *et al.*, 2011) and DE lesions (Kitano *et al.*, 2007; Noel *et al.*, 2010). Remarkably, the circulating let-7d levels in women with endometriosis is found to be marginally decreased (Cho *et al.*, 2015) and the let-7a levels in mice with induced endometriosis is significantly reduced (Seifer *et al.*, 2017). The involvement of IGF-1 in endometriosis has been long suspected (Sbracia *et al.*, 1997) and its stimulating role in inducing ERβ and aromatase also has been recently demonstrated (Zhou *et al.*, 2016). Thus, the increased local oestrogen concentration may suppress let-7 microRNAs and induce fibrogenesis through the ERα/IGF-1 pathways.

The overexpression of ERβ in endometriosis (Xue *et al.*, 2007) could also result in elevated expression of collagen I and III in endometriotic stromal cells and thus increased ECM products, as in rat fibroblasts (Dworatzek *et al.*, 2019). Of course, while the finding is reported in male rats (Dworatzek *et al.*, 2019), it should be noted that so far all the reports on ERα/ERβ aberration in endometriosis have not evaluated their phosphorylation status, and it is well documented that phosphorylation at a particular site on both ERα and ERβ is critical for nuclear translocation and transcriptional activation upon oestrogen treatment(Lannigan, 2003; Sanchez *et al.*, 2010).

Moreover, ERβ regulates platelet-derived growth factor (Patrone *et al.*, 2003), a cytokine known to regulate the growth and the differentiation of fibroblasts into myofibroblasts (Kilarski *et al.*, 2005). Alternatively, cytosolic ERα and ERβ can efficiently activate transcription at AP-1 sites in response to oestrogen stimulation (Bjornstrom and Sjoberg, 2004). AP-1 is known to regulate fibroblast activation and proliferation (Gagliardi *et al.*, 2003) and myofibroblast activation (Fitzner *et al.*, 2004). Interestingly, genes in the AP-1 family are known be involved in the early stage of endometriosis (Hastings and Fazleabas, 2006) and are also implicated in endometriosis development (Beste *et al.*, 2014).

Taken together, the increased local oestrogen production can induce mutagenesis and CAMs. Coupled with elevated ERβ expression and the expression of ERα in the smooth muscle cell component of lesions, it may also facilitate lesional fibrogenesis.

Just as the genetic alterations, including CAMs, can contribute to tumorigenesis, epigenetic alterations are also known to contribute to cancer initiation and development (Kanwal *et al.*, 2015; Cavalli and Heard, 2019). The frequency of somatic mutations can be influenced by DNA methylation (Poulos *et al.*, 2017) and the nucleosome orientation (Pich *et al.*, 2018). In some cases, malignant transformation can occur without apparent CAMs (Green *et al.*, 2014). In pancreatic ductal adenocarcinoma, it is reported that metastasis does not seem to involve any apparent CAMs; instead, large-scale epigenetic reprogramming appeared to be responsible (McDonald *et al.*, 2017), underscoring the importance of epigenetic aberrations in malignant transformation.

Similarly, epigenetic aberrations also are involved in fibrogenesis (Moran-Salvador and Mann, 2017; Aseem and Huebert, 2019). While it is recognized that endometriosis is an epigenetic disease (Guo, 2009a) and an epigenetic drug, valproic acid, has been used in treating adenomyosis with promising results (Liu and Guo, 2008; Xishi *et al.*, 2010), one area that so far has attracted little attention is the epigenetic changes during lesional development and fibrogenesis. As endometriotic lesions undergo EMT, FMT, SMM and fibrogenesis, they also acquire and accumulate epigenetic aberrations, such as the changes in gene and protein expression of DNA methyltransferases (DNMTs), histone deacetylases and histone lysine methyltransferases and demethylases (Wu *et al.*, 2007; Ding *et al.*, 2014; Zhang *et al.*, 2017). Indeed, inflammation and oxidative stress can lead to epigenetic alterations concomitant with fibrogenesis (Morgado-Pascual *et al.*, 2018; Shrishrimal *et al.*, 2019). In particular, chronic inflammation and prolonged transcriptional suppression, which occur in endometriosis, are known to cause gene hypermethylation and silencing (Hsieh *et al.*, 1998; Issa *et al.*, 2001; Song *et al.*, 2002; Stirzaker *et al.*, 2004). Indeed, prolonged stimulation with tumour necrosis factor-α induced a partial methylation at the promoter of progesterone receptor isoform B (PR-B) in endometriotic epithelial cells (Wu *et al.*, 2008), which may account for PR-B hypermethylation in endometriosis (Wu *et al.*, 2006). This area is still evolving, and more research is warranted.

A scheme showing possible drivers of CAMs and fibrogenesis is depicted in Figure 2.

Limitations of published studies and future research

Owing to the still scanty data on the mutational profiles in normal endometrium, future studies are badly needed in order to catalogue all somatic mutations, profile its mutational landscape, mutational signatures (what kind of mutations, which can be used to delineate the underlying mechanism of mutational processes), copy number changes and structural variants (large-scale indels), and also to distinguish cancer-driver and passenger mutations (Hess *et al.*, 2019).

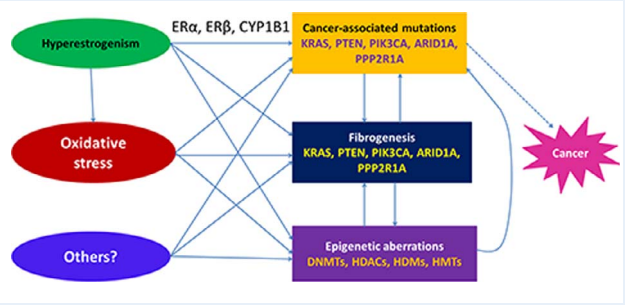

Figure 2 Possible drivers of CAMs, fibrogenesis and epigenetic aberrations in endometriotic lesions. Hyperestrogenism, oxidative stress and perhaps other factors yet to be identified, jointly drive CAMs, fibrogenesis and epigenetic aberrations in endometriotic lesions. CAMs and epimutations also promote fibrogenesis, and the process of fibrogenesis as a whole may generate enormous selection pressure so that cells with CAMs and/or epimutations have higher fitness. Epigenetic aberrations may also promote CAMs. See text for more details. ARID1A: AT-rich interactive domain-containing protein 1A; CYP1B1: cytochrome P450 family 1 subfamily B member 1; DNMT: DNA methyltransferase; ER: oestrogen receptor; HDAC: histone deacetylase; HDM: histone demethylase; HMT: histone methyltransferase; KRAS: Kirsten rat sarcoma; PIK3CA: phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit α; PPP2R1A: protein phosphatase 2 scaffold subunit Aα; PTEN: phosphate and tension homology deleted on chromsome ten.

In view of the ubiquitous age-related somatic mutations in normal tissues, and the roles of oestrogens, BMI, reproductive history, history of oral contraceptive (OC) or intrauterine device (IUD) and hormone use in driving mutations in the endometrium (Lin *et al.*, 2009; Mutter *et al.*, 2014; Westin *et al.*, 2015; Busch *et al.*, 2017), future studies are warranted to catalogue the mutational profiles in endometrium epithelium in women of different ages, BMI, reproductive history and history of OC/IUD use. Since age at menarche also determines when the endometrial epithelium starts cell division, its impact on the mutational profiles also needs to be evaluated.

The study by Suda *et al.* (2018) nicely demonstrated the power of deep sequencing in delineating the relationship between different cells. The cheaper and more accurate sequencing methods in the future should afford sequence data for the tissues of interest. With these data, it will be feasible to establish phylogenetic relationships between or among different tissues in the same patient based on the molecular clock (Siegmund *et al.*, 2009). This can be used to infer the relationship between clear-cell and endometrioid OVCA and its associated OE (Guo, 2015), particularly the time it takes from transformation of benign endometriotic lesions to malignancy. In addition, the same methods could be used to delineate the relationship in the reported link between focal adenomyosis and deep endometriosis (Chapron *et al.*, 2017; Marcellin *et al.*, 2018) and between adenomyosis and myomas (Filip *et al.*, 2019). The methods also can be used to see whether adenomyotic lesions also harbour CAMs, and, if so, their frequency and their relationship, if any, with the location and 'age' (Liu *et al.*, 2018).

One conspicuous area that has so far received little attention is the global epigenetic landscape in different subtypes of endometriosis and in comparison with that of eutopic endometrium since epigenetic alterations or epimutations have also been recognized as important in the development of endometriosis (Guo, 2009a,b; Naqvi *et al.*, 2014) and tumorigenesis (Aldiri *et al.*, 2017). Of relevance, the transcriptional inactivation of the MLH1 gene through promoter hypermethylation is often associated with microsatellite instability (Leung *et al.*, 1999), which is reported to be associated with the malignant transformation of endometriosis (Amemiya *et al.*, 2004; Ali-Fehmi *et al.*, 2006; Ren *et al.*, 2012). Promoter hypermethylation of MLH1 has been found in ~4% (2/46) of endometriotic lesions (Martini *et al.*, 2002). Moreover, PR-B has been reported to be hypermethylated in ectopic endometrium (Wu *et al.*, 2006a,b,c) and partially methylated in eutopic endometrium from women with endometriosis (Rocha-Junior *et al.*, 2019), which may account for reduced PR-B expression and subsequent progesterone resistance. Although there have been reports on genome-wide analysis of DNA methylation in endometriosis (Wang *et al.*, 2019), so far there has been no epigenetic study to match the scope, depth and methodological rigor of sequencing studies such as that of Suda *et al.* (Suda *et al.*, 2018).

More broadly, changes in the levels of DNMTs, the enzymes responsible for initiating and/or maintaining DNA methylation, are intimately associated with the TGF-β signalling pathway (Koh *et al.*, 2016), suggesting that the culprit causing CAMs and fibrogenesis in endometriosis also may actuate DNMTs and may thus be responsible for epigenetic aberrations as well. Incidentally or not, overexpression of DNMTs has been reported in endometriosis (Wu *et al.*, 2007). In addition, the TGF-β signalling pathway may crosstalk with the focal adhesion kinase and PI3K/Akt pathways to increase DNMT expression via a transcription-independent mechanism involving an increase in phosphorylation and inactivation of glycogen synthase kinase-3β (Koh *et al.*, 2016). Perhaps more uncannily, overexpression of DNMTs has been reported in several fibrotic diseases (Neveu *et al.*, 2015; Page *et al.*, 2016; Wu *et al.*, 2017), suggesting that there should be a global change in the epigenetic landscape in endometriosis. Future studies are needed in this area. The culprits responsible for CAMs and fibrogenesis, such as aging and oxidative stress, also happen to be responsible for epigenetic aberrations (Barcellos-Hoff and Dix, 1996; Jiang *et al.*, 2014; Jones *et al.*, 2015; Ito *et al.*, 2017) (Fig. 2).

Of course, epigenetics encompasses a plethora of various histone modifications, along with an array of writers, erasers and readers and/or effectors of these modifications as well as a kaleidoscopic of DNA methylation, long non-coding RNA and microRNA involvement. A complete understanding of the epigenome in both ectopic and eutopic endometrium should hold the key to understand the pathogenesis and pathophysiology of endometriosis - a daunting task but a must in order to unravel the mystery of endometriosis.

Summary answers

From the vista of the reported CAMs in ectopic, eutopic and histologically normal endometrium as well as the above review, some plausible answers to the questions raised in the Introduction can be provided, as found below.

Why is there such a wild discrepancy in reported mutation frequencies? How can we reconcile such a discrepancy?

The discrepancies found so far can be attributable mostly to the following factors: first, whether a specific cell type (e.g. epithelial cells, which

necessitates the use of microdissection techniques) or the mixture of different cells (e.g. endometriotic tissues) was used for mutation detection; second, different mutation detection methods (essentially boils down to the use of error-correction NGS technology or not); and third, whether targeted sequencing, or whole-genome sequencing was used. Older studies that extracted DNA from the whole tissues tend to report much lower mutation frequencies since the mixture of different cell types would obscure the signals in epithelial cells and thus reduce the signal-to-noise ratio. Older mutation detection methods cannot mutations with lower frequencies, and thus miss them. Targeted sequencing detects mutations in a pre-defined set of genes, and, by nature, cannot detect mutations in genes that are not in the set. Thus, only those studies using microdissection procedures and error-corrected NGS technologies can detect low-frequency somatic mutations, even in histologically normal endometrium.

Why does ectopic endometrium have a higher mutation rate than that of eutopic endometrium?

Eutopic and ectopic endometrium are known to have different gene expression profiles (Wu *et al.*, 2006a). This may be attributable to the difference in their respective microenvironments, with ectopic endometrium experiencing higher local oestrogens and more oxidative stress, thus increasing ectopic endometrium exposure to DNA-damaging agents, and thus a higher mutation rate.

Would the occurrence of CAMs in endometriotic lesions increase the risk of cancer in their carriers?

Cancers do not arise overnight. Extensive studies in tumorigenesis indicate that pre-cancerous tissues/organs acquire and accumulate CAMs and progress gradually to cancer. Therefore, once endometriotic lesions harbour CAMs that invariantly confer advantages of clonal expansion, they may further acquire and accumulate more CAMs and, as such, eventually complete the malignant transformation if left intact. To reduce the risk of malignant transformation, it is thus of vital importance to remove the lesions completely or to keep the lesions at bay by inducing lesional atrophy and/or dormancy so as not to give them more opportunity to acquire and accumulate more CAMs. Hence, the occurrence of CAMs in endometriotic lesions will render an increased risk of cancer to their carriers if left untreated.

How often do CAMs occur?

CAMs occur as a result of increased cumulative cell turnover (increased number of cell divisions as in aging and, for eutopic or ectopic endometrium, reproductive history such as pregnancy and lactation), exposure to various mutagenic factors such as excessive oestrogens, inflammation and ROS. Thus, even histologically normal endometrium may harbour CAMs. Due to increased local oestrogen production, inflammation and abundant ROS, the ectopic endometrium faces a harsher microenvironment than the eutopic or normal endometrium, and may thus experience a higher mutation rate. Given that mutations are nearly ubiquitous in physiologically normal tissues, including endometrium, and in view of a more hostile lesional microenvironment, ectopic endometrium may harbour more CAMs than its eutopic counterpart. Beyond that, however, it is still unclear how often CAMs occur in endometriotic lesions, at what pace, and what combination of CAMs is needed to complete the malignant transformation. That said, it is reasonable to expect that the chance of CAM occurrence is proportional to the extent of lesional fibrosis (Guo, 2018a). Future studies are needed to elucidate the mutational burden, signature and frequency of CAMs in relation to the extent of lesional fibrosis.

Will all patients with endometriosis, deep or otherwise, have CAMs in the lesions sooner or later?

The answer is a categorical yes, as long as the ectopic endometrium has the time and opportunity to acquire CAMs. In other words, if a lesion is not removed by surgical means or kept dormant, it will have the opportunity to acquire and accumulate CAMs. However, it may take years to accumulate the right type and combination of CAMs to complete malignant transformation.

OE is now well documented to be linked with OVCA, but why does extraovarian endometriosis seldom lead to cancer?

First of all, malignancy induced by extraovarian endometriosis has been reported, although such cases are rare. Patients with OE do have a higher risk of developing certain histotypes of OVCA, mainly endometrioid and clear-cell OVCA. However, the increased risk is fairly modest, with the odds ratio typically ranging from 1.3 to 3, hence the absolute risk of developing ovarian cancer is still small (Kim *et al.*, 2014; Guo, 2015). The difference in the risk of malignant transformation between OE and extraovarian endometriosis is attributable to several reasons. First, aside from the difference in tissue vulnerability and sensitivity, ovarian tissues have a much higher oestrogen concentration than extraovarian sites, and this hyperestrogenism, in conjunction with increased lesional expression in CYP1B1 (especially in OE lesions), results in a microenvironment that is conducive for mutagenesis and thus increased CAMs. The higher ERβ expression (linked to inflammation) in OE lesions than other extraovarian endometriosis may also indicate higher mutagenic pressure in OE lesions. Thus, endometriosis-induced malignancy in extraovarian sites may take longer than that induced by OE, resulting in a seemingly higher malignancy rate in women with OE than those with extraovarian endometriosis. Since women with DE are often older than those with OE and since aging can decrease cell division rate, DE lesions may have a reduced chance of CAM occurrence than OE lesions, resulting in a lower incidence of malignant transformation.

What clinical implications, if any, do the CAMs have for the bearers?

While lesions harbouring CAMs are not synonymous with malignancy, they can acquire and accumulate more CAMs and undergo malignant transformation if not removed. Thus, early diagnosis and subsequent removal of endometriotic lesions should greatly reduce and even eliminate the risk of malignancy. Indeed, women with endometriosis who had radical extirpation of all visible lesions are reported to have a substantially reduced risk of OVCA (Melin *et al.*, 2013). While

surgery can remove lesions, it should be used sparingly and judiciously (Chapron *et al.*, 2019) since surgery increases the risk of adhesion, organ damage and premature ovarian failure, and may also promote the development of residual lesions (Guo and Martin, 2019). However, when surgery is performed, whenever possible it should remove all lesions. Drug-induced lesional dormancy that essentially suppresses cell divisions may be a good alternative for the prevention of occurrence of, or further acquirement, of CAMs.

Lesions carrying CAMs may pose a challenge to treatment by non-surgical means since the mutations are unlikely to be eliminated. This is especially true when there is extensive intra-lesion mutational diversification. One silver lining is that neoantigens may result from the presence of lesional CAMs, which may be harnessed for immunotherapy and for better diagnosis. This, of course, will require more research.

When a patient with endometriosis is found to have CAMs, should she be concerned or worried?

Not necessarily. First of all, a tissue harbouring CAMs is not synonymous with cancer or pre-cancer. It needs to acquire and accumulate the right type as well as certain combination of CAMs in order to complete the malignant transformation. Therefore, if lesions harbouring the CAMs can be completely and thoroughly removed, then the chance of malignant transformation is effectively nil. Alternatively, if the lesion harbouring the CAMs can be tamed through drug-induced dormancy and/or atrophy, then the chance of further malignant transformation can also be reduced substantially.

Do these CAMs tell us anything about the pathogenesis and/or pathophysiology of endometriosis?

Recent reports of deep sequencing not only of ectopic endometrium but also eutopic and histologically normal endometrium have greatly facilitated our understanding of the pathogenesis and/or pathophysiology of endometriosis. The finding that histologically normal endometrium can still carry CAMs essentially casts doubt that endometriosis may originate from CAM-harbouring eutopic endometrium. The report that CAMs are found predominantly in the epithelial component of endometriotic lesions but not in the stromal component corroborates the finding that molecular processes such as EMT, MMT, EndoMT, and possibly others, all occur in endometriotic lesions. Thus, epithelium and stroma of endometriotic lesions do have different developmental trajectories, and this raises the possibility of targeting specifically these molecular processes, either individually or collectively, as an interventional measure. The various CAMs reported in eutopic and ectopic endometrium also highlight the challenge in the management of endometriosis-harbouring CAMs.

Are there any limitations in these studies?

The reports of CAMs in both ectopic, eutopic and histologically normal endometrium shed some much needed light on the pathogenesis and pathophysiology of endometriosis. However, each of the studies reveals certain aspects of endometriosis. We still do not know the real pathogenesis of endometriosis, nor do we know the tempo and pace of each and every CAM identified so far, or how many (or what CAM repertoire) are needed to complete the malignant transformation. We do not how if there are any factors that can either promote or hinder the occurrence of CAMs. Nor do we know whether there is any difference in type and frequency of CAMs among different subtypes of endometriosis or among different patients with different symptomatology and severity, as well as different life history. In particular, none of the published studies evaluated the relationship between CAM frequency and the extent of lesional fibrosis, which should be correlated (Guo, 2018a). Hopefully, future studies can address these issues.

What kind of future research is needed so that we can build upon our knowledge and further unveil some long-standing mysteries and conundrums in endometriosis?

There are still many unknowns. Future studies are needed to understand how many and what additional CAMs are required to complete the malignant transformation, especially in extraovarian endometriosis. From what is known already, the cancerous tissues have massive mutations. Is there any trigger(s) that promotes the apparent benign endometriotic lesions to malignant transformation? Can we catalogue all CAMs in endometriotic lesions or in endometrium? What additional CAMs or even molecular events are needed in order to complete the malignant transformation?

What are the mutational loads, signatures and landscapes for CAMs in eutopic and ectopic endometrium? Is there any difference in CAM repertoire among different subtypes of endometriosis? What is the pace of endometriosis-induced malignant transformation for a given CAM? Do adenomyotic lesions have similar CAMs as in endometriosis? If lesions carrying CAMs are not removed, which drug can effectively induce lesional atrophy or dormancy to reduce the risk of further CAMs?

So far the major focus has been on the genomic mutations, including CAMs. However, it is well known in tumorigenesis that there are also epigenetic aberrations. Compared to our increasing knowledge of CAMs, so far we know little, if anything, about epigenetic aberrations, especially in eutopic and histologically normal endometrium. Future studies are warranted to catalogue and gain insight into the landscape of epigenetic aberrations in ectopic, eutopic and histologically normal endometrium. Moreover, since the epigenetic aberrations may be closely linked with genetic aberrations, future studies are needed to delineate their inter-relationship and their respective drivers.

Conclusions

The recent reports of CAMs in endometriosis have generated a great deal of enthusiasm for the use of the next-generation sequencing methods to unveil the pathogenesis and pathophysiology of endometriosis. Indeed, these studies have shed some new light on the pathophysiology of endometriosis, giving a rare glimpse at its underlying genetic landscape. Since somatic mutations are widespread even in normal tissues and accumulate with age, most endometriotic lesions harbouring CAMs, especially extraovarian ones, do not necessarily lead

to malignancy, at least not immediately, due to manifested symptoms that prompt subsequent surgical removal of lesions or medication that induces lesional dormancy or perhaps atrophy. However, the CAMs-carrying lesions may eventually undergo malignant transformation if left intact or untreated.

As endometriotic lesions are wounds undergoing ReTIAR and, as a result, fibrogenesis, the CAMs in endometriosis discovered so far are mostly involved also in fibrogenesis. Several common denominators, such as age and oxidative stress, drive both CAMs and fibrogenesis as well as epigenetic changes. The distinct developmental trajectories of endometriotic stroma and epithelium highlight distinct but somehow dependent and co-evolved developmental processes in the two cellular components and underline the importance of lesion microenvironment in shaping the lesion destiny.

The finding of CAMs in endometriosis adds another layer of complexity for its management since pharmacological means are not likely to rectify mutations. While more research is warranted, as of now it seems that long-term care is needed when lesions are not physically removed. The possibility of malignancy, though remote, should be still taken into consideration in patient management, and early intervention seems to be advisable given the progressive nature of endometriosis.

While these studies have, collectively, illuminated the genetic landscape of endometriosis, the pathogenesis still remains a mystery. Future sequencing studies may shed more light on the pathogenesis, and to infer the phylogenetic and tempospacial relationship between OE lesions and EAOC, as well as the relationship between focal adenomyosis and DE. This will call for future profiling studies of the mutational burden, signatures and other structural variants in normal endometrium in women of different ages, BMI and reproductive history, as well as history of hormonal medication, OC or IUD use, perhaps with and without endometriosis, adenomyosis, uterine fibroids and other gynaecological conditions.

The same methodologies can be employed for adenomyosis, a disease closely related to endometriosis but which is seemingly under researched. In addition, one area in need of more research with the scope, depth and rigor that match the published CAMs studies is the epigenetic landscape of both ectopic and eutopic endometrium. When the genetic and epigenetic landscapes of ectopic, eutopic and normal endometrium are sufficiently understood, we should have a much better understanding of the pathogenesis and pathophysiology of endometriosis, and the day may come when this dreadful disease can be managed effectively.

Post scriptum note

After this manuscript was accepted for publication, the author became aware of a paper by Suda *et al.* (2019) based on targeted sequencing of 11 OE and 10 normal endometrial samples. It reports that there was no shared mutation between epithelial and stromal cells in OE tissues and the normal endometrium; in OE lesions, the ratio of CAMs per sample was significantly higher in the epithelium than in the stroma, even though there was no difference in the frequency of CAMs between the two components (Suda *et al.*, 2019). In addition, there was no significant difference in the mutation frequency between epithelial and stromal cells in OE tissues and the normal endometrium, and apparently between OE tissues and normal endometrium (Suda *et al.*, 2019). These findings lend firm support for the views expressed in this review, in that the constituents of the endometriotic stromal component comprise recruited and transformed cells from various sources (Fig. 1); somatic mutations are ubiquitous, not only in endometriotic tissues but also in normal endometrial tissues; there is no data to support the view that women with endometriosis have pre-existing CAMs before the genesis of endometriosis; that is, it is endometrium harbouring CAMs that causes endometriosis. In a nutshell, we are now still in square one as far as the pathogenesis of endometriosis as concerned. However, through these sequencing studies, we have gained more understanding of the pathophysiology of endometriotic lesions, in that perhaps EndoMT, MMT, recruitment of BMDSCs and other molecular processes that so far we know very little about are just as important, if not more important, than EMT. In particular, ascertaining the source of myofibroblasts in lesions is now becomes a pressing issue, since this would be crucial for intervention owing to the pivotal role of myofibroblasts in lesional fibrogenesis.

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Conflict of interest

The author declares no conflict of interest.

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