Research Highlights

Highlights from the latest articles in epigenomics of endometriosis

Pathogenesis of endometriosis and its relationship to gynecological cancers

Evaluation of: Vestergaard AL, Thorup K, Knudsen U *et al.* Orogenic events associated with endometrial and ovarian cancers are rare in endometriosis. *Mol. Hum. Reprod.* doi:10.1093/molehr/gar049 (2011) (Epub ahead of print).

Endometriosis, the presence of ectopic endometrial tissue primarily in the ovaries, peritoneum and rectovaginal septum affects 10% of women at reproductive age [1]. While endometriosis is considered benign, it is associated with an increased risk of ovarian cancer, predominantly of clear cell and endometrioid subtypes [2]. Epidemiological data indicate no association between endometriosis and cancer of the uterine endometrium [2]. Molecular studies with microsatellite markers suggest that ovarian endometrial cysts, typical manifestations of endometriosis, have a monoclonal origin characteristic of neoplasia [3,4]. Furthermore, identical changes have been found in endometriosis and concurrent ovarian clear cell or endometrioid carcinoma (e.g., *PTEN* mutation and loss [5], *PIK3CA* mutation [6], and *ARID1A* mutation and absent expression [7]), suggesting that such changes may represent early events in transforming endometriosis into ovarian cancer. Observations of a clonal relationship between endometriosis and ovarian cancer support the hypothesis of extraovarian origin of at least endometrioid and clear cell epithelial ovarian carcinoma [8].

Vestergaard *et al.* [9] analyzed DNA from ectopic endometriosis lesions from 23 women and found that genetic and epigenetic changes common in endometrial and ovarian carcinomas were absent, with one exception (*KRAS* codon 12 mutation detected in one case). Mutations in nine genes and promoter methylation in six

genes were tested. Obviously, the possible existence of molecular changes in genes not tested or alterations beyond the reported sensitivity of the methods (2–5%) cannot be excluded. Moreover, the procedure of tissue procurement may critically affect the likelihood of detecting molecular alterations. The so-called superficial endometriosis (e.g., ovarian endometrioma) is associated with secondary changes due to repeated hemorrhage. As a result, the epithelium may be reduced to a single layer and the stroma is scant; frequently, these components are completely lost and replaced by fibroblastoid proliferation and diffuse infiltration of histiocytes. Even in ‘deep endometriosis’ (e.g., rectovaginal disease), the proportion of endometriotic tissue in a typical biopsy specimen rarely exceeds 25%. Studies cited above which report identical changes in endometriosis and adjacent malignancies [5–7] used laser capture microdissection to isolate epithelial cells of endometriotic cysts. However, Amemiya *et al.* [10], who used laser capture microdissection as well, detected *KRAS* mutation and microsatellite instability in ovarian endometrioid carcinoma but not in normal or atypical endometriosis bordering the cancer, thus conforming to Vestergaard *et al.* [9].

Clearly, the molecular pathways of endometriosis including those that lead to ovarian cancer remain to be defined by additional studies, which should be based on sufficiently large series of relevant clinical specimens combined with new *in vivo* and *in vitro* models [11,12].

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Endometriosis as an epigenetic disease

Evaluation of: Kawano Y, Nasu K, Li H *et al.* Application of the histone deacetylase inhibitors for the treatment of endometriosis: histone modifications as pathogenesis and novel therapeutic target. *Hum. Reprod.* 26(9), 2486–2498 (2011).

Endometriosis is a common gynecologic disease ascribed to hormonal and immunological factors, and possibly to genetic and environmental factors as well [1,2]. Epithelial cells of endometriotic cysts reveal genetic alterations with variable frequencies (reviewed in the accompanying research highlight on Vestergaard *et al.* [3] above). Epigenetic mechanisms have recently emerged as possible mediators of genetic effects as well as endogenous or exogenous influences coming from the environment [4]. Endometriosis cells may show hypermethylation of gene promoters including those encoding progesterone receptor- β and other steroid receptors [5], overexpression of DNA methyltransferase genes [6], and mutations in genes responsible for chromatin remodeling [7]. Similar changes may occur in a variety of malignancies including ovarian cancers synchronous to endometriosis

[7], suggesting that such changes may contribute to the pathogenesis and malignant potential of endometriosis.

Kawano *et al.* [8] observed that endometriotic cyst-derived stromal cells showed decreased levels of acetylated histones H3 and H4, accompanied by repressive chromatin. The changes could be reversed by histone deacetylase inhibitors in cultured cells.

The findings are significant from two main points of view. First, they add a new facet to endometriosis as an epigenetic disease and show that not only epithelium but stroma, too, is an important target of molecular alterations in this disorder. Since pronounced secondary changes (fibrosis and chronic inflammation) are often present in older endometriotic lesions, it is necessary to use cell type-specific markers to verify the tissue of origin. The markers used by Kawano *et al.* to characterize the cell cultures (cytokeratin, vimentin, factor VIII and leucocyte common antigen [9]) exclude epithelial, endothelial and inflammatory cell contamination. CD10, an established relative marker of endometrial stromal cells, was also used as a positive marker [8]. As myoepithelial cells, too, may express CD10 [10], other positive markers (estrogen receptor- α and progesterone receptor) and negative

markers (smooth muscle actin) might have been worthwhile additions.

Second, a possible new mode of treatment for endometriosis is proposed by demonstrating that histone deacetylase inhibitors induced the accumulation of acetylated histones in the promoter regions of cell cycle regulator genes, suppressed cell proliferation and induced apoptosis of cultured endometriotic cyst stromal cells [8]. Various translational applications of epigenetic changes may be even more pertinent in ovarian cancer to which endometriosis may develop [11]. However, a number of important concerns relevant to any solid tumors in this context (e.g., transport to the affected organ, pleiotropic effects, and long-term safety and efficacy [2,12]) first need to be successfully addressed and solved in clinical trials before epigenetic drugs can become part of clinical treatment regimen in endometriosis.

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Epigenetic events in endometrial tumorigenesis

Evaluation of: Dewdney SB, Rimel BJ, Thaker PH *et al.* Aberrant methylation of the X-linked ribosomal S6 kinase RPS6KA6 (RSK4) in endometrial cancers. *Clin. Cancer Res.* 17(8), 2120–2129 (2011).

Endometrial cancer is the most common malignancy of the female genital tract. Type I (endometrioid and estrogen-dependent) tumors account for 80% and type II (nonendometrioid and nonestrogen-dependent) tumors for 20% of the cases [1]. Type I tumors occur in premenopausal and younger postmenopausal women, are of low-grade and localized, and have a favorable outcome, whereas type II tumors occur in older postmenopausal women, are of high grade and more advanced stages, and are associated with a worse prognosis.

The different clinical behavior is paralleled by distinct genetic alterations. Type I tumors exhibit frequent microsatellite instability and mutations in *PTEN*, *PIK3CA*, *KRAS* and Wnt pathway genes, whereas type II tumors display *TP53* mutations and chromosomal instability [2]. Type I and type II endometrial cancers essentially differ relative to their epigenetic profiles as well [3]. The CpG island methylator phenotype, CIMP, is characteristic of type I but not type II endometrial cancers [3] and may in part be

explained by altered DNA methyltransferase gene expression which is elevated in type I and decreased in type II tumors compared with normal endometrium [4].

Dewdney *et al.* [5] provide a specific example of a differential involvement of a candidate tumor suppressor gene in type I versus type II tumors. They found that the promoter of the *RSK4* gene encoding a putative suppressor of the ERK signaling pathway showed hypermethylation in a majority of primary endometrioid (type I) endometrial tumors and hypomethylation in serous (type II) tumors relative to normal endometrium. Furthermore, among endometrioid tumors, *RSK4* methylation declined along with decreasing differentiation (i.e., towards higher grade).

The results by Dewdney *et al.* [5] are in agreement with a recent investigation showing that high-grade (grade 3) carcinomas of type I exhibit morphological and immunohistochemical features of both type I (e.g., lack of p53 overexpression) and type II endometrial carcinomas (e.g., low estrogen receptor- α and progesterone receptor expression) [6]. It is possible that in type I tumors with low-grade, increased ERK signaling due to silencing of *RSK4* by promoter hypermethylation is important, together with previously established alterations such as hyperestrogenism, whereas type I tumors with high grade and type II tumors develop through other

mechanisms that are less well understood. These observations as a whole underline the important notion that in analogy to ovarian cancer, endometrial cancer comprises distinct clinicopathological subtypes which should be appropriately taken into account in the design of diagnostic and therapeutic strategies.

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