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Coates edited 18-49.R1 US spelling INVITED COMMENTARY

Distinct developmental trajectories of endometriotic epithelium and stroma: implications for the origins of endometriosis†

Running title: Distinct developmental patterns of endometriotic epithelium and stroma

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/path.5136

†Invited commentary for Noë *et al.* Independent development of endometrial epithelium and stroma within the same endometriosis. *J Pathol* 2018; **245:** 265-269.

Conflicts of Interest: *No conflicts of interest were declared.*

Abstract:

Endometriosis is a common gynecologic disease characterized by the ectopic growth of endometrial-like tissue. Despite the widespread prevalence of endometriosis, its pathogenesis remains poorly understood. A recent study by Noë *et al*. provides evidence that the epithelium and stroma within the same endometriotic lesions follow distinct and independent developmental trajectories. They used droplet digital PCR analysis of laser-captured epithelial-enriched and stromal-enriched endometriosis tissue and found that all 19 somatic passenger mutations analyzed were enriched exclusively in the epithelial compartment. These findings are consistent with the clonal expansion of epithelial cells, whereas stromal cells may be continuously regenerated or recruited over the course of disease. Further findings of differing allelic frequencies among passenger mutations within the epithelium of the same endometriotic lesions are suggestive of subclonality or the existence of multiple clones in some cases. Overall, the authors' observations of clonally-dominant somatic passenger mutations in the epithelium and not the stroma of endometriosis add to the recent description of cancer-associated mutations in such lesions and provide clues to the pathogenesis of endometriosis. Further studies to determine where and when these mutations occur and

whether they can be used to develop the first biologically-informed classification system for endometriosis are warranted.

Keywords: Endometriosis, passenger mutations, somatic mutations, clonal evolution, epithelium, stroma, pathogenesis

Endometriosis is a chronic, estrogen-dependent gynecologic disease defined by the growth of endometrial-like epithelium and stroma outside of the uterus [1]. Endometriosis affects an estimated 176 million women worldwide [2] – however, despite the prevalence of endometriosis among women of reproductive age, the pathogenesis of the disease remains elusive. Specifically, many theories have emerged to explain the developmental processes underlying endometriosis including the retrograde menstruation, coelomic metaplasia, and stem cell theories. In the retrograde menstruation theory, endometrial cells shed from the uterus are refluxed through the fallopian tubes and into the pelvic cavity and ultimately give rise to ectopic endometriotic lesions [3]. The coelomic metaplasia theory proposes that normal cells lining the peritoneum can undergo metaplasia into endometrial-like cells and establish lesions [3]. Lastly, the stem cell theory asserts that a stem cell/progenitor cell(s) (which may originate from neonatal/adult endometrium, bone marrow, or elsewhere in the body) differentiates to establish cells comprising endometriotic lesions [3]. Although circumstantial evidence exists to support each of these various theories, endometriosis is immensely heterogenous in its clinical presentation and no single theory has proven sufficient to explain every incident case of endometriosis. For example, whilst retrograde menstruation is the most direct explanation for peritoneal endometriosis it can not explain the rare cases of pulmonary endometriosis and either blood or

lymphatic borne metastasis or one of the independent mechanisms mentioned above would need to be invoked.

The current study by Noë *et al*. is an extension of work published by this team in 2017 [4,5]. By focusing analysis on passenger mutations within endometriotic lesions, this study presents intriguing findings that shed insight into the origins of this complex disease [4]. From the perspective of the stem cell theory, Noë *et al*. sought to elucidate whether epithelial and stromal cells from the same endometriosis lesions are clonally-derived from same progenitor/stem cells [4]. Previously, this group had performed whole-exome sequencing of mixed endometriotic lesions (consisting of epithelium and stroma) and identified various passenger mutations (synonymous and missense mutations) within lesions [5]. They subsequently designed droplet digital PCR assays for 19 different passenger mutations and determined all these mutations to be enriched in the epithelial component of endometriosis lesions [4]. Interestingly, none of the passenger mutations assessed were enriched in the stromal compartment of lesions (low mutant allele frequencies detected in the stroma were likely a result of contamination with trace amounts of epithelial cells).

Passenger mutations – as distinguished from driver mutations – are randomly-occurring mutations that have no effect on the fitness of a clone. However, because such passenger mutations are inherited by daughter cells following clonal expansion, these mutations may be utilized as genetic markers in which to infer clonality or the

genetic relatedness of cells. In consideration of the genetic relatedness of epithelial cells and stromal cells within the same endometriotic lesions, three possible scenarios could exist, as illustrated in Fig. 1.

Noë *et al*. findings are consistent with Fig. 1D, therefore providing evidence that the epithelial cells and stromal cells of endometriotic lesions follow independent developmental trajectories with clonally-related epithelial cells and no detectable relationship between the stromal cells. They detected all passenger mutations in the epithelial compartment exclusively [4]; as noted by the authors, this finding suggests that the epithelium demonstrates clonality, whereas the stroma does not [4]. Passenger mutations present in cells that ultimately give rise to the endometriotic epithelium achieve detectable levels as a result of clonal expansion, whereas passenger mutations that may be present in cells giving rise to endometriotic stroma remain at undetectable levels (presumably because the clone does not expand massively). Overall, these findings suggest that whereas epithelial cells may establish themselves at an ectopic site through a seeding/metaplastic event with subsequent clonal expansion, stromal cells may be continuously regenerated or recruited to the site of endometriotic lesions in an ongoing process.

Another interesting finding was that each of the six endometriotic lesions examined harbored multiple passenger mutations [4]. Although all passenger mutations were enriched in the epithelium, the mutant allele frequencies (MAFs) of such mutations

differed from one another. This finding is evident in Case 2 – Noë *et al*. reported MAFs of 17% for *CD300E* and 35% for *OR7E24* within the epithelial compartment [4]. The authors postulate that such findings indicate subclonality within some endometriotic lesions [4]. In the clonal expansion of epithelial cells already harboring a passenger mutation (such as *OR7E24*), one of the proliferating cells may have acquired a second passenger mutation (such as *CD300E*) and therefore a subset of cells would be positive for both passenger mutations. Although subclonality represents a plausible explanation for the discrepancies in MAFs for multiple passenger mutations with the same lesion, as noted by the authors, their experiments do not exclude the possibility of the existence of multiple clones or even copy number changes that distort the MAFs [4]. In other words, it is possible that two (or more) independent clonal expansion events occurred at the same ectopic site and gave rise to epithelial cells harboring different passenger mutations. This suggestion is supported by a previously published observation of the presence of two distinct *KRAS* hotspot mutations in the epithelium of the same endometriotic lesion [5]. Regardless, both possibilities are thought-provoking and reveal the complexity of endometriosis development.

Studies focusing on the presence of somatic mutations have demonstrated immense utility in answering key questions surrounding endometriosis in recent years. Previously, the detection of somatic mutations in *ARID1A* or *PIK3CA* in clear cell and endometrioid ovarian cancers and concurrent endometriosis helped establish endometriosis as the

precursor of these cancers [6,7]. Furthermore, in a recent study, one patient harbored the same *KRAS* gain-of-function mutation in three anatomically-separated lesions of endometriosis, thereby suggesting that: 1) driver mutations may arise early in the pathogenesis of endometriosis and 2) it is possible that some benign, deep infiltrating endometriosis lesions can spread or metastasize [5,8]. The findings of Noë *et al*. further enhance our understanding of the pathogenesis of endometriosis by highlighting the independent and distinct developmental patterns of the epithelium and stroma within the same endometriotic lesions. Consistent with the observation of driver mutations exclusively in the epithelial compartment in the study by Anglesio *et al*. [5], Noë *et al*. observed passenger mutations at detectable levels in the epithelium only [4]. Such findings suggest that the clonal expansion of 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 endometrial-like stroma. Moreover, evidence from other studies suggest that inflammatory processes at the site of endometriosis could potentially lead to the recruitment of bone marrow-derived mesenchymal stem cells to such sites [9]. These stem cells may subsequently contribute to stromal regeneration [10].

It is important to note that the study by Noë *et al*. does not assess the validity of stem cell theory over other theories and the origins of endometriosis remain contentious. Particularly, although the authors show that the development of epithelial and stromal cells is likely independent, it is unclear by which mechanisms these cells arise at ectopic sites. Other theories such as retrograde menstruation and coelomic metaplasia, in conjunction with stem cell theory, may offer explanations to this phenomenon. Furthermore, do the same patterns of stromal and epithelial cell development hold true for other forms of endometriosis? We hope that future work by the authors and other research groups will help clarify the sources of both epithelial and stromal cells and how they come to establish endometriotic lesions. In closing, Noë *et al*. reveal the complexity of the genesis of endometriosis and future experimental work on this disease should note whether observations made are relevant to epithelial or stromal cells within endometriosis lesions, as these cells likely arise from dissimilar sources and play differing yet complimentary roles within this complex disease. In addition, somatic mutations are the first easily measurable features of endometriosis and could potentially be used to create a biologically informed classification for this disease. Such a goal would require rigorous analysis of large numbers of clinically-annotated lesions and adherence to a stepwise biomarker development pathway [11].

Author contributions statement:

VL and DGH reviewed the literature and jointly wrote the commentary.

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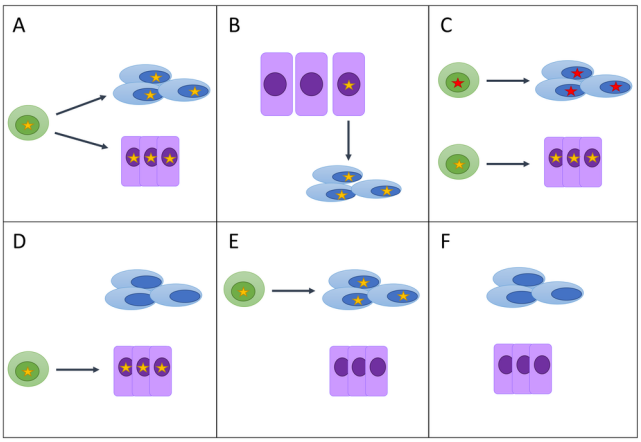
Figure Legend:

Figure 1. Possible developmental pathways leading to specific inheritance patterns of passenger mutations. (A) The same stem/progenitor cells (in green) differentiate into both epithelial cells (in purple) and stromal cells (in blue). Passenger mutations (denoted by a yellow star), if present in the originating cell, would be detected in both epithelial and stromal cells. (B) Epithelial cells transdifferentiate into stromal cells (or *vice versa*). The same passenger mutations would be detected in a subset of both epithelial and stromal cells (albeit to differing frequencies). (C) Epithelial cells and stromal cells arise independently from the clonal expansion of different progenitor cells. Unique passenger mutations (denoted by either yellow or red stars) would be exclusive to either epithelial or stromal compartments. (D) Progenitor cells clonally expand to give rise to epithelial cells, whereas stromal cells arise without clonal expansion. (E) Progenitor cells clonally expand to give rise to stromal cells, whereas epithelial cells arise without clonal expansion. (F) Cells giving rise to either epithelial cells or stromal cells do not undergo clonal expansion. No passenger mutations are detectable in either compartment.



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