Endometriosis and the neoplastic process

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Abstract

Endometriosis is a frequent disorder that commonly presents with infertility and pelvic pain. Although the precise aetiology of endometriosis is unclear, it is generally considered to involve multiple genetic, environmental, immunological, angiogenic and endocrine processes. Genetic factors have been implicated in endometriosis but the susceptibility genes remain largely unknown. Although endometriosis is a benign disorder, recent studies of endometriosis suggest endometriosis could be viewed as a neoplastic process. Evidence to support this hypothesis includes the increased susceptibility to develop ovarian clear-cell and endometrioid cancers in the presence of endometriosis, and molecular similarities between endometriosis and cancer. In this article we discuss (i) the evidence suggesting that endometriosis might be viewed as a neoplastic process, and (ii) the implications of this hypothesis for elucidating the pathogenesis of endometriosis and developing novel methods of diagnostic classification and individualised treatments.

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What is endometriosis?

Endometriosis is defined as the implantation of endometrium-like glandular and stromal cells outside their normal location in the uterus. Endometriotic lesions are usually identified at laparoscopy localised to ovaries and the pouch of Douglas (Fig. 1). Endometriosis is diagnosed in 30% of cases referred for infertility investigations (Lapp 2000) and in 10–70% of women with pelvic pain (Lapp 2000). Overall, studies estimate that endometriosis may affect around 7–15% of women of reproductive age, thus making this a common condition.

Endometriosis has been considered a 'disease' because it is often identified when investigating women with infertility, pelvic pain, dyspareunia (pain on intercourse) and dysmenorrhoea (painful periods). Traditionally the classification of endometriosis has been made by anatomical (surgical staging by revised American Fertility Society score) and histopathological (atypical and non-atypical endometriosis) criteria (Roberts & Rock 2003). However, this combined approach of classification does not correlate closely with pelvic pain or reproductive outcome, and is prone to inter-observer error. Furthermore, the emphasis on targeting the endometriotic lesion, by surgical removal or hypo-oestrogenic inactivation, does not necessarily correct the aberrant underlying molecular mechanism(s). This explains why current endometriosis

treatment does not alleviate clinical symptoms in all cases, and recurrence is common (Donnez *et al.* 2002).

These disparities suggest that endometriosis may not be a true 'disease' but a heterogeneous entity with differing subtypes. One subtype may be capable of causing symptomatic disease directly consequent to endometriotic pathology (e.g. ovarian endometriomas, pelvic adhesions). While another subtype may be associated with symptoms without an obvious endometriotic-lesion basis. Another subtype may be clinically asymptomatic and its presence be considered a normal 'non-pathogenic' phenomena. Consequently the current focus on treating the endometriotic lesion should be reconsidered, and efforts to understand the pathogenesis of endometriosis, and its temporo-spatial relationship with symptomology, should be increased.

Endometriosis and the neoplastic process

For some time, endometriosis research has focused on comparisons of various physiological processes in the endometrium (ectopic vs eutopic) of women affected by endometriosis, against unaffected women (Sharpe-Timms 2001). This has identified multiple anomalies in genetic, environment, angiogenic, endocrine, metabolic and immunological mechanisms. Some of these correlate with the severity of endometriosis and/or associated clinical

Figure 1 Typical uterosacral/pouch of Douglas endometriosis lesion.

sequelae implying a causative rather than simply associative role. However, the major obstacle has been the difficulty in discriminating between processes fundamental to endometriosis aetiopathogenesis and epiphenomena. Importantly, these physiological differences are multi-compartmental (endometrium, peritoneal fluid, follicular fluid and blood) and not just localised to the site of the endometriotic lesion, implying a fundamental widespread alteration in reproductive tract function. This multifactorial multi-compartmental pathogenesis, coupled with the clinical heterogeneity, has created a confusion of data, with little consensus on a unifying mechanism.

Since Sampson first reported in 1925 (Sampson 1925) that endometriosis may give rise to malignant change, and proposed criteria for diagnosis of malignancy arising in endometriosis, extensive evidence for an association between endometriosis and cancer (especially ovarian) has accumulated. A recent landmark publication, ‘Hallmarks of cancer’ (Hanahan & Weinberg 2000), defined seven critical features of the cancer phenotype (Table 1). To evaluate the hypothesis that endometriosis may be viewed as a neoplastic process, we review the (i) clinicopathological and (ii) molecular and genetic features of endometriosis in relation to the framework suggested by Hanahan. In addition, we outline how the hypothesis provides a basis for elucidating the pathogenesis of endometriosis and the prospect of using ‘molecular signatures’ for the classification and treatment of endometriosis.

Clinicopathological similarities of endometriosis and cancer

The histopathological and epidemiological evidence demonstrating the strong association between endometriosis and ovarian cancer is given in the following subsections. There are two hypotheses that could explain the link: (i) endometriotic implants may directly undergo malignant transformation, perhaps through an atypical endometriosis transition phase; and (ii) both endometriosis and cancer

Table 1 The hallmarks of cancer. The listed capabilities are mostly acquired directly, or indirectly, through changes in the genome of cancer cells.

Self-sufficiency in growth signals	Mitotic growth signals are needed for cells to move from a quiescent state into an active proliferative state. These signals are transmitted into the cell by transmembrane cell-surface receptors that bind to: diffusible growth factors, extracellular matrix (ECM) components, cell-to-cell adhesion interaction molecules
Insensitivity to anti-proliferative signals	Growth inhibitory signals (soluble or immobilized in ECM and on surfaces of nearby cells) are received by transmembrane cell-surface receptors coupled to intracellular signalling circuits
Resistance to apoptosis	Evasion mechanisms of programmed cell death
Limitless replicative potential	Disruption of intrinsic cell-autonomous programme that limits their multiplication. This program operates independently of the cell-to-cell signalling pathways described above
Sustained angiogenesis	Virtually all cells in a tissue are obligated to reside within 100 µm of a capillary blood vessel to allow adequate permeation of oxygen and nutrients crucial for cell survival. The cells within aberrant proliferative lesions initially lack angiogenic ability, but in order to progress, incipient neoplasias must develop angiogenic ability
Tissue invasion and metastasis	This enables cancer cells to escape the primary tumour mass and colonise new sites where, at least initially, nutrients and space are not limited
Genomic instability	Mutations or inactivation/activation of TSGs, oncogenes, DNA monitoring and repair enzymes, checkpoint systems at mitosis. These are carried out by intragene (e.g. mutation, deletion) and epigenetic (e.g. promoter hypermethylation) mechanisms

share common antecedent mechanisms and/or predisposing factors (e.g. genetic susceptibility, immune/ angiogenic dysregulation, environmental toxin exposure), with obvious divergence in molecular pathways downstream.

Histopathology

Like malignancy, endometriosis displays features of atypia, adherence, invasion and metastases. Atypical endometriosis is characterised histologically by endometrial glands with cytological or architectural atypia (LaGrenade & Silverberg 1988), and has been observed in 12–35% of ovarian endometriosis (Seidman 1996, Nishida *et al.* 2000, Bayramoglu & Duzcan 2001). Around 60–80% of cases of endometriosis-associated ovarian cancer (EAOC) occur in the presence of atypical ovarian endometriosis (Fukunaga *et al.* 1997, Ogawa *et al.* 2000, Oral *et al.* 2003). Of these cases, 25% show direct continuity of the atypical ovarian endometriosis with ovarian cancer (Fukunaga *et al.* 1997), underlying a potential ‘premalignant’ transition spectrum of non-atypical to atypical and malignant variants.

Morphometry

Morphometric analysis of cancer, used for assessing mitotic activity and grading, has been shown to correlate with clinical prognosis (Kronqvist *et al.* 2002). Although morphometric analysis of non-atypical endometriosis showed no difference between active (red) and inactive (black or white) lesions, it has yet to be studied in atypical endometriosis (Regidor *et al.* 2002). Nonetheless, mild cytological atypia in the glandular epithelium of endometriotic cysts has been associated with normal DNA diploid patterns, whereas severe atypia may be associated with aneuploidy (Ballouk *et al.* 1994).

Ovarian malignancy may arise directly from ovarian endometriosis

Around 60% of EAOs occur with the cancer adjacent to endometriosis or arising directly from ovarian endometriosis, with the remaining 40% occurring with distant endometriotic disease (Erzen & Kovacic 1998, Modesitt *et al.* 2002). Clear-cell and endometrioid carcinomas are the commonest EAOs with ovarian endometriosis, while clear-cell adenocarcinoma and adenosarcoma are the commonest EAOs in extra-ovarian endometriosis (Erzen & Kovacic 1998, Stern *et al.* 2001, Zaino *et al.* 2001). The risk of direct malignant transformation of ovarian endometriosis has been estimated as 0.7–1.6% over an average of 8 years (Seidman 1996, Nishida *et al.* 2000). Interestingly, there is a common unexplained left-sided predominance for endometriotic cysts, and ovarian endometrioid and clear-cell cancers (Vercellini *et al.* 2000, Al Fozan & Tulandi 2003).

Increased risk of ovarian cancer in women with endometriosis, irrespective of whether endometriosis is distant or adjacent to ovarian tumour

The age-standardised incidence of ovarian cancer in women in the UK is 21.9 per 100 000 (0.02%), with around 75% of cases diagnosed in postmenopausal women (National Statistics 2003). If there were no association between cancer and endometriosis then the incidence of endometriosis in women with ovarian cancer would be similar to that in the general population. However, the incidence of endometriosis in women with ovarian cancer is 8–30% (Fukunaga *et al.* 1997, Ogawa *et al.* 2000, Oral *et al.* 2003). This compares with a background incidence of endometriosis of 7–15% in women of reproductive age, and less than 2% in postmenopausal women (Lapp 2000). These data correlate with the finding from a Swedish population study, where the risk of ovarian cancer was increased 4.2-fold (95% confidence interval 2.0–7.7) in the presence of endometriosis (Brinton *et al.* 1997).

Furthermore, the histology of EAO (40–55% clear-cell, 20–40% endometrioid and <10% serous and mucinous subtypes) (Fukunaga *et al.* 1997, Yoshikawa *et al.* 2000, Modesitt *et al.* 2002) differs considerably from that

seen in all ovarian cancers (FIGO 1998 annual report 55% serous, 13% mucinous, 14% endometrioid, 6% clear-cell) (Pecorelli *et al.* 1998).

Increased risk of synchronous endometrial and ovarian cancers, especially endometrioid type, in the presence of endometriosis

Simultaneously detected endometrial and ovarian carcinomas are most often associated with endometrioid subtypes, and ovarian endometriosis was identified in around 30% of these cases (Erzen & Kovacic 1998, Stern *et al.* 2001, Zaino *et al.* 2001).

Clinical behaviour and prognosis of EAO differs from matched ovarian cancer subtypes not associated with endometriosis

EAO compared with ovarian cancer without endometriosis, presents at a less-advanced stage, lower grade, predominantly endometrioid and clear-cell type, and has a better overall survival (Erzen *et al.* 2001, Modesitt *et al.* 2002).

Increased risk of extra-ovarian cancers

Around 80% of intraperitoneal cancers associated with endometriosis relate to ovarian cancer, with the remainder extra-ovarian (Modesitt *et al.* 2002). A separate study showed an increased risk of extra-pelvic cancers (breast and non-Hodgkin's lymphoma) in women with endometriosis (Brinton *et al.* 1997).

Molecular similarities of endometriosis and cancer

In the following subsections we review the molecular and genetic features of endometriosis in relation to the characteristics of cancer suggested by Hanahan & Weinberg (2000) (see Table 1).

Self-sufficiency in growth signals

Like uterine and breast cancer, endometriosis behaves as an oestrogen-dependent neoplasm. Endometriosis has specifically adapted to oestrogen-induced signalling by:

- i) Increased local production of oestrogen through increased expression of aromatase cytochrome P450 expression but deficient 17 β -hydroxysteroid dehydrogenase type 2 expression (which impairs inactivation of potent oestradiol to less-potent oestrone (Bulun *et al.* 2000b)).
- ii) Increased responsiveness to oestrogen. There is increased oestrogen receptor (ER- α) expression in active (red lesions) compared with inactive (black lesions) endometriosis (Matsuzaki *et al.* 2001b).
- iii) Inherited genetic polymorphisms in oestrogen and progesterone receptors (PRs), which predispose to

endometriosis (Kitawaki *et al.* 2001, Wieser *et al.* 2002).

- iv) Inherited genetic polymorphisms in drug-metabolising enzymes (CYP1A1, CYP19 and GSTM1) which predispose to endometriosis (Baranova *et al.* 1999, Hadfield *et al.* 2001, Nakago *et al.* 2001, Arvanitis *et al.* 2003) and ovarian endometrioid and clear-cell cancers (Baxter *et al.* 2001). These alterations may induce endometriosis or cancer by altering a dioxin-induced oestrogen growth signal. Dioxins are environmental contaminants, and have been shown to induce endometriosis-like and oestrogen-dependent tumours in animal models (Birnbau & Cummings 2002). Of importance, there is a doubled risk of developing endometriosis amongst women with high serum dioxin levels (Eskenazi *et al.* 2002). Activation of ERs in endometriosis may occur indirectly through up-regulated CYP1A1 activity, which causes increased aromatase P450 and oestrogen production (Bulun *et al.* 2000a), or directly by dioxin-activated aryl hydrocarbon receptor (Ohtake *et al.* 2003).

Other growth factors, such as transforming growth factor- α and insulin-like growth factor-I (IGF-I) have also been implicated in endometriosis and cancer development (Lebovic *et al.* 2001). IGF-I signalling is required for cell-cycle progression and appears to be a pre-requisite for malignant transformation and implantation. A higher risk for cervical, ovarian and endometrial cancer is related to high IGF-I levels in post- and premenopausal women. Plasma IGF-I levels are higher in cases of severe endometriosis; however, in endometriosis IGF-I levels locally in the endometrium are reduced (Druckmann & Rohr 2002).

Insensitivity to anti-proliferative signals

Cell division relies on the activation of cyclins (e.g. cyclin D1), which bind to cyclin-dependent kinases (cdk) to induce cell-cycle progression towards S phase and later to initiate mitosis. Since uncontrolled cdk activity is often the cause of human cancer, their function is tightly regulated by cdk inhibitors (e.g. p21 and p27 Cip/Kip proteins). For example, increased expression of cyclin D1 and cdk occurs in breast cancer and is associated with poor outcome.

At the cellular level, differences in expression of p27Kip1 protein (cdk inhibitor) in active and inactive endometriotic lesions (Matsuzaki *et al.* 2001a), coupled with increased p21 expression in endometriomas compared with benign and malignant ovarian tumours (Fauvet *et al.* 2003), suggest a role for increased cdk activity through reduced cell-cycle inhibitor activity, which is an imbalance frequently seen in cancer.

At the tissue level, endometriosis may resist the anti-proliferative effect of progesterone by the predominant expression of the inhibitory PR-A isoform instead of the stimulatory PR-B isoform (Attia *et al.* 2000).

Resistance to apoptosis

Malignancy commonly displays overexpression of anti-apoptotic (Bcl-2), under-expression of pro-apoptotic (BAX) factors, and inactivation of p53 gene (p53 is a tumour suppressor gene (TSG) whose protein (TP53) is pro-apoptotic) through mutation. Similarly, endometriotic lesions have also evolved strategies to evade apoptosis by: (i) increased Bcl-2, and decreased BAX (Meresman *et al.* 2000); (ii) up-regulation of survivin and matrix metalloproteinases (MMPs) (Ria *et al.* 2002, Ueda *et al.* 2002a); (iii) elevated soluble Fas ligand (FasL) and interleukin (IL)-8 in endometriotic peritoneal fluid (the increased FasL expression by IL-8 may induce apoptosis of T lymphocytes and thus enable endometriosis to evade immune-mediated cell death (Garcia-Velasco *et al.* 2002)); and (iv) germline (Chang *et al.* 2002) and somatically acquired (Bischoff *et al.* 2002) inactivating mutations of p53 gene.

Limitless replicative potential

With each replicative cycle, telomeres (repetitive DNA sequences capping each chromosome) become progressively shorter, eventually resulting in cell senescence and cell death. Tumours commonly express the enzyme telomerase, which protects the telomeres from shortening and thus preventing 'cell ageing'. Oestrogen and progesterone stimulate, while tamoxifen and wild-type (normal variant) p53 inhibit, telomerase activity in breast and endometrial cancer cells (Vidal *et al.* 2002, Wang *et al.* 2002). Although there are no published studies examining telomerase function in endometriosis, it is notable that estrogen-dependent neoplasms are potentially susceptible to telomerase control.

Sustained angiogenesis

Pathological angiogenesis, immune cell suppression and immune cell activation co-exist in endometriosis and cancer processes (Folkman 2002, Gazvani & Templeton 2002). Genetically transmitted or environmentally induced (e.g. exposure to dioxins) alterations in the angiogenic and/or immune response may predispose women to the ectopic implantation of endometrial cells, transported into the peritoneal cavity by retrograde menstruation, which thereby leads to endometriosis. Significantly, both cancer and endometriosis share some of the mediators implicated in this 'inflammatory angiogenesis' model. Furthermore, the genes of these mediators exhibit genetic polymorphisms that predispose either to endometriosis (e.g. intercellular adhesion molecule-1, IL-6 and IL-10 gene promoters) (Kitawaki *et al.* 2002, Vigano *et al.* 2003, Wieser *et al.* 2003) or to cancer (e.g. IL-6, IL-8, tumour necrosis factor (TNF)- α , NF κ B-1, and peroxisome proliferator-activated receptor- γ genes) (Landi *et al.* 2003).

Anti-angiogenic therapy involves the inhibition of pro-angiogenic factors (e.g. anti-vascular endothelial growth factor (VEGF) monoclonal antibodies) or activation of

endogenous inhibitors of angiogenesis (e.g. endostatin and angiostatin). Pre-clinical studies have shown that endostatin effectively inhibits tumour growth and shrinks existing tumour blood vessels. Phase 1 clinical cancer trials of endostatin and angiostatin are ongoing, and preliminary results show minimal toxicities. Similarly, anti-angiogenic strategies for treating endometriosis exist, but are still at the experimental phase (Hull *et al.* 2003). Soluble truncated receptor (Flt-1) and an affinity-purified antibody to human VEGF-A, significantly inhibited the growth of endometrial explants in a mouse *in vivo* model of endometriosis by disrupting the vascular supply. Gene transfection (using a replication-deficient adenovirus vector AdAngiostatin) of the endogenous angiogenesis inhibitor angiostatin to the peritoneum of a mouse was successful in treating a mouse *in vivo* model of endometriosis (Dabrosin *et al.* 2002).

Tissue invasion and metastasis

The ability to invade through the basement membranes characterises the transition from non-invasive to invasive cancer. Tumours secrete proteases (e.g. MMPs) to degrade the basement membrane and surrounding stroma. Expression of MMP-2 and MMP-9 is correlated with grade and stage of many cancers. Likewise, MMP activity is up-regulated in endometriotic lesions (Mizumoto *et al.* 2002).

De-regulation of cell adherence signalling involving integrins, β -catenin, E-cadherin and P-cadherin has been demonstrated in the genesis of a number of malignancies (Morin 1999), and has also been implicated in endometriosis aetiopathogenesis (Scotti *et al.* 2000, Witz *et al.* 2000, GT Chen *et al.* 2002, Ueda *et al.* 2002b). β -Catenin mutations have been identified in endometrial and ovarian endometrioid cancers (Palacios & Gamallo 1998, Moreno-Bueno *et al.* 2001) but have not been looked for in endometriosis. Cytokeratin-positive and E-cadherin-negative endometriotic cells have an invasive phenotype in an *in vitro* collagen invasion assay similar to metastatic carcinoma cells (Starzinski-Powitz *et al.* 1999).

Genomic instability

The classic model of malignant transformation of the cell involves the stepwise acquisition of multiple genetic alterations, which confers a clonal selective advantage at each step, predisposing to the next step (Lengauer *et al.* 1998). This is often accompanied by activation of proto-oncogenes to oncogenes (transformation of normal cellular growth, proliferation and differentiation genes) and inactivation of TSGs (genes that encode for proteins which inhibit excess cellular proliferation and malignant transformation). The genetic alterations can occur at different levels and include single nucleotides, small stretches of DNA (microsatellites), whole genes, chromosomal components or whole chromosomes. The genetic alterations

can be intragene or epigenetic (e.g. gene silencing by promoter hypermethylation). Six principal genetic mechanisms have been identified to contribute to genomic instability in cancer, but only the first three have been looked for in endometriosis:

- i) Gain in oncogenic activity
- ii) Inactivation of TSG (loss of both gene copies of allele confers functional loss), or inactivation of haploinsufficient TSG (loss of only a single gene copy of allele confers functional loss)
- iii) Anomalies in DNA mismatch repair enzymes, identified by microsatellite instability (MSI)
- iv) Inactivation of genes that monitor genomic instability at cell cycling (e.g. mitotic spindle assembly checkpoint genes)
- v) Telomere dysfunction (provokes chromosomal aberrations initiating carcinogenesis) and telomerase-mediated telomere maintenance (enables cells to achieve a fully malignant endpoint and metastasis)
- vi) Hypermethylation

These mechanisms often act in synergy to promote genomic instability and tumour cell proliferation. For example, deficiency of the TSG p53 alters the cellular response to DNA damage, in that it leaves cells with attenuated DNA damage checkpoint controls and a reduced propensity for apoptotic cell death. Thus, although the DNA repair capacity of these cells is reduced, survival is increased. This promotes genomic instability and contributes to the resistance of p53-deficient cells to cytotoxic agents.

Importantly, pre-malignant lesions display similar genetic aberrations to established cancer. Loss of mismatch repair enzyme activity, and loss of PTEN (phosphatase and tensin homologue gene) and p53 TSGs frequently occurs in premalignant and malignant stages of breast, endometrial and ovarian carcinomas (Obata *et al.* 1998, Codegoni *et al.* 1999, Saito *et al.* 2000, Laloo & Evans 2001, Mills *et al.* 2001). Furthermore, epithelial–stromal interactions are important in the tumour microenvironment and tumour development. Mutually exclusive germline mutations in PTEN and TP53 have been reported in epithelial and stromal cells of breast cancer underlying the co-dependence of these two cell types in tumourigenesis (Kurose *et al.* 2002).

In a similar manner, endometriosis demonstrates somatically acquired genetic alterations analogous to those found in cancer, resulting in the clonal expansion of genetically abnormal cells. The genetic evidence supporting the 'pre-neoplastic' state of endometriosis involves the following:

Monoclonality

Most neoplasms are monoclonal in origin and evidence for monoclonality of endometriosis has been demonstrated in several studies (Jimbo *et al.* 1997, Tamura *et al.* 1998,

Wu *et al.* 2003), although these findings have been challenged recently (Mayr *et al.* 2003).

Comparative genomic hybridisation (CGH)

CGH has shown over-representation (increased copy-number) of chromosomes 1, 2, 3, 5, 6p, 7, 16, 17q, 20, 21q and 22q in an endometriosis cell culture line FbEM-1, while chromosomes 5p, 6q, 9q, 11p, 12, 13q, 18 and X were under-represented. CGH repeated in endometriotic tissue revealed loss of DNA copy number on 1p, 22q and chromosome X, while gain on 6p and 17q. Fluorescent *in situ* hybridisation (FISH) analysis confirmed that the gain at 17q includes amplification of the proto-oncogene HER-2/neu (Gogusev *et al.* 1999, 2000).

FISH

FISH analysis of late-stage endometriotic lesions showed monosomy of chromosome 17, and loss of TP53 (17p13.1) locus. Because not all endometriotic cells displayed this genetic alteration it was suggested that this was a somatically acquired mutation, perhaps occurring in mainly advanced endometriosis states (Kosugi *et al.* 1999, Bischoff *et al.* 2002).

Loss of heterozygosity (LOH)

LOH commonly indicates regions of TSG inactivation, and has been identified in endometriosis and endometriosis-derived cell lines at 5q, 6q, 9p, 10q, 11q, 22q, p16 (Ink4), galactose-1-phosphate uridylyl transferase, p53 and apolipoprotein AII (Jiang *et al.* 1996, Obata & Hoshiai 2000, Thomas & Campbell 2000, Goumenou *et al.* 2001). Importantly, cases with ovarian cancer adjacent to endometriosis or arising from endometriosis showed common genetic LOH alterations in the endometriosis and cancer, indicating a possible malignant genetic transition spectrum between endometriosis and cancer (Jiang *et al.* 1998, Campbell & Thomas 2001).

MSI

Hypermethylation of hMLH1 (whose gene product is a component of the DNA mismatch repair pathway), with concurrent absence of hMLH1 protein expression, is noted in 8.6% of endometriotic lesions (Martini *et al.* 2002).

Somatic mutations in TSGs

Mutations of PTEN, a TSG, were identified in 20% of ovarian endometrioid carcinomas (EAO and sporadic) and 20% of solitary endometrial cysts, suggesting that inactivation of the PTEN is an early event in the malignant transformation of endometriotic implants (Sato *et al.* 2000). A separate study identified reduced PTEN protein expression in 15% of endometriosis cases (Martini *et al.* 2002).

Germline mutations in TSGs

As stated earlier, there are germline (Chang *et al.* 2002) and somatically acquired (Bischoff *et al.* 2002) inactivating mutations of the p53 gene.

Evidence from EAOs arising from endometriosis

Endometrioid EAOs arising from endometriosis show higher expression of p53 and c-erbB-2 oncoproteins than similar ovarian endometrioid cancers without endometriosis (Prefumo *et al.* 2003). The different pattern of expression in the two groups suggests different molecular pathways and could explain variations in cancer subtype and prognosis between the two groups (Erzen *et al.* 2001, Modesitt *et al.* 2002).

Implications of the 'endometriosis is a neoplastic process' hypothesis

In summary, there is extensive clinicopathological, molecular and genetic evidence supporting the hypothesis that endometriosis is a neoplastic process with a potential for malignant transformation. Like sporadic cancer (Balmain *et al.* 2003), endometriosis may be considered to arise by complex interactions between inherited germline polygenic low-penetrance alleles (polymorphisms) (Hadfield *et al.* 2001, Zondervan *et al.* 2001), somatically acquired genetic alterations (Campbell & Thomas 2001), environmental factors (Birnbau & Cummings 2002), and the processes of the hallmarks of cancer described earlier. A visual summary of the main pathways of this hypothesis is shown in Fig. 2. Acceptance of this hypothesis permits the use of investigative strategies that have proved successful in defining the molecular processes that underlie cancer: genomic, transcriptomic and proteomic profiling. Thus the analysis of genetic changes associated with normal endometrium, non-atypical, atypical and EAO lesions could define the sequential changes involved in the initiation, proliferation and malignant transformation of endometriosis.

Genomic profiling of endometriosis

CGH studies have been undertaken in endometriosis, but could only detect relatively large-scale deletions or duplications. Improved sensitivity and throughput could be achieved by the use of high-throughput matrix array-based CGH (Veltman *et al.* 2003). Rapid detailed LOH analysis could also be achieved using single nucleotide polymorphism (SNP) microarray-based chips (Hoque *et al.* 2003, Marnellos 2003).

Transcriptomic profiling of endometriosis

Gene expression microarray analysis has been demonstrated to provide better subclassification and prognostic predictions than conventional histopathology in many

Figure 2 Proposed model for endometriosis pathogenesis based on cancer framework.

cancers (van de Vijver *et al.* 2002, Liu 2003). Notably, DNA microarrays have been recently undertaken in endometriosis (Eyster *et al.* 2002, Kao *et al.* 2003), endometrial cancer (Mutter *et al.* 2001, Risinger *et al.* 2003), normal endometrium (Borthwick *et al.* 2003), and endometrium of women with endometriosis (H-W Chen *et al.* 2002). Indeed, the combination of CGH and expression microarrays (Bayani *et al.* 2002, Tay *et al.* 2003), or SNP microarrays could be valuable for prioritising the analysis of candidate TSGs and proto-oncogenes (Dobrin & Stephan 2003).

Proteomic profiling of endometriosis

This is a complementary approach to genomic profiling. Immunohistochemistry can be used to provide a limited proteomic profile, but recently developed high throughput proteomic technology (e.g. mass spectrometry, matrix-assisted laser desorption and ionisation time-of-flight, surface enhanced laser desorption and time-of-flight, and

protein microarrays) promise the capacity to define a wide-ranging proteomic profile (Dobrin & Stephan 2003).

Molecular re-classification of endometriosis

Genomic and proteomic profiling should provide new insights into the pathogenesis of endometriosis and malignant transformation. A better understanding of reproductive tract molecular pathology, and the temporo-spatial relationship of endometriosis with clinical sequelae, allows the development of novel therapies targeted to the aberrant process at the molecular level, rather than focussing solely on endometriotic lesion eradication. Examples of agents under investigation for endometriosis and cancer using the cancer hallmark model are listed in Table 2. However, it should be noted there is considerable overlap in the endocrine, metabolic, angiogenic, immunological roles of the mediators implicated in endometriosis and cancer. Thus great care should be taken in delivering

Table 2 Individual therapeutic approaches to endometriosis according to cancer cell hallmarks. Consequent to endometriosis heterogeneity, the exact contribution of each hallmark component may vary between individuals and clinical symptoms. Nevertheless, therapies can be designed to target predominant categories following endometriosis molecular classification (expression signature).

Cancer cell hallmark	Targets under evaluation in endometriosis based on agents used in cancer trials	Targets/ putative targets under investigation in cancer
Self-sufficiency in growth signals	Aromatase inhibitors, selective ER (e.g. Arzoxifene) and PR modulators, Mirena coil, gonadotrophin-releasing hormone antagonists (e.g. cetrorelix)	Inhibitors of: mitogen-activated protein kinase inhibitors, HER-2 receptor (trastuzumab), IGF-I receptor, EGFR (erbitux), EGFR tyrosine kinase (gefitinib), farnesyl transferase, Bcr-Abl tyrosine kinase (imatinib mesylate)
Insensitivity to anti-proliferative signals	—	Proteasome inhibitors (bortezomib), cdk inhibitors (flavopiridol)
Resistance to apoptosis	Angiostatin gene transfer, transfection with pro-apoptotic (e.g. BAX) gene; COX-2 inhibitors	COX-2 inhibitor (celecoxib), thalidomide, apoptosis inducers (exisulind inhibits cGMP). Immunotherapy by genetically modified tumour vaccines (e.g. HER-2 peptide vaccination) or humoral factors (e.g. immunokines like IL-12, TNF antagonists; monoclonal antibody to CA-125 (ovarex), recombinant immunotoxin to mesothelin)
Limitless replicative potential	—	Telomerase modifiers
Sustained angiogenesis	Anti-VEGF monoclonal antibody (bevacizumab), VEGF receptor tyrosine kinase inhibitors	Angiozyme (cleaves mRNA for Flt-1, the main receptor for VEGF), protein kinase C-beta inhibitor (LY317615), COX-2 inhibitor, thalidomide, lysophosphatidic acid inhibitors
Tissue invasion and metastasis	—	Inhibit/modify catenin/cadherin signalling, selective MMP inhibitors
Genomic instability	—	Gene therapy to deliver therapeutic or corrective gene to alter oncogenes/TSG balance. Genes may be delivered by infectious (adenovirus) or non-infectious (liposome) vectors. Examples: <ul style="list-style-type: none"> • Adenoviral E1A (oppose HE-2/neu oncogene), • Adenovirus transfection of wild-type p53 (restore TSG) • Transfect viral suicide genes like HSV thymidine kinase (sensitises to ganciclovir cytotoxicity) • Antisense oligonucleotides (targeting proto-oncogenes, oncogenes like <i>c-myc</i>, protein kinase C-alpha (affinitak) • Tribozymes (cleave oncogene transcripts)

EGFR, epidermal growth factor receptor; COX-2, cyclooxygenase-2.

the agent and selecting the mechanism to be targeted, in order to avoid systemic compromise, or impairing other critical aspects of reproductive tract function (e.g. fertility).

Genomic and proteomic profiling may resolve the heterogeneity of endometriosis into around 10–20 distinct endometriotic 'signatures'. Each molecular 'signature' may correlate more closely to: location (ovarian, pouch of Douglas), invasiveness, atypical compared with non-atypical histology, infertility compared with pelvic pain, susceptibility to malignant transformation, sensitivity or resistance to various therapies. Thus clinical endometriosis treatments could be individualised to the specific expression 'signature'. Furthermore, testing for specific 'signatures' in accessible cells (e.g. serum, endometrial and endometriotic biopsies) could form the basis of a sensitive population-wide screening method. Although, in the case of predicting endometriosis malignant transformation susceptibility, research would be needed to assess how these relatively expensive and labour-intensive tests compare against simpler and cheaper immunohistochemistry and/or computerised nuclear morphometry.

Conclusion

Endometriosis possesses many features of a benign neoplastic process with the potential for malignant transformation. Application of strategies used in the investigation of cancer should provide new insights into the classification and molecular pathogenesis and of endometriosis.

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