Thesis for doctoral degree (Ph.D.)
2016

Endometriosis - involvement of stem cells and clinical impact

Karin Andersson Di Claudio

Karolinska Institutet

From the Department of Women's and Childrens Health
Karolinska Institutet, Stockholm, Sweden

ENDOMETRIOSIS -INVOLVEMENT OF STEM CELLS AND CLINICAL IMPACT

Karin Andersson Di Claudio

Karolinska Institutet

Stockholm 2016

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by E-print
Cover image: “Studio per la testa di Leda” Leonardo da Vinci (Firenze 1505).

ISBN 978-91-7676-224-0

ENDOMETRIOSIS – INVOLVEMENT OF STEM CELLS AND CLINICAL IMPACT

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Karin Andersson Di Claudio

Principal Supervisor:
Kristina Gemzell-Danielsson,
Professor, MD, PhD
Karolinska Institutet
Department of Women's and Children's Health
Division of Obstetrics and Gynecology

Co-supervisor(s):
Lalit Parameswaran Grace Kumar, PhD
Karolinska Institutet
Department of Women's and Children's Health

Opponent:
Dharani Hapangama, MD, PhD
Reader in Gyneacology
University of Liverpool
Institute of Translational Medicine

Examination Board:
Matts Olovsson, Professor, MD, PhD
Uppsala University
Department of Women's and Children's Health

Kenny Rodriguez-Wallberg, MD, PhD
Karolinska Institutet
Department of Oncology and Pathology

Mats Brännström, Professor, MD, PhD
Sahlgrenska Academy/University of Gothenburg
Department of Obstetrics and Gynecology

”*There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle.”* – Albert Einstein (1879-1955)

Per Louise, Ellen e Paolo

ABSTRACT

Introduction: Endometriosis is a common gynaecological disease affecting up to 10% of women of reproductive age. The women suffer from severe abdominal pain and infertility as a consequence of the chronic inflammation. The disease has also been associated with an increased risk of cancer, in particular endometrial and ovarian cancer. Endometriosis represents an important socioeconomic burden as the condition is associated with productivity loss, medical and surgical treatments including assisted reproduction, and a compromised quality of life. The pathophysiology of endometriosis is not fully understood, and as of today we are unable to identify women at risk for cancer development and offer them a tailor-made prophylactic treatment.

Aims: The overall aim of this thesis is to explore some of the mechanisms that have an important influence on clinical impact, in particular infertility and the risk of developing endometriosis-associated cancer. The mechanisms enabling endometriotic lesion establishment are explored in an in vitro experimental model and the methylation profile of the fertility-regulating gene HOXA10 is investigated in eutopic and ectopic endometrium. This study also attempts to identify the molecular link between endometriotic stem cells and the development of ovarian cancer by exploring CSC-specific markers and their molecular signatures, and gene expression profile of cancer-correlated molecules in different endometrial compartments.

Results: Significant changes were found in the endometrium of women with endometriosis compared to healthy controls. The first study demonstrated the expression of ApoE, ITGB2, ITGB7, LAMC1, CD24, and JAM-1 in women with and without endometriosis. Also, some of the molecules showed a significant altered expression upon comparing endometrium from women with and without endometriosis, as well as eutopic and ectopic endometrium of women with endometriosis. ApoE and JAM-1 were decreased in both proliferative and secretory phase in endometrium from women with endometriosis, and mRNA expression of LAMC1 was reduced in endometrium from endometriosis patients compared with controls in the proliferative phase. CD24 expression was significantly expressed in eutopic and ectopic endometrium in women with endometriosis. In the second study, we found a significant hypermethylation of the HOXA10 gene in eutopic secretory endometrium in women with endometriosis compared with controls. When comparing the methylation profile in patients suffering from ovarian endometriosis with patients presenting extra-ovarian disease, we could not demonstrate any significant correlation between methylation status and stage of disease. The third study demonstrated that mesenchymal endometrial stem cells from women with endometriosis showed an active S-phase as well as an up-regulation of PTEN, VEGF-α, and decreased BCL2 gene-expression compared to controls. A subset of potentially ‘high-risk’ patients could be identified showing a significant up-regulation of genes involved in reprogramming SOX2, NANOG; cancer metabolism TP53, K-ras; and epithelial-

mesenchymal transition genes TGF-α and SNAI1. TP53 turned out to play the role of a master regulator. When comparing monolayer to 3D spheroid cultures, an increased co-expression of CSC surface markers CD44 and CD133 was seen, and the chemo-sensitivity assay performed in a 3D-tumour microenvironment revealed increased tumour invasion in the 'high-risk' group. In the fourth study, we found a significant difference in the expression of genes that correlated with endometrial malignant transformation in both endometrial stromal and glandular compartments in endometriosis patients compared with controls.

Conclusions: Our results shed light on the molecular linkage to the etiology of endometriosis and malignant transformation of endometriosis, as well as providing useful information relevant to endometriosis-associated infertility and pathogenesis.

LIST OF SCIENTIFIC PAPERS

I. Sundqvist J, Andersson KL, Scarselli G, Gemzell-Danielsson K, Lalitkumar PG. “Expression of adhesion, attachment and invasion markers in eutopic and ectopic endometrium: a link to the aetiology of endometriosis”. *Hum Reprod*. 2012 Sep;27(9):2737-46.

II. Andersson KL, Bussani C, Fambrini M, Polverino V, Taddei GL, Gemzell-Danielsson K, Scarselli G. “DNA-methylation of HOXA10 in eutopic and ectopic endometrium”. *Hum Reprod*. 2014 Sep;29(9):1906-11.

III. Vignesh-Srinivasan S, Andersson KL, Jo Varghese S, Green R, Nister M , Gemzell-Danielsson K, Lalitkumar PGL. “Identification of distinct cell population with cancer stem cell characteristics in endometrium and endometrioma of a subset of women with endometriosis”. *Manuscript.*

IV. Andersson KL, Naven H, Boggavarapu NR, Lalitkumar PGL, Gemzell-Danielsson K. “Study on global expression of endometrial genes reveals a possible link between endometriosis and endometrial cancer in a subgroup of women”. *Submitted.*

CONTENTS

LIST OF ABBREVIATIONS

ABCG2	ATP-binding cassette sub-family G member 2
ApoE	apolipoprotein E
ALDH1	aldehyde dehydrogenase 1
ARID1A	AT-rich interactive domain-containing protein 1A
BFGF	basic fibroblast growth factor
BLC2	gene B-cell lymphoma 2 protein
BrdU	bromodeoxyuridine (5-bromo-2'-deoxyuridine)
CD	cluster of differentiation
CDKN2BAS	cyclin-dependent kinase inhibitor 2B antisense RNA
cDNA	complementary deoxyribonucleic acid
C/EBP	CCAAT-enhancer-binding proteins
CHD	cornary heart disease
c-myc	myc avian myelocytomatosis viral oncogene
CNV	copy number variants
CpG site	cytosin guanin rich region
CSC	cancer stem cell
CT	threshold cycles
CTNNB1	catenin cadherin-cssociated protein beta 1
DC	dentritic cell
DNA	deoxyribonucleic acid
DNMT	DNA methyltrasferases
DR6	death receptor 6 (tumor necrosis factor receptor superfamily member 21; TNFRSF21)
E2	estradiol
EAC	endometriosis associated cancer
EAOC	endometriosis- associated ovarian cancer
ECM	extracellular matrix
EGF	epidermal growth factor
EHF	ETS homologous factor

EMT	epithelial-mesenchymal transition
EnSC	eutopic endometrial stem cells
EndoSC	ectopic (endometrioma) endometrial stem cells
EPCAM	epithelial cell adhesion molecule
EQ	EuroQol
ER	estrogen receptor
EZR	ezrin
FACS	fluorescence-activated cell sorting
Fas	fatty acid synthase
FF	follicular fluid
FGF	fibroblast growth factor
FIGO	International Federation of Gynecology and Obstetrics
GPER1	G protein-coupled estrogen receptor 1
GWAS	genome-wide association studies
γ-H2AX	phosphorylated H2A histone family, member X
H-EnSC	endometrial stem cell in healthy control
HIF	hypoxia-inducible factor
HGF	hepatocyte growth factor
HNF	hepatic nuclear factor
HOXA	homeobox gene, cluster A
ICAM1	intercellular adhesion molecule 1
IGF	insulin growth factor
IHC	immunohistochemistry
IL	interleukin
IPA	Ingenuity Pathway Analysis
ITGB	integrin β
IVF	in vitro fertilization
JAM-1	junctional adhesion molecule-1
JUN	jun proto-oncogene
K-ras	kirsten rat sarcoma viral oncogene homologue
LAMC1	laminin γ-1

LCM	laser capture micro-dissection
LIF	leukemia inhibitory factor
LH	luteal hormone
LIMMA	linear models for microarray
MAPK	mitogen-activated protein kinases
MFR	monthly fecundity rate
MMPs	matrix metalloproteinases
MSCs	mesenchymal stem cells
mRNA	messenger ribonucleic acid
Nanog	homeobox gene named ’Tir Na Nog,’ the mythologic Celtic land of the ever young (Omim)
NFE2L3	nuclear factor, erythroid 2-Like 3
NK	natural killer
NO	nitric oxid
Notch	neurogenic locus notch
Oct 4 (POU5F1)	octamer-binding transcription factor 4 (POU class 5 homeobox 1)
OPLS-DA	orthogonal partial least squares-descriptive analysis
PAR	proteinase-activated receptor
PCA	principle component analysis
PCO	polycystic ovarian syndrome
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
P-EnSC	patient eutopic endometrial stem cell
P-EndoSC	patient ectopic (endometrioma) endometrial stem cell
PERP	TP53 apoptosis effector
PDGF	platelet-derived growth factor
PF	Peritoneal fluid
PLIER	probe logarithmic intensity error estimation
PR	progesterone receptor
PTEN	phosphatase and tensin homolog

RA	rheumatoid arthritis
RANTES	regulated on ractivation, normal T-cell expressed and secreted
RNA	ribonucleic acid
ROS	reactive oxygen species
RR	relative risk
RVS	recto vaginal septum
RT-PCR	real time polymerase chain reaction
SAM	significance snalysis of microarrays
SCID	severe combined immuno deficiencies
SDF-1	stromal cell-derived factor 1
sec	sequence
SF-1	steroidogenic factor 1
SLE	systemic lupus erythematosus
SMO	smoothened, frizzled class receptor
SLC34A2	solute carrier family 34 member 2
SLPI	secretory leukocyte peptidase inhibitor
SNAI1	zinc-finger transcription factor
SNPs	single nucleotide polymorphisms
SOX	sex determing region
SS	Sjögren Syndrome
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinase
TNF-α	tumour necrosis factor α
T53/p53	tumour protein 53
VEGF	vascular endothelial growth factor
Wnt	wingless-type MMTV integration site family
WT1	wilms tumor protein

POPULÄRVETENSKAPLIG SAMMANFATTNING

En av tio kvinnor i reproduktiv ålder drabbas av endometrios med negativ påverkan på livskvalitet och barnafödande som följd. Livmoderslemhinnan som spridits utanför livmodern och bildat små ”härdar”, vanligast på äggstockarna och på bukväggen,”menstruerar” varje gång kvinnan har sin mens och kan då skapa svåra buksmärtor och kronisk inflammation. Sjukdomen innebär för de drabbade kvinnorna ofta långa perioder av sjukskrivning, upprepade kirurgiska ingrepp och/eller infertilitetsbehandlingar. I sällsynta fall kan endometrios innebära en ökad risk för cancerомvandling av livmoderslemhinnan eller äggstockscystor.

Sjukdomens orsaker är fortfarande ofullständigt kända. Många teorier har framförts under decennier av forskning inom området och sannolikt är det ett samspel mellan genetiska, immunologiska och miljöfaktorer som ligger bakom sjukdomens uppkomst. Den teori som genom tiderna har ansetts som den viktigaste är den som bygger på att kvinnor med endometrios anses ha ett bakåtflöde vid menstruation (retrograd menstruation) vilket medför att blod hamnar i buken via äggledarna. Modern forskning har visat att stamceller skulle kunna spela en roll i uppkostmekanismen av endometrios. Stamceller besitter unika egenskaper som gör att de kan utvecklas till olika celltyper och skulle därför kunna förklara varför livmoderslemhinnan via retrograd menstruationsflöde lyckas ”invadera” och etablera sig utanför sitt ursprungsorgan. Vi har studerat olika stamcellsmarkörer i livmodern och i endometrios ”härdar” för att kartlägga deras förekomst hos kvinnor med och utan endometrios. Förekomsten av dessa molekyler (ApoE, ITGB2, ITGB7, LAMC1, CD24 and JAM-1) kan påverka enodometriecellernas förmåga att fästa och invadera och därmed möjliggöra uppkomsten av en endometrioshärd.

Vi har också studerat hur gener relaterade till infertilitet och cancerutveckling uttrycks i livmoderslemhinnan hos kvinnor med och utan endometrios. Vad beträffar infertilitet har vi tittat på en gen som är viktig för implantation av det befruktade ägget, HOXA10. Tidigare forskning har visat att denna gen är otillräckligt uttryckt hos kvinnor med endometrios. Vi har tittat närmare på orsaken till detta förändrade genuttryck och sett att det beror på dna-förändringar genom så kallad metylering. Vi har visat att livmoderslemhinnan hos kvinnor med endometrios har en mycket högre metylering av denna gen jämfört med kvinnor utan endometrios. Metylering är en dna-förändring som är reversibel och vi hoppas att vår forskning kan bidra till utveckling av behandlingsmetoder som kan återställa uttrycket av denna gen och därmed bidra till förbättrad fertilitet hos kvinnor med endometrios.

I två studier har vi också undersökt möjliga orsakssamband mellan endometrios och risken för cancerutveckling i livmodern och i äggstockarna. Vi har med olika molekylära tekniker såsom cellodlingstekniker, cellsortering och studie av cellcykelfasen undersökt mesenkymala stamceller i livmoderslemhinnan hos kvinnor med och utan endometrios. I denna studie användes en cellodlingsmodell där olika typer av cytostatika tillsattes för att bekräfta att de celler som har tumörliknande egenskaper uppvisade resistens mot cytostatika. Vi kunde i

denna studie identifiera en undergrupp av endometriospatienter som uppvisade ett genuttryck liknande den som kan påträffas i cancer.

I studien där vi undersökte de bakomliggande orsakerna till endometriosassocierad livmodercancer kunde vi genom microarray-teknik påvisa att elva gener kopplade till cancerutveckling var signifikant annorlunda uttryckta i gruppen av kvinnor med endometrios jämfört med kontrollgruppen.

Då man ej klarlagt sjukdomens exakta uppkomstmekanismer kan man tyvärr fortfarande inte erbjuda kvinnor med endometrios en botande eller förebyggande behandling.

Våra studier bidrar till att utöka kunskapen om sjukdomens bakomliggande orsaker, dess påverkan på fertilitet och den möjliga kopplingen till cancerutveckling. Vår förhoppning är att dessa resultat kan bidra till fortsatt forskning inom området med målet att förbättra möligheten till patientcentrerad, ”skräddarsydd”, behandling och identifiera när det är nödvändigt att även erbjuda förebyggande åtgärder för att minska risken för cancerutveckling.

1 INTRODUCTION

Around one out of ten women of fertile age suffers from endometriosis, a disease characterized by many unresolved questions regarding its pathophysiology, despite decades of research dedicated to better understand the complexity of the disease.

Many aspects still remain poorly understood, a fact that affects the possibilities of curative treatment and prevention. What remains beyond doubt is that this group of patients suffers from many compromised health aspects, including infertility, chronic pain, and risk of endometriosis-associated cancer.

1.1 PATHOGENESIS OF ENDOMETRIOSIS

Endometriosis has sometimes sarcastically been nominated "the disease of the theories", elucidating the fact that the disease has a multifactorial origin and its pathogenic complexity is yet not fully defined.

1.1.1 Retrograde menstruation – coelomic metaplasia and implant survival

The theory of retrograde menstruation, first described by Sampson (Sampson 1927) explains endometriosis as a consequence of peritoneal dislocation of endometrial implants.
Taken together with the theory that endometriosis is induced through a metaplastic process in the peritoneal mesothelium called coelomic metaplasia (Matsuura et al., 1999), these have been the leading presumptions for many decades. But it's also known that the prevalence of endometriosis is far less than the occurrence of tubal reflux menstruation in women. This could possibly be explained by the co-existence of molecular and/or immunologic defects in endometriosis (Lucidi et al., 2005). The theory of coelomic metaplasia could still be supported for ovarian endometriosis development, as the coelomic epithelium lining the peritoneum and ovary can undergo metaplasia (Vercellini et al., 2013).

The implant survival could then be explained by an altered endometrial gene transcription and an increased endometrial invasion induced by the early endometriotic lesion (Nair et al., 2008), and by failure of the immune system to clear implants from the peritoneal surface (Giudice and Kao 2004).

Another requirement for survival is an hypoxic microenvironment, which supports the attachment and implantation of ectopic endometrium with the support of pro-angiogenic factors. Hypoxia promotes the expression of downstream genes involved in implantation and persistence of ectopic endometrium. Recently published data show a high expression of HIF-1α, HIF-2α, VEGF-α, PAR-1, and PAR-4 in patients with ovarian endometriosis (Filippi et al., 2015; Lu et al., 2014).

Together with the involvement of immune clearance escape, neuroangiogenesis, matrix degradation, this helps lesions survive. Attachment and invasion to ectopic sites may then be facilitated by up-regulation of adhesion molecules (Burney 2013).

1.1.1.1 *Sustained cell proliferation and apoptosis avoidance*

Cumulating evidence suggests that apoptosis regulation in ectopic lesions is supported by an up-regulation of anti-apoptotic genes and a coordinated down-regulation of genes involved in apoptotic pathways (Sourial et al., 2014).
Recent in vivo data emerged in a baboon-model of endometriosis shows that ectopic implant survival is facilitated by an overexpression of pro-proliferative markers such as telomerase, nucleolin and proliferating cell nuclear antigen (PCNA) and loss of γ-H2AX expression (phosphorylated H2A histone family, member X) (Hapangama et al., 2010).
As a consequence endometrial proliferation is sustained by affected DNA-repair recognition and evading of apopotosis, in particular in the initial establishment of the disease (Hapangama et al., 2010).

1.1.2 Role of endometrial stem cell implantation

Gargett CE and collaborators have shone a light on the relatively new field of stem cell research, and their findings suggest that endometrial stem/progenitor cells could be involved in eutopic and ectopic endometrial regeneration and differentiation (Gargett and Masuda 2010). In the hypothesis first described by Leyendecker *et al.*, more of the basalis layer, which contains the endometrial stem/progenitor cells required for the monthly endometrial self-renewal, is shed in women with endometriosis. Together with one of the established theories of retrograde menstruation, this has led to further research focusing on how abnormally-shed endometrial stem/progenitor cells establish ectopic peritoneal implants (Gargett 2006, 2007; Leyendecker et al., 2002; Sasson and Taylor 2008).

Clonogenic cells with stem/progenitor properties have been identified in ectopic endometriotic lesions. Chan *et al.* investigated the colony-forming activity of endometriotic epithelial and stromal ovarian endometrioma, and observed a greater proportion of clonogenic stromal cells in the proliferative phase. This suggested that endometriotic lesions possess a hormone-dependent cell population that under hormonal stimulation in the early cycle phase can proliferate and differentiate (Chan et al., 2011). Ectopic endometrial mesenchymal stem cells (endometrial MSCs) have been clearly shown to have a greater capacity for cell migration and angiogenesis when compared to eutopic endometrial MSCs in an *in vivo* mouse transplant model (Kao et al., 2011).

Further studies in the field focused upon the aspect of neonatal progesterone-withdrawal bleeding and subsequent onset of early pre-menarcheal endometriosis (Brosens et al., 2013; Gargett et al. 2014). However, no studies to date have generated direct evidence of the role of endometrial stem cells in the pathogenesis of endometriosis (Gargett et al., 2016).

1.1.3 Impact of immune system

The association between inflammation and endometriosis is well known and involves local vascularization, somatic cells, and immunocytes. An overproduction of prostaglandins and metalloproteinases is seen in women with endometriosis (Bulun 2009).

It has been debated, however, that endometriosis is a consequence of inappropriate immune defence response, or that the pelvic and peritoneal inflammation is a consequence of the disease. At present, most evidence supports the latter (Kyama et al., 2003).

The vast majority of women have some degree of retrograde menstruation (75-90%) (Burney and Giudice 2012), but most will never develop the disease. This could partly be explained by the fact that an unsatisfactory immune vigilance fails to clear cell/tissue implants from the peritoneal surface. The local pelvic inflammatory process with its altered function of immune cells in the peritoneal environment is considered to play a pivotal role in evolution of the disease.

Several immune aspects are thought to be involved.

1.1.3.1 *Cell-mediated immunity*

The main function of NK (natural killer) cells is to eliminate infected cells as well as tumour cells. In women with endometriosis, local and systemic variation in NK cell function as well as a decrease in NK-mediated cytotoxicity have been shown (Thiruchelvam et al., 2015). These changes contribute to a clearance of defective endometrial cells located in the pelvis and correlate to some extent with disease severity.

Uterine NK cells (uNKs) have a definite NK cell population dedicated to the eutopic endometrium undergoing cyclical changes, and which persists and proliferates in case of successful implantation. Studies have shown that NK cells present an altered phenotype with high expression of the cytotoxic cell surface receptors CD16+ and NKp46+ in women with endometriosis, a fact that might play a role in endometriosis-associated infertility.

However, a recent systematic review and meta-analysis of the potential benefits of immune therapy in case of high levels of NK cells and infertility confirmed that there is yet no conclusive data to allow evidence-based conclusions (Seshadri and Sunkara 2014).

An increase in number and activation of peritoneal macrophages has been demonstrated in women with endometriosis (Eisenberg et al., 2012). Also, pro-inflammatory chemo-attractant cytokines for monocytes, macrophages, and granulocytes have been detected in the peritoneal fluid in women with endometriosis.

Interleukin-1 β has been designated an angiogenetic potential throughout VEGF and IL-6 activation (Lebovic et al., 2000).

Several mechanisms and factors are involved to enhance the establishment of the endometriotic cells that have escaped the immune surveillance. Among them are ICAM-1 that conciliates immunity related cell-to-cell synergies and the Fas-Fas ligand system, which mediates cell death of activated immune cells in a pro-inflammatory environment, such as the peritoneal fluid in women with endometriosis (Eisenberg et al., 2012).

1.1.3.2 *Humoral-mediated immunity*

Increased B-cell activity and presence of autoantibodies have been shown in women with endometriosis. Various autoantibodies have been noted, such as phospholipid antibodies and also tissue-specific anti-endometrial and anti-ovarian antibodies. Some authors have in fact proposed the investigation of the presence in serum of anti-endometrial antibodies as a diagnostic tool (Randall et al., 2007). What is always important to consider is that the presence of autoantibodies is not synonymous with autoimmune disease (Lleo et al., 2010).

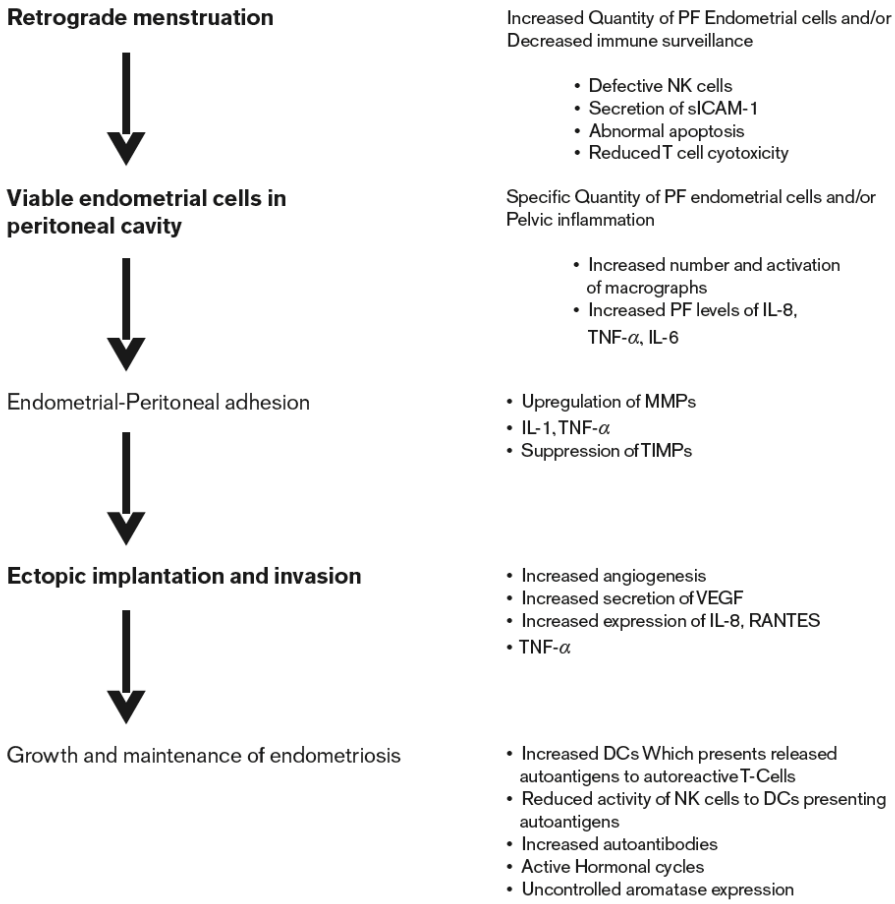

Figure 1 summarizes the role of the immune system in developing and maintaining the disease (modified from Kyama et al., 2003, with the author's kind permission).

1.1.4 Steroid metabolism dysfunction – attenuated progesterone action

Traditionally, endometriosis has been considered predominantly an oestrogen-dependent disease, but a more recent consensus is that the hormonal dysfunction is also related to progesterone regulation and incompetence. Several target genes crucial for successful implantation have been reported as deregulated in women with endometriosis (Kao et al., 2003), many of them correlated with progesterone metabolism and progesterone receptor function. The activated progesterone receptor plays a major role in regulating the tissue remodelling that the uterus undergoes during menses and pregnancy. A dysfunction of the progesterone-regulatory processes, induced by the chronic inflammatory state caused by endometriosis, leads to the condition termed progesterone resistance or attenuation (Burney et al., 2007). Progesterone resistance can involve the progesterone receptor isoforms (PR-A and PR-B) as well as downstream molecules such as TGF, retionoic acid, c-myc, or the co-activators of the receptor itself (Burney et al., 2007). In endometriosis tissue, a remarkable reduction of PR-A and PR-B levels has been shown (Bulun 2009).

1.1.5 Genetics

The role of genetics and epigenetics has in recent years become a hot topic due to efforts to better understand the mechanisms of the pathophysiology of endometriosis and its impairment on fertility. Endometriosis is clearly heritable, with a sevenfold risk of developing the disease in women with an affected mother or sister (Simpson and Bischoff 2002). Studies on monozygotic twins demonstrate a correlation to disease stage (Hadfield et al., 1997). Genes involved in cytokine-related inflammation, steroid and hormone receptors, and matrix degradation have been reported to be differentially expressed in women with endometriosis (Burney 2013). Even though various genes have been proposed, no robust candidates have come to light (Rahmioglu et al., 2012). Genetic association studies, and more recently, genome-wide association studies (GWAS) have brought new insights to the field. Several GWAS have been conducted on Japanese and European populations and the first one, reported in 2010, identified a significant association between endometriosis and rs10965235 located on the CDKN2BAS gene (cyclin-dependent kinase inhibitor 2B antisense RNA) on chromosome 9p21 (Uno et al., 2010). The same study also reported a locus on chromosome 1p36 containing Wnt4 as a candidate locus for endometriosis (rs 7521902).

Shortly after, another large GWAS announced an associated signal (rs12700667) on chromosome 7p15.2 in an intergenic region near the genes HOXA10 and NFE2L3 (Painter et al. 2011). These authors confirmed the results in a larger, independent (and geographically different) cohort. Nyholt *et al*. conducted a meta-analysis that helped confirm the findings of of the previous GWAS and also reported five new signals associated with endometriosis in European and Japanese populations: rs13394619, rs10859871, rs4141819, rs7739264, and rs1537377 (Nyholt et al., 2012). The latest large GWAS identified three new SNPs of significance: rs1519761, rs6757804, and rs2235529, which reside near Wnt4 (Albertsen et al., 2013).

Recently, Sapkota *et al*. performed an independent replication and meta-analysis for endometriosis risk loci for nine of the above mentioned SNP loci (rs7521902, rs13394619, rs4141819, rs6542095, rs1519761, rs7739264, rs12700667, rs1537377, and rs10859871). The findings provided supporting evidence for associations of the implicated SNP loci with endometriosis (Sapkota et al., 2015).

1.1.5.1 *Epigenetics*

Epigenetics is one of the most expanding fields in bio-molecular research. It is characterized by a reversible condition, influenced by age and lifestyle factors, that underlies a wide range of pathologies. The theory of endometriosis as an epigenetic disease is now well-established (Guo 2009). The most frequent and well-documented epigenetic mechanism is DNA methylation followed by histone modification and regulation of chromatin modifications. Commonly, promoter hypo- and hyper-methylation is related to gene expression and silencing, respectively. The first documentation of epigenetic alteration in endometriosis was associated with the HOXA10 gene, which showed a hypermethylation in the endometrium of women with endometriosis (Wu et al., 2005). The same research group demonstrated further that DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b) are highly expressed in endometriotic lesions (Wu et al., 2007). These enzymes, by catalysing the process of DNA-methylation in the endometrium, could affect transcriptional activation or silencing of genes crucial for apoptosis and proliferation regulation.

In their recent GWAS, Naqvi *et al*. demonstrated 129 genes with altered methylation (59 hypermethylated and 61 hypomethylated), and with confirming RT-PCR, the authors showed several new genes with an altered expression and methylation in endometriosis patients (O-6-methylguanine-DNA methyltransferase, dual specificity phosphatase 22, cell division cycle associated 2, inhibitor of DNA binding 2, retinoblastoma binding protein 7, bone morphogenetic protein receptor, type 1B, tumour necrosis factor receptor 1B, zinc finger protein receptor 681, immunoglobulin superfamily, member 21, and tumour protein 73) (Naqvi et al., 2014).

Regarding histone modifications, several genes in women with endometriosis have presented an altered histone acetylation status, such as ER-α, GPER1, SF-1, HOXA10, C/EBP α, HIF-1 α, DR6, and E-cadherin (Nasu et al., 2014). The authors also pose the question of many researchers involved in the field of epigenetics: To what extent are the alterations a cause or a consequence of the disease?

Thus, the concept of endometriosis as an epigenetic disease is still fairly novel and many pieces of the puzzle are still missing. Future research in the field could shed light on risk factors, pathogenesis, early diagnosis, prognostic markers, and new treatment strategies.

1.2 CLINICAL IMPACT OF ENDOMETRIOSIS

For a woman affected with endometriosis the clinical impact is considerable.
The first aspect regards the diagnostic delay, which is still far too long even though medicine and society now have greater knowledge and understanding of the disease (Simoens et al., 2012; Staal et al., 2016).

Other aspects include infertility, chronic pain, adverse psychological conditions, affected sexual life, and reduced quality of life as a consequence of all these (Moradi et al., 2014).
In this chapter, the focus will fall on two major aspects that negatively influence the health of the woman: reduced reproductive capacity and risk of endometriosis-associated cancer.

1.2.1 Infertility – the mechanisms

Endometriosis negatively affects fertility (Giudice 2010). A normal monthly fecundity rate (MFR) is 15-20% (MFR 0.15-0.2), while women with untreated endometriosis have an estimated MFR less than 0.05 (2-10%) (Holoch and Lessey 2010).

Up to 50% of infertile women can address endometriosis as the origin of fertility impairment (Meuleman et al., 2009). Although it has been debated over the years, the stage of disease is correlated with severity of fertility impairment, and it appears that women with severe disease are more likely to suffer infertility (Macer and Taylor 2012).

The infertility that is related to endometriosis is multifactorial and can be attributed to processes acting independently or in synergy, with these processes taking place in diverse compartments such as the peritoneal cavity, uterus, and ovaries.

1.2.1.1 *Pelvic cavity*

The pelvis is an origin of endometriosis-related infertility based upon several observations. Chronic inflammation generates a favourable environment for adhesion development, and eventual surgical treatment could aggravate this. Peritubal adhesions may also cause ovarian entrapment and decreased tubal motility and as a consequence, sub-optimal ovum transport (Liakakos et al., 2001). Such adhesions might also be an important explanation for increased infertility in severe stages of endometriosis compared to mild disease involvement.

The peritoneal fluid in women with endometriosis is an important element because it is the site of various aspects of inflammation-induced alterations. Some of these are correlated to the aberrant cell-mediated immunity with an inflammatory cascade induced by the increased number of activated macrophages, while others are correlated to the presence of cytokines and growth factors that are overrepresented in the peritoneal environment due to increased leucocyte presence.

Peritoneal fluid concentrations of RANTES (regulated upon activation, normal T cell expressed and secreted), IL-6, IL-1, TNF-α, and VEFG have all been reported to be increased in women with endometriosis and to affect sperm survival and capacitation (Gupta et al., 2008; De Ziegler et al., 2010).

Macrophages and leucocytes present in the peritoneal environment generate large amounts of reactive oxygen species (ROS), which also are assigned a role in endometriosis-associated infertility (Gupta et al., 2006).

1.2.1.2 *Ovary*

A potentially reduced ovarian tissue volume following eventual surgery is a cause of reduced fertility (Somigliana et al., 2014). An endometrioma has a negative impact on ovarian fertility capacity by generating an inflammatory process (Holoch and Lessey 2010), besides compromising the available ovarian tissue by space-occupying effects.

There is evidence that women with endometriosis present an altered folliculogenesis with a slower follicular growth and a reduced dominant follicle size, in part depending upon alterations in granulosa cell kinetics and apoptosis, and with the negative influence of oxidative stress (Gupta et al., 2008).

Moreover, concentrations of a large number of molecules appear increased in the follicular fluid (FF) in affected women. These include TNF-α (Falconer et al., 2009); the interleukins IL-6, IL-1β, IL-8, IL-1α, and IL-10; VEGF; and NK-cells, macrophages, and B-lympocytes (Gupta et al., 2008).

The role of nitric oxide (NO) and its influence on the follicular microenvironment has also been assigned an important role in adequate folliculogenesis, and a deregulated NO has been discovered in the FF of endometriosis patients (Goud et al., 2014). These findings support the hypothesis that a dysregulation of NO affects follicular health negatively with an increased granulosa cell apoptosis and reduced oocyte quality as a consequence.

As previously mentioned, aromatase activity plays a role in the pathogenesis of the disease (Bulun et al., 2005), and the increased level of IL-6 found in the FF cause a decreased aromatase activity across the mitogen-activated protein kinases (MAPK) pathway.
The final result is a decreased level of E2 in the FF, negatively affecting fertilizing capacity (Gupta et al., 2008).

1.2.1.3 *Impact on the endometrium*

The healthy endometrium manages to sustain an equilibrium that permits ovulation, implantation, and menstruation to be established with a balanced steroid action of oestrogen and progesterone. An imbalance between these hormones, with an oestrogen dominance and progesterone resistance associated with chronic inflammation, appears to be crucial in the pathogenesis of endometriosis (Lessey and Young 2014). Evidence supports that endometriosis influences the eutopic endometrium even though the mechanisms on the molecular level are poorly understood (Macer and Taylor 2012). Endometrial receptivity is also altered due to aberrant expression of cell adhesion molecules, among them integrins. In particular, the αvβ3 integrin expression, required during the implantation window, has

been reported to be deficient in women with endometriosis (Macer and Taylor 2012; Garrido et al., 2003).

Other molecules relevant to endometrial receptivity and altered in endometriosis involve L-selectin ligand and LIF. A reduced LIF expression supports a harmful NO release with negative effects on endometrial receptivity-related biomarkers as a consequence (Lessey and Young 2014), while a reduced L-selectin expression compromises the adhesion of the embryo to the endometrium (Lessey et al., 2013). In addition, the extracellular matrix ligand of αvβ3 integrin, IL-11, ICAM and osteopontin, is aberrantly expressed in women with endometriosis (Hapangama et al., 2012; Lessey et al., 2013). The role of Wnt7a has also recently been emphasized. The Wnt7a protein product is important for development of the female genital tract, but also for maintenance of adult uterine plasticity expression. Up-regulation of Wnt7a might interfere with the mechanisms essential for successful implantation (Macer and Taylor 2012). It's also been known since the late 1990's that HOX genes from the A cluster present an aberrant expression in eutopic endometrium in women with endometriosis, and a lack of HOXA10 and HOXA11 mRNA increase during the implantation window, which could be one of the possible mechanisms of infertility in these patients (Taylor et al., 1999). DNA-methylation has been attributed as responsible for the reduced expression of HOXA 10/11 genes. A significant hypermethylation of the promotor of the HOXA 10 gene in eutopic mid-secretory endometrium in women with endometriosis has recently been demonstrated (Fambrini et al., 2013).

Thus, cumulative evidence supports that endometriosis negatively influences the fertility properties of the endometrium, but many mechanisms behind this still remain unclear. Future research in the field is necessary to better understand the linkages at the cellular and molecular level in order to prevent implantation failure in these women.

1.2.2 Endometriosis and cancer – molecular links and role of cancer stem cells

Endometriosis has been associated with gynaecological as well as non-gynaecological cancer. Evidence for the latter is yet not satisfactory and is often associated with conflicting results. The non-gynaecological cancers that have been reported as correlated with endometriosis are cutaneous melanoma (Kvaskoff et al., 2007; Melin et al., 2007), brain cancer (Melin et al., 2006, 2007), non-Hodgkin lymphoma (Kvaskoff et al., 2015), thyroid and renal cancer (Melin et al., 2006).

In literature, ovarian cancer is the most represented of the endometriosis-associated gynaecological cancers first described by Sampson in 1925 (Kvaskoff et al., 2015). The phenomenon has since been studied extensively, and many different types of studies confirm that women with endometriosis are exposed to an increased risk of certain histologic subtypes of ovarian cancer (Aris 2010; Heidemann et al., 2014; Pearce et al., 2012). Of the five sub-groups of ovarian cancer (high- and low-grade serous, clear-cell, endometroid, and mucinous) an increased risk for clear-cell and endometroid invasive ovarian cancer has been

shown in women with endometriosis (Aris 2010; Kobayashi et al., 2008; Zafrakas et al., 2014). As recently reported in a pooled analysis of more than 13,000 women, there is also an increased risk of low-grade serous invasive ovarian cancer (Pearce et al., 2012). Studies have also reported a better survival rate among women with ovarian cancer and co-existing endometriosis, suggesting that women with endometriosis, in countries where health care access is guaranteed, might undergo a higher number of ultrasound scans and thereby increase their chance of earlier diagnosis (FIGO I-II stage) (Heidemann et al., 2014). Whether parity actually helps lower the risk of ovarian cancer has been investigated and a positive trend can be seen even if statistical significance was not reached (Melin et al., 2007).

Regarding breast cancer, the available evidence suggests a modest increase in risk even though no studies have been conducted with stratification for breast cancer type, molecular subtype, or hormone receptor status (Kvaskoff et al., 2015).

Epidemiological studies have demonstrated a decreased risk of cervical cancer (Melin et al., 2006, 2007) and the same authors have also reported an increase in endometrial cancer risk (Melin et al., 2006). Many studies that did not show an increased risk of endometrial cancer had a low sample size (Kvaskoff et al., 2015) and further studies are required before establishing a lack of association. However, a study including 454 women demonstrates a clear linkage between endometriosis and endometrial cancer (Zucchetto et al., 2009) and there's evidence of a risk of endometrial cancer in women with adenomyosis (Baba et al., 2016; Koike et al., 2013; Kok et al., 2015).

1.2.2.1 *Molecular links*

Endometriosis shares many characteristics with cancer such as the capacity to avoid apoptosis, self-regulation of proliferation, and properties that generate angiogenesis (Pollacco et al., 2012).

In terms of apoptosis, the focus has been put on B-cell lymphoma 2 protein (BCL-2) and protein 53 (p53), as the first is an anti-apoptotic regulatory protein and the latter is a DNA-repair regulator and signals apoptosis when required (Pollacco et al., 2012). BCL-2 is overexpressed in ovarian carcinoma and one study demonstrated an overexpression in 23% of endometriotic cysts. The same authors could not find any up-regulation of p53 in any of the benign cysts, while an up-regulation was seen in the ovary affected by endometriod ovarian cancer (Nezhat et al., 2002). However, the sample size was rather limited and other authors have confirmed an overexpression of p53 in atypical endometriosis and EAOC (Saintz De La Cuesta et al., 2004).

Another crucial aspect for malignant transformation of an endometriotic cyst is the loss of ARID1A function. The lack of expression of this tumour suppressor has been considered a key event in early molecular malignant transformation leading to endometriosis-associated ovarian cancers. (Ayhan et al., 2012).

Other molecular events with potential influence on malignant transformation of endometriosis tissue include PTEN silencing (Catasús et al., 2004; Cho et al., 2009; McConechy et al., 2014; Pardal et al., 2003; Sato el al., 2000), K-ras and CTNNB1 mutations (Amemiya et al., 2004; McConechy et al., 2014), and HNF-1β activation (Gadducci et al., 2014; Kato et al., 2006).

1.2.2.2 *Role of cancer stem cells*

Cancer stem cells (CSCs), a subpopulation of cancer cells possessing tumour-initiating capability, have been identified in a variety of carcinomas using different combinations of cell-surface antigens and intracellular proteins, and are considered a critical population for tumour progression. Recent reports underline the importance of CSCs in tumour progression, recurrence, and drug resistance (Ye et al., 2014).

Following the first discovery of the cancer stem cell role in initiating human acute myeloid leukaemia after transplantation into SCID mice in the mid 1990's (Lapidott et al., 1994), CSCs have so far been identified in solid tumours such as breast (Al-Hajj et al., 2003; Ginestier et al., 2007), brain (Singh et al., 2003), prostate (Collins et al., 2005), colon (Dalerba et al., 2007; O'Brien et al., 2007; Ricci-Vitiani et al., 2007), lung (Eramo et al., 2008), liver (Yang et al., 2008), and ovarian cancer (Ben-Porath et al., 2008; Peng et al., 2010; Rizzino 2009). Recently cancer stem cells have been reported in endometrial cancer (Mirantes et al., 2013).

Regarding markers for CSCs, in ovarian cancer early progenitor cells are associated with specific surface markers like CD133 and CD117 (Bapat 2010; Curley et al., 2009; Kusumbe et al., 2009; Liu et al., 2010). Aldehyde dehydrogenase 1 (ALDH1) has been considered reliable in investigating various human cancer stem cells (Deng et al., 2010; Ma and Allan 2011). In association with the expression of the CD133 antigen, ALDH1 represents a useful ovarian cancer stem cell marker (Kryczek et al., 2012).

Oct 4 (POU5F1) is a known transcription factor with functions relevant to pluripotency and cell survival, and is associated with several somatic tumours such as lung, gastric, colorectal, rectal, bladder, breast, prostate, and ovarian cancers (Ben-Porath et al., 2008; Cheng et al., 2007; Peng et al., 2010; Rizzino 2009).

Other CSC markers are SOX2 (Liang et al., 2012) and NANOG (Wang et al., 2013), while stem cell signalling markers are represented by notch (Shah et al., 2013), CTNNB1 (McConechy et al., 2014), and SMO (Chen et al., 2007). Musachi-1 has been attributed to asymmetric division (Götte et al., 2008), while TGF-β (Lamouille et al., 2014), SNAI1 (Dang et al., 2011; Lamouille et al., 2014), and HIF1α (Liang et al., 2012) are involved in epithelial-mesenchymal transition (EMT) associated pathways. E-cadherin represents another molecule with a pivotal role in morphogenesis, tumour genesis, signal transduction, and EMT (Gumbiner 2005; Lamouille et al., 2014; Zohn et al., 2006) In addition, VEGF, known for decades to play a crucial role in angiogenesis (Leung et al., 1989), has lately been recognized as very important for tumour initiation and function (Goel and Mercurio 2013).

CSCs reside in niches, which are distinct anatomical regions within the tumour microenvironment. The niches are essential for CSCs as they guarantee a shelter from the immune system vigilance as well stemness phenotype preservation (Plaks et al., 2015). The niches, with an altered cytokine network and extracellular matrix cross-talk, enable the processes involved in tumour initiation and progression, finally contributing to a metastatic potential. Among the molecules involved in these mechanisms are VEGF, TGF-β, MMPs, Wnt, PDGF, IGF, SDF-1, FGF, EGF, HGF, Wnt, Notch ligand, and Hedgehog ligands, all produced by cells in the microenvironment (Ye et al., 2014). These niche molecules are suggested to support CSC plasticity and self-renewal, as well as offer a barrier for drug delivery (Ye et al., 2014).

1.2.3 Chronic disease development

1.2.3.1 *Autoimmune diseases*

Besides cancer, endometriosis has been associated with inflammatory and autoimmune diseases. Studies have revealed an increase in incidence of systemic lupus erythematosus (SLE), multiple sclerosis, rheumatoid arthritis (RA), and Sjögren Syndrome (SS) (Harris et al., 2015; Sinaii et al., 2002). In a Danish study of 37,661 women with endometriosis, a significant risk of SLE, SS, and MS was observed (Nielsen et al., 2011). A large Swedish cohort study demonstrated a significant association between endometriosis and celiac disease (Stephansson et al., 2011), and another Danish study also presented evidence for a positive association between endometriosis and inflammatory bowel diseases (ulcerative colitis and Crohn's disease) (Jess et al., 2012).

Features similar to autoimmune disease include polyclonal B lymphocyte activation and T/B-lymphocyte immunological activation as well as elevated levels of cytokines and decreased apoptosis (Nothnick 2001).

In terms of autoantibodies, it's important to first state that their presence is not synonymous with autoimmune disease, as they are also found in healthy subjects and in many conditions including cancer (Lleo et al., 2010). Further, natural antibodies may play a role in inflammation prevention and their role in autoimmunity is yet to be defined (Lleo et al., 2010).

1.2.3.2 *Other health conditions related to endometriosis*

The existing literature on allergies and asthma associated with endometriosis is scarce, but the available evidence shows an increased risk of atopic allergy and asthma in women with endometriosis compared to controls (Bungum et al., 2014).

Regarding cardiovascular diseases, a recent study (Nurses' Health Study II) found an increased risk of myocardial infarction (RR 1.52), angina pectoris, and coronary artery by-pass surgery (Kvaskoff et al., 2015). An hypothesis for this increased risk of coronary heart

disease (CHD) is the chronic inflammation condition and oxidative stress present in endometriosis patients, as well as altered levels of low-density lipoprotein (Kvaskoff et al., 2015).

In relation to pregnancy and pregnancy related complications, a recent systematic review reveals that there's evidence for an increased risk of miscarriage, preterm birth, and shorter gestational age, as well as an elevated risk for placenta previa (Leone et al., 2016).
The authors emphasise that current findings do not require any changes in surveillance of women with endometriosis during their pregnancy, but physicians should be aware of the risk of placenta previa.

1.2.4 Socio-economic impact and quality of life aspects

Women affected by chronic pelvic pain are at high risk of psychological stress and the condition might interfere considerably with daily life activities, including causing anxiety and depression (Weijenborg et al., 2007). Many studies on the quality of life of women affected by endometriosis provide clear evidence that their quality of life is compromised on many fronts, such as severe dysmenorrhea and dyspareunia, infertility, and altered work capacity with lost days at work, as well as surgery-related problems. The overall annual cost for endometriosis in Europe due solely to lost days at work has been estimated at 30 billion Euros (D' Hooghe and Hummelshoj 2006).

Diagnostic delay, which is about 6.7 years in symptomatic women (Nnoaham et al. 2011), is considered to be a consequence of either patient or doctor responses. (Ballard et al., 2006). At the patient level, there's a tendency to normalise the symptoms as well as a fear of being unable to cope. Doctors' reasons for delaying diagnosis are due to normalization or trivialization of pain symptoms, intermittent suppression of symptoms caused by contraceptives, or misdiagnosis due to lack of knowledge (Ballard et al., 2006). For assessing quality of life in relation to health status, a commonly used instrument is the EQ-5D, developed by EuroQol Research Foundation (http://www.euroqol.org). This survey methodology defines the health-related quality of life in five dimensions (mobility, self-care, usual activity, pain/discomfort, and anxiety/depression). Every dimension is further rated at three levels: 'no problem', 'some problem', or 'major problem'. A recent, prospective multicentre study conducted in ten countries has evidenced the aspects of costs and quality of life related to endometriosis (Simoens et al., 2012). The study showed that 56% reported pain and discomfort problems, 36% expressed problems with anxiety/depression, and 29% reported obstacles with usual activities.

In terms of the economic burden of endometriosis on healthcare, the EndoCost Study conducted by the World Endometriosis Research Foundation looked at the cost of treating women in referral centres in ten different countries. The results showed that endometriosis is an economic burden (with annual direct health care costs of €3113 per patient), similar to other chronic disease such as diabetes, RA, and Crohn's disease. Further, indirect costs (mainly related to productivity loss) were double the direct health costs (Simoens et al.,

2012). In that study, quality of life was the most decisive predictor of direct health care costs and total costs.

In conclusion, we can state that endometriosis carries a considerable socioeconomic burden. The reasons for this are many, including the chronic nature of a disease characterized by insufficient knowledge of its complex and multifactorial pathogenesis, further aggravated by a significant diagnostic delay.

The need for future research in this field is critical. We need to develop efficient diagnostic therapeutic strategies and thereby improve the quality of life for this vast patient group, and at the same time reduce the substantial economic burden that endometriosis represents to society.

2 AIMS

The overall aim of this thesis is to explore some of the mechanisms that have an important influence on clinical impact, infertility, and risk of malignancy.

The specific objectives were to:

- Study the invasion of endometrial stem cells and establishment of endometriotic lesions in an *in vitro* experimental model.
- Investigate the methylation profile of the HOXA10 gene in eutopic and ectopic endometrium.
- Identify the molecular link between endometriotic stem cells and the development of ovarian cancer by exploring CSC-specific markers and their molecular signatures.
- Explore the gene expression profile of known cancer-correlated molecules in different endometrial compartments in order to better understand the potential malignant transformation of the eutopic endometrium in patients with endometriosis.

3 MATERIALS AND METHODS

More detailed description of the materials and methods is provided in the original articles (Study I-II) and manuscripts (Study III-IV).

3.1 SUBJECTS

All subjects in the studies were between 18-42 years old. All women had regular menstrual cycles (25-32 days), and any hormonal treatment or intrauterine device three months prior to sample collection was an exclusion criteria. Endometriosis patients in the study had previously been diagnosed for endometrioma.

3.2 ETICHAL PERMITS

The studies included in this thesis were approved by the regional ethics committee in Stockholm (2008-1566-31/3 and 2013/1960-31/4). Written informed consent was obtained from all participating subjects.

3.3 GENERAL METHODS

3.3.1 Endometrial biopises

Endometrial biopsies were obtained in all four studies using Randall curette (Stille, Stockholm, Sweden), Pipelle curette (Cooper Surgical, Trumbull, USA), or Endoram device (RI-MOS, Modena, Italy). In Study I, the samples were obtained both in the proliferative phase and secretory phase; in Study II and IV only in the luteal phase; while Study III did not require any timed biopsy for the patient sample when luteal phase biopsy (LH+6) was used for controls.

Endometrial samples were processed with RNA-later (Study I and II). In Study III, the tissues were transported to the cell culture lab in HAMF10 (Gibco®Life Technologies, Sweden) with antibiotics. For Study IV, part of the endometrial tissue was immediately frozen in liquid nitrogen. Endometrial dating was performed histopathologically to verify the phase of the menstrual cycle according to Noyes criteria (Noyes et al., 1975).

3.3.2 RNA extraction and cDNA preparation (Study I, III and IV)

RNA extraction was performed by using TRIZOL®reagent (Invitrogen, Carlsbad, CA, USA) in Study I. Picopure RNA extraction kit (Arcturus® PicoPure®Applied Biosystems, Foster City, CA, USA) was used for RNA extraction in Studies III and IV. Extracted RNA was then treated with RQI RNase-free DNase (Promega Biotech AB, Stockholm, Sweden) and subsequently reverse-transcribed using Superscript™ II RNase H-Reverse Transcriptase Kit (Invitrogen). In Study III, Purelink® micro RNA isolation kit was used to isolate RNA, and SuperScript® VILOTM (Life Technologies, Sweden) was used for cDNA synthesis.

3.3.3 Real-time PCR (Study I, III, and IV)

For real-time PCR analysis, Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was used with 18S as an internal housekeeping gene. StepOne Plus Instrument (Applied Biosystems) was used for Study III and IV. Taqman gene expression assays were applied to obtain ΔCT values. The details of primers used are given in Supplementary Table 1 (Manuscript Study III) and in Supplementary Table 1 (Manuscript Study IV).

The mean CT value of 18S was subtracted from the CT value of the target gene from the respective sample. The differential expression for the specific gene (ΔΔCT) was calculated by subtracting the mean ΔCT of the housekeeping gene from the ΔCT of the target gene (Schmittgen and Livak 2008). Mean ΔC_T differences from all samples were then converted to scientific format ($2^{-\Delta CT}$) according to current *Nature* protocol published for analysing real-time PCR data analysis using comparative C_T method (Schmittgen and Livak 2008).

3.3.4 Analysis of molecular interaction and biofunctional pathways (Study III and IV)

Ingenuity® Pathway Analysis software (IPA, Qiagen Inc. USA) was used to explore the molecular interactions and biological pathway analysis of the altered genes relevant to ovarian cancer development within the EnSC and EndoSC patient groups (Study III) and of genes associated with malignant transformation of the endometrium (Study IV).

3.4 STUDY I

3.4.1 Immunohistochemistry

Endometrial tissues were fixed in paraformaldehyde and the paraffin sections were used for immunohistochemistry (IHC). MACH3™ Mouse-Probe HRP-polymer kit or MACH3™ Rabbit-Probe HRP-polymer kit (Biocare Medical, CA, USA) were used for all immunohistochemical stainings and counterstained with Mayer's haematoxolyn. Negative mouse IgG isotype control (N1698, Dako, Carpinteria, CA, USA) or ChromPure Rabbit IgG (011-000-003, Jackson Immunoresearch, West Grove, PA, USA) were used.
Two independent observers scored the slides blindly according to the following staining intensity criteria: 0= no staining, 1= weak staining, 2= moderate staining, and 3= strong staining. The percentage of stained cells was graded as follows: 0= no staining, 1= <10%, 2=11-50%, 3= 51-80%, and 4= >81%. The two scores were then multiplied to generate the final score.

3.4.2 Statistical analysis

The Kruskal-Wallis test was used to compare the independent groups, followed by multiple comparisons with Dunn's correlation. A P-value less than 0.05 was considered statistically significant.

3.5 STUDY II

3.5.1 DNA-extraction and sodium-bisulfite DNA modification

BioRobot EZ1 (QIAGEN, Hilden, Germany) was used for genomic DNA extraction from endometrial samples. Incubation with high-bisulfte salt concentration converted unmethylated cytosine into uracil, while methylated cytosines remained unaffected according to the manufacturer's protocol (EpiTect Bisulfite Kit, Qiagen, Germany).

3.5.2 PCR amplification/Pyrosequencing analysis

DNA amplification of a CpG-rich fragment within the HOXA10 gene promoter in the 5' region up-stream of the exon 1 (F1) was used, as previously described (Wu et al., 2005). The amplified region was analysed using real-time DNA-sequencing technology (Pyrosequencing Biotage, Westborough, MA, USA). Two sequencing primers designed through Assay Design software 1.0 (Biotage) were used: sequences 1 and 2, identifying 11 and 8 CpG sites respectively: seq 1: GAAATTAAATTGGGAGT, and seq 2: TTTTGGTTTATTAATATAGA.

Methylation analysis and quantification were carried out using the PyroMark ID pyrosequencing system and Pyro Gold reagents (Biotage). The methylation profile was then expressed as the percentage of average methylated CpG sites in the amplified region.

3.5.3 Statistical analysis

Student's t-test was used to compare methylation status between the groups. P-values less than 0.05 were considered significant.

3.6 STUDY III

3.6.1 Endometrial mesenchymal stem cell sorting

Endometrial cell isolation was performed according to our standardized protocol (Lalitkumar et al., 2013). Fibrous endometriotic tissue was incubated with Collagenase type III (Worthington Biochemical Corporation, Lakewood, NJ, USA), Dispase II (Sigma-Aldrich, St. Louis, MO, USA) and DNase I (Sigma-Aldrich) in PBS (Gibco®). Cells were plated onto T25 flasks (Corning, Thermo Fischer Scientific Inc., USA) and cultured with DMEM/F12 (Gibco®) with 10% MSC qualified FBS (Gibco®) in a 37°C, 5% CO2 humidified incubator. Isolated cells were expanded for two generations and stained for MSC markers with an antibody mix of CD90-FITC (Abcam, UK), CD73-APC (BD Pharmingen, USA), and CD105-PE (Abcam, UK), and then sorted using the MoFLOW® XDP flow cell-activated cell sorter (Beckman Coulter, USA). MSCs from all patient and control groups were characterized for their differentiation into MSC lineages (adipocytes, osteocytes, and chondrocytes) using Stem Pro® Osteogenesis, Chrondrogenesis, and Adipogenesis differentiation kits (Gibco®). Results were visualized with immunofluorescence using

antibodies against Osteocalcin and Agreccan markers (R&D Systems, Sweden) and HCS LipidTOXTM green neutral lipid reagent (Molecular Probes® Life Technologies, Sweden).

3.6.2 Cell proliferation and cell cycle analysis

Expanded endometrial MSCs from endometrium from controls (H-EnSC), cases (P-EnSC), and from endometrioma (P-EndoSC) were evaluated for their proliferative activity and cell distribution within different phases of cell cycle. Cancer cell lines SKOV3 and Ishikawa were used as positive and negative controls, respectively, in the presence and absence of BrDU. Cell distributions were categorized by gating for different phases in cell cycle using FACS pseudodot plots. Real Time PCR (RT-PCR) using Taqman® gene expression assays for proliferation and apoptosis markers confirmed results.

3.6.3 Spheroid cultures

Monolayer MSCs from all groups were plated on Corning® ultra-low attachment 6 well plates (Thermo Fischer Scientific Inc., USA) with tumour sphere conditioning medium containing EGF, BFGF, B27 supplement and insulin. They were allowed to grow into second generation spheres the size of >50 um for 10-12 days. They were used in studies with RT-PCR, FACS characterization, and immunofluorescence for comparing markers of MSCs.

3.6.4 Classification of a potential high-risk subgroup of patients

Univariate and multivariate models with SIMCA 14 software (Umetrics AB, Sweden) were used to assess intragroup variability. Principle component analysis (PCA) was used to achieve the highest possible predictability, and with scatter plot distribution of patients, we sub-classified those patients who were close to or away from a 95% confidence interval (CI) in both EnSC and EndoSC as potential ‘high-risk’ or ‘low-risk’ patient subgroups.

Gene-loading plots revealed a distribution of specific genes that contribute to ‘high-risk’ status by showing a distribution analogous to that of patients in the scatter plot. Identified potential risk groups and their regulated genes were further defined for higher predictability at the multi-parametric level using an orthogonal partial least squares-descriptive analysis (OPLS-DA).

To confirm aberrantly-regulated crucial pathways among the sub-groups, heat maps were generated by GENE-E software version 3.0.224 (Broad Institute Inc. Cambridge, MA, USA.). Trend curves were then created to compare expression trends between potential ‘high-risk’ and ‘low-risk’ patients in comparison with validated cancer cell lines and healthy volunteers.

3.6.5 Flow cytometry characterization

All MSC groups were analysed for surface expression of stromal markers CD146-PerCP-cy5.5, SUSD2/W5C5-APC (Biolegend, San Diego, CA, USA), CD10-FITC (Miltenyi Biotec, Bergisch Gladbach, Germany); epithelial markers SSEA1-Alexa fluor 488 (Santa

Cruz biotechnology, CA, USA), EPCAM-PE (Miltenyi Biotec), cytokeratin18-PE (BD Horizon, Piscataway, NJ, USA); as well as hematopoietic lineage markers CD45-APC, CD34-APC, CD20-PerCP, and CD3-FITC (BD Pharmingen, Franklin Lakes, NJ, USA). Their expression was compared in terms of median fluorescent intensity (MFI) with respect to their unstained controls, and results were rendered in histograms using FlowJo data analysis software (LLC, Oregon).

Monolayer MSCs were compared with their respective in vitro-generated spheroid MSCs from all patient and healthy controls for CSC markers ALDH1 by Aldefluor assay (Stem cell technologies, Vancouver, Canada) and anti-human antibodies CD133-1-APC (Miltenyi Biotec), CD44-PE (Biolegend), CD117-PE-cy7 (Biolegend), and ABCG2-PerCP-cy5.5 (Biolegend). Co-expression of CSC markers between monolayer and spheroid MSCs were represented in pseudodot plots, while the individual marker expression of both cultures were shown in histograms.

3.6.6 Co-localization of CSC marker proteins

Dual colour immunofluorescence was used for observation of co-expression of CSC marker proteins in spheroid MSCs. Anti-human OCT3/4 antibody (Santa Cruz Biotechnology, TX, USA), rabbit polyclonal PROM1/CD133 (Biorbyt, Cambridge, UK), and CD44variant 6 (Molecular Probes® Life Technologies) were used as primary antibodies. As a positive control, SKOV3 ovarian cancer line was used. Stained spheres were incubated overnight and tagged with secondary antibodies; donkey anti-mouse alexa fluor 488 (Molecular Probes® Life Technologies) and goat anti-rabbit Abberior® STAR633 (Abberior, Göttingen, Germany).

Co-localization of CSC markers was visualized in the following combinations: OCT3/4 and CD133, CD44v6 and CD133. Images were captured using Zeiss LSM 700 confocal microscopy (Carl Zeiss, Tokyo, Japan) and for construction, a co-localization dot plot created with Huygens software (Scientific Volume Imaging, Hilversum, Netherlands) was used.

3.6.7 Chemo-sensitivity and tumour invasion assay

To assess the potential invasiveness and drug-resistance capacity among endometrium of patients (P-EnSC and EndoSC), a 3D-tumour invasion model was designed according to protocols provided by Cultrex® 3D Spheroid Fluorometric Proliferation/viability assay kit and Cultrex® 3D Spheroid Invasion assay kit (Trevigen Inc. Gaithersburg, MD, USA).

Harvested cells were suspended, seeded in a 96 well low attachment plate (Corning), and incubated under hypoxic conditions (2% O2) for 72 hours. The MSC drug resistance capability was assessed by subjecting cultures to increasing doses of Paclitaxel (0.1, 1, 10nM) and Cisplatin (0.1, 1, 10µM). At the end of the treatment, one-tenth volume of Resazurin was added and resorufin was read at 590nm. In addition, invasion was assessed in response to chemo-resistance by performing the above steps until spheroid expansion, then invasion media along with addition of chemo-attractants MCF-1. Media containing presence/absence

of Paclitaxel (10nM) or Cisplatin (10μM) was later added and incubated for 8 days. Images were captured from the time of adding invasion media using live cell instrument (Leica) and assessed every other day. Images were processed for calculating invasion area using ImageJ software.

3.6.8 Statistical analysis

Groups from both monolayer and spheroid endometrial MSCs were checked for Gaussian distribution using Shapiro-Wilk's normality test, and homogeneity of variance using Bartlett's Test. Groups that had $P>0.05$ in both tests were considered parametric.

For comparing EnSC and EndoSC, a paired T- test was used, while the Wilcoxon Signed T-test was used if groups were paired and non-parametric. Similarly, for unpaired groups, either an unpaired T-test or Mann-Whitney test was performed. For chemo-sensitivity assay, a two-way Annova test was used.

Statistical software GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA) was used.

3.7 STUDY IV

3.7.1 Laser capture micro-dissection (LCM)

Fresh frozen sections (9 μm) were placed and fixed on a membrane slide (Membrane Slide NF 1.0 PEN, Carl Zeiss Microimaging GmbH, Germany) and subjected to UV and a subsequent dehydration process including repeated ethanol washings. PixCell II LCM System (Arcturus, Plaisir, France) was used to isolate glandular epithelial cells and stromal cells from the endometrium. Microdissected cells were collected on optically transparent LCM Macro caps and stored in -70°C until RNA isolation.

3.7.2 mRNA microarray analysis

The Whole Human Genome Oligo Microarray (Agilent Technologies, CA, USA) was used to perform microarray analysis. First strand cDNA was transcribed from 300 ng of total RNA using T7-Olige(dT) promoter primer. Samples were transcribed in vitro and Cy-3-labelled by using a Quick-AMP labelling kit (Agilent Technologies). cRNA was further fragmented into pieces ranging from 35 to 200 bases in size, and approved using Agilent 2100 Bioanalyzer technology. Fragmented cRNA samples were hybridized onto chips by means of 17 hours of incubation at 65°C with constant rotation, followed by a two-step microarray wash of one minute in two washing buffers (Agilent Technologies). Hybridized microarrays were scanned in an Axon 4100A scanner (Molecular Devices, Sunnyvale, CA, USA).

3.7.3 Data Analysis

3.7.3.1 *Preprocessing and quality assessment*

The gene level signals were extracted from CEL files by using Affymetix Expression Console™ 1.3v software using Probe Logarithmic Intensity Error Estimation (PLIER) summarization algorithm. The normalization was performed by the Global Median method.

In the initial step, the raw data was checked for any significant outliers. Any sample having more than three times the standard deviation was considered an outlier. The quality assessment of the raw data and filtered data was assessed by MA plots, Density plots, Hierarchical clustering, Quantile-Quantile plots, and Principal Component Analysis (PCA) plots.

3.7.3.2 *Data filtering and differential expression analysis*

Data filtering of PLIER data was performed by using a gene filter package in R 3.1.2v (Therneau and Ballman, 2008). A non-specific filter was applied with a hybridization signal ≥ 40, yielding a total of 2625 genes in the stromal compartment and 3548 genes in the epithelial compartment.

To analyse the differential expression, Linear Models for Microarray (LIMMA) and Significance Analysis of Microarrays (SAM) analysis were performed by using one ChannelGUI package in R software (R Core Team, 2012). The data was log2 transformed for the differential analysis.

3.7.4 Statistical analysis

An unpaired t-test was performed to compare differences in the groups. A P-value less than 0.05 was considered significant and a minimal change of 1.5-fold was applied to select up-regulated and down-regulated genes.

4 RESULTS AND DISCUSSION

4.1 STUDY 1

It is well known that up to 90% of women experience some degree of retrograde menstruation, but only 10-15 % of women develop endometriosis. To establish endometrial implants in ectopic sites, many factors are involved such as altered peritoneal environment, genetic factors, and reduced immune surveillance, together with an increased angiogenesis property and an enhanced capacity in adhesion and attachment of the shed endometrial cells.

Endometrial cells with phenotypes relevant to stemness, attachment, adhesion, and migration can reach the peritoneal cavity with retrograde menstruation and are able to adhere and establish endometriotic implants/lesions.

In this paper, we have investigated the expression of a set of molecules with a possible involvement of adhesion, attachment, and invasion of endometrial cells: apoprotein E (ApoE), integrin-β-2 (ITGB2), laminin-y-1 (LAMC1), CD24 molecule, and junction adhesion molecule-1 (JAM-1).

As shown in Fig 1 (Study I), endometrium from controls and women with endometriosis expressed ApoE, ITGB2, ITGB7, LAMC1, CD24, and JAM-1. Gene expression of ApoE and JAM-1 was decreased in both the proliferative and secretory phase in endometrium of women with endometriosis compared with the controls. Also, mRNA expression of LAMC1 was reduced in the endometrium from endometriosis patients compared with controls in the proliferative phase. An altered gene expression of CD24 was seen between the endometrium in endometriosis patients and endometriomas in the secretory phase. The ITGB2 protein expression was altered in epithelial cells between the endometrium from healthy volunteers and endometriosis patients in the secretory phase.

The aetiology of endometriosis still remains debatable and the processes involved in the invasion mechanism of the endometrial cells are still poorly understood. Our study revealed for the first time an expression of ApoE, ITGB2, ITGB7, and LAMC1 in endometriomas and in eutopic endometrium.

Collective cell migration is a phenomenon important in cancer biology (Friedl and Gilmour 2009) and might play an important role in endometriosis development as well, even though the underlying concepts still have to be refined (Donnez et al., 2015). Traditionally, the molecules thought to facilitate cell-cell adhesion and cell-ECM adhesion in endometriosis have been members of the families of integrins, cadherins, laminin, and fibronectin (Béliard et al. 1997).

The in vivo endometriotic invasion model developed by Kao et al. elucidates how ectopic endometrial mesenchymal stem cells clearly demonstrate a superior capacity for cell migration and invasion (Kao et al., 2011).

In an experimental baboon model, more recently developed, molecules that have been nominated as crucial in the initial attachment/invasion include Ki-67, E-cadherin, and β-catenin.

How these and other molecules related to the initial establishment of ectopic endometrium are influenced by the oestrogen metabolites in the peritoneal fluid is, however, not reported so far. Studies focusing on this might contribute and complete the missing links.

Our study showed an aberrant expression of CD24 in endometriosis patients where the cyclical appearance pattern was lacking compared to controls, a fact that could affect the endometrial receptivity recognized in this patient group (Brosens et al., 2012).

Further, some of the genes explored in this study are also associated with malignancy. Elevated ApoE concentrations have been found in malignant ovarian cyst fluids, suggesting

an association with deregulated lipoprotein metabolism and ovarian cancer (Podzielinski et al., 2013). A dramatic up-regulation of the same protein has also been seen in poorly differentiated endometrial adenocarcinomas (Huvila et al., 2009), while a negative correlation can be observed with JAM-1 expression and endometrial cancer grade (Koshiba et al., 2009). CD24 has been associated with cancer progression and development and an enhanced expression has been seen in patients with endometrial carcinoma (Kim et al., 2009).

There's an urgent need to better understand the molecular linkage to malignant transformation in endometriosis to avoid unnecessary apprehension as well as potential overtreatment. This study contributes by providing new information on potential aetiological components as well as useful data on molecules correlated with malignant transformation.

4.2 STUDY II

The clinical expression of endometriosis varies significantly from patient to patient, suggesting that the nature of the pathology only partly depends on localization, duration, and the patient's genetic predisposal.

Accumulating evidence suggests that endometriosis is an epigenetic disease, which could explain the many features of the disorder and the difficulties in correlating the stage and clinical outcome in terms of pain and fertility-related aspects. The concept of an epigenetic disease opens up a broader understanding of the complexity of the disease mechanisms, but could also yield difficulty or confusion in diagnostic/therapeutic management of patients.

In this paper, the epigenetic mechanism DNA-methylation of HOXA10 gene was analysed in different compartments of endometriosis as well as eutopic endometrium in cases and controls.

The eutopic endometrium of women with endometriosis was significantly more methylated in comparison to controls (sequence 1: 8.68% in cases and 6.25% in controls: p=0.037, sequence 2: 11.89% in cases and 9.25% in controls: p=0.032). Eutopic endometrium was also significantly more methylated than ectopic tissue in the endometriosis patients (mean difference -3.6 sequence 1: p=0.001 and -6.0 sequence 2: p=0.0001).

Case	Methylation ectopic tissue sequence 1 (%)	Methylation ectopic tissue sequence 2 (%)	Methylation endometrium sequence 1 (%)	Methylation endometrium sequence 2 (%)
1. Cyst	7	10	5	9
2. Cyst	10	8	13	16
3. Cyst	5	7	10	14
4. Cyst Vaginal node	5 7	6 13	17	16
5. Cyst RVS	3 3	6 9	7	15
6. Muscle	6	10	8	16
7. RVS	4	7	11	15
8. Cyst	8	9	7	9
9. RVS	3	4	15	18
10. Cyst	4	5	11	13
11. Peritoneal nodule	5	6	11	13
12. Peritoneal nodule	4	4	6	7
13. Cyst	5	5	11	11
14. Cyst	6	2	6	7
15. RVS	1	3	5	7
16. Peritoneal Nodule	4	5	5	8
17. Cyst Peritoneal nodule	5 5	3 10	5	8
18. Cyst	5	6	4	11

Table 1. Methylation (%) of the hoxa10 gene in eutopic and ectopic endometrium in endometriosis patients.

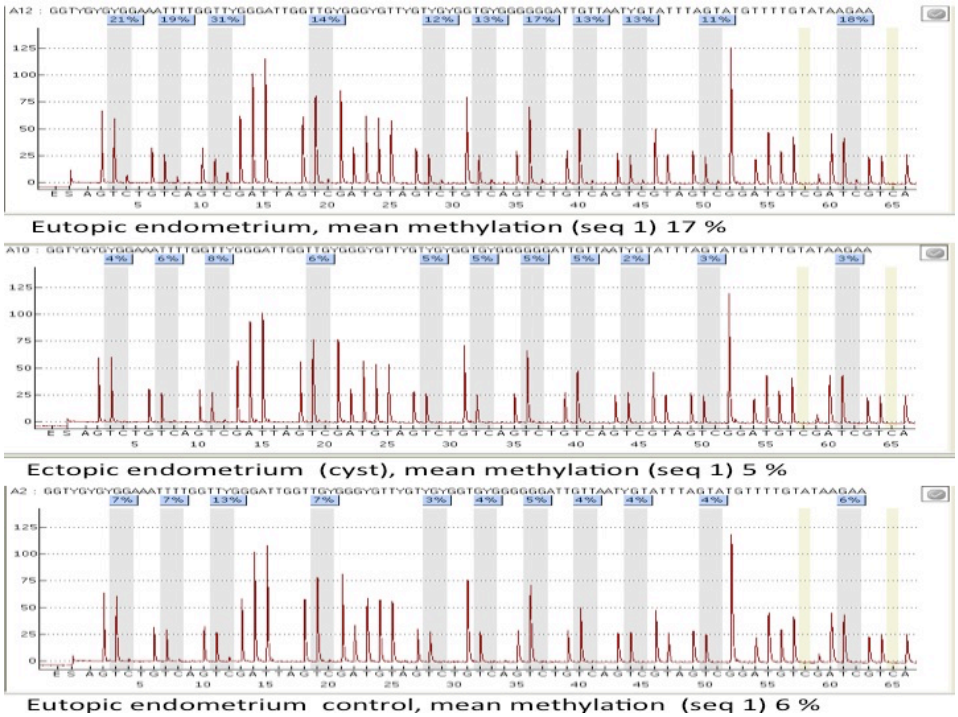

Figure 2. Chromatograms showing CpG island methylation (%) in eutopic endometrium and ectopic endometrium (cyst) in endometriosis patients (case nr 4) and eutopic endometrium of a healthy control.

These results support the fact the endometrial function in endometriosis is altered and in this case negatively influencing the fertility capacity of the secretory phase.

This study is the first to present data on endometrial HOXA10 methylation from different ectopic sites.

Another aim of the study, besides investigating if the epigenetic mechanism of methylation was responsible for the aberrant expression of the gene previously described in endometriosis patients, was to explore whether an extra-ovarian involvement would correspond to a higher methylation status. It's known that the disease stage is not correlated with the severity of pain and a correlation between a more severe stage of disease (extra-ovarian disease) and a higher extent of methylation could not be found.

These results suggest that the eutopic endometrium represents the nucleus of the alteration, which is not then conserved and transferred in the ectopic atmosphere. In the literature, there's a lack of data on the differences in molecular signature between ectopic and eutopic endometrium. Recently, a higher expression of HIF-1/2α, PAR-1/4, and VEGF-A has been described in ovarian endometriomas when compared to deep, infiltrating endometriosis

lesions (Filippi et al., 2015). This fact supports the idea that endometriosis is not just one disease but might be several diseases.

The sample size of this study is restricted because it was not easy to find suitable subjects with endometriosis, as hormonal treatment prior to surgery was an exclusion criterion. Also, the fertility status was known for only 7 of the cases, as many hadn't yet initiated an IVF itinerary or attempted pregnancy.

An altered HOXA10 expression has also been associated with polycystic ovarian syndrome and hydrosalpinx (Du and Taylor 2015). For this study, these diseases didn't represent an exclusion criterion and were not screened for. The women included in the study, cases as well as controls, had a regular menstrual cycle, which is presumably why cases of PCO were not included among the subjects.

Our study reveals new and interesting data regarding the methylation of an important fertility-regulating gene HOXA10 where we can find eutopic endometrium significantly more methylated in endometriosis patients, but with a non-correspondence between the stage of disease and methylation level of the ectopic endometrium.

4.3 STUDY III

Even though endometriosis-associated cancer (EAC) is a rare event, occurring in 0.7-2.5% of endometriosis patients, endometriosis is still considered a precursor of certain histological subtypes of ovarian cancer, such as ovarian endometrioid and clear cell ovarian carcinoma (Gadducci et al., 2014). The importance of a better understanding of the carcinogenic linkage with this common gynaecologic disorder is undebatable. In the context of stem cell origin of EAC, endometrial MSCs may undergo de-differentiation or reprogramming into endometrial cancer stem cells (CSCs) due to an altered microenvironment such as hypoxia and inflammation leading to tumour initiation (Ye et al.,2014).

In this study, we aimed to investigate the molecular link between endometriotic and endometrial stem cells and the development of ovarian cancer by exploring CSC-specific markers, and its molecular signatures that were previously known with stem cell and cancer signalling, pluripotent functions, and asymmetric division.

Selected populations positive for CD90, CD73, and CD105 (5-10%, endometrial stem cells) were characterized by higher expression of W5C5, EPCAM, CD44, and CD146. Differential potential was then assessed and a successful differentiation of MSCs into adipocytes, osteoblasts, and chondrocytes could be documented.

The cell cycle analysis demonstrated a greater proliferative capacity and a reduced expression of apoptopic genes such as BCL-2 ($p<0.05$) (Fig. 2, Manuscript 2.)

Moreover, we could see that EndoSC exposed less proliferation potential compared with its paired eutopic sample, indicating a MSC niche alteration that could play a role in the onset mechanisms of endometriosis.

RT-PCR assay could further outline an overexpression of genes involved in cancer metabolism, EMT, and re-programming (such as genes PTEN, MMP3, and TNF-α) when compared with the MSCs of patients and controls. Because the incidence of diverse genes did not reach significance, presumably explained by the high variability among the patients, a categorized univariate PCA and multivariate OPLS-DA modelling was used to investigate the biological importance of the molecular alterations and identify a subset of potentially 'high-risk' samples in the P-EnSc and EndoSC groups.

This characterization affirmed that the 'low-risk' subgroup presented a gene expression profile comparable to that of controls, while the 'high-risk' subgroup showed an expression pattern more similar to that seen in the positive controls (cancer cell line SKOV3).
An up-regulation of genes involved in cancer metabolism, EMT, and re-programming could be distinguished (TP53, KRAS, TGF-alfa-1, SNAI1, SOX2, and NANOG), and TP53 turned out to be a master regulator behind the alterations found in the endometriosis patients (Fig. 3, Manuscript 3).

The generation of 3D spheroids from sorted MSCs, identifying enrichment of pluripotent and self-renewal genes during comparison of sphere versus monolayer cultures, has generated significant data in our study. Spheroids from eutopic and ectopic endometrium showed a significant presence of CSC markers and pluripotent self-renewal genes compared with monolayer cultures. In particular, a distinction between 'low-risk'/healthy groups and 'high risk' individuals was observed in relation to cells expressing SOX2, NANOG, CD44, and CD133. SOX2 has been recognized as responsible for reprogramming of MSCs into CSCs (Herreros-Villanueva et al., 2013), and a similar trend could be seen in our spheroid cultures.

The analysis of CSC markers demonstrated in spheroids an increased expression compared to monolayer, and CD44+CD133+ and CD44+/CD133/ABCG2+ cells were significantly different between P-EnSC and EndoSC. Thus, we suggest that CD44/CD133 and/or ABCG2 positive cell populations should be used to additionally recognise CSCs in endometriosis patients for further investigations in vivo.

Tumour invasion assessment in a chemo-sensitivity assay in a 3D-tumour microenvironment revealed a MSC invasion in seven P-EnSC and six EndoSC out of the 11 paired samples, even though the difference in invasion potential was less marked between the endometriosis samples and the positive controls. This fact reminds us that we are studying a predominantly benign disease and not a clear premalignant condition. When further investigating the chemo-sensitivity at an individual level through a viability assay, we could see in patient 36 a sensitivity for Paclitaxel in both P-EnSc and EndoSC, while Cisplatin sensitivity could be seen only in the endometrium sample (Fig. 7, Manuscript 3).

In this study, we identified a high-risk group of endometriosis patients that exhibited a significant up-regulation of some of the cancer stem cell markers and important genes involved in cancer metabolic pathway in their endometrial and endometriotic MSCs. However, further confirming studies that follow up such patients are necessary before we can categorize endometriosis patients as high or low risk for developing ovarian cancer based on the gene profile in the eutopic and ectopic endometrium.

4.4 STUDY IV

This study's aim was to explore the gene expression profile of known cancer-correlated molecules in different endometrial compartments in order to better understand the potential links to malignant transformation of the eutopic endometrium in endometriosis patients.

The following genes turned out to be significantly deregulated in the stromal compartment (Fig. 2, Manuscript 4): EHF (ΔΔCt -4.11; p=0.0001), PERP (ΔΔCt -3.16; P=0.005), JUN (ΔΔCt 4.24; p=0.012), WT1 (ΔΔCt 2.44; p=0.016), and in glands (Fig. 3, Manuscript 4): EZR (ΔΔCt 2.53; p=0.0001), SLPI (ΔΔCt -2.10; p=0.0002), PERP (ΔΔCt -2.11; p=0.002), and SLC34A2 (ΔΔCt -2.00; p=0.002).

The impact of endometriosis on reproductive health and endometrial function is well known. The association with an increased risk of ovarian cancer is also well documented and the ovaries represent the far most common location for the malignant transformation.

The literature has reported the eutopic endometrium as a rare transformation locus (Baba et al., 2016). On the other hand, the association between adenomyosis and endometrial cancer has been better established (Baba et al., 2016; Koike et al., 2013). Adenomyosis, as well as endometrial adenocarcinoma, is oestrogen-dependent and could possibly provide a model for better understanding of oestrogen-dependent malignant transformation.

The reports on endometrial cancer in endometriosis patients have so far been conflicting and the carcinogenic linkage still remains poorly understood. Also, many epidemiological studies conducted on the association between endometriosis and endometrial cancer have had a limited sample size, which could explain why no significant correlation between the disorders has been found.

In this study, we showed that several genes crucial in promoting endometrial tumourgenesis were significantly deregulated in the endometrium of endometriosis patients, namely EZR, SLPI, and EHF. EZR is known to be involved in the biology of cancer and plays a specific role in facilitating the indicators necessary for metastasis initiation. Also, SLP1 expression levels have been correlated with the malignant potential of cells (Devoogdt 2004) and a significant aberrant expression of these genes in the endometrium provides important information.

This study reveals important data inherent to the molecular linkage that could contribute to the malignant transformation of the endometrium in endometriosis.

In the glandular compartment, the network between pathways involves cellular movement, cellular growth, and proliferation. Among the top canonical pathways in the stromal compartment is the TP53 signalling pathway, and among top diseases/bio functions, cancer occupies first place. The corresponding involvement in the glandular compartment is inflammatory disease and endocrine system disorders (Fig 1 a,b, Manuscript 4).

This fact underscores the importance of observing the endometrial compartments separately, as their function and regulation can be markedly diverse. The samples in this study were all obtained on the same cycle day (LH+6-7), which is why a potential hormonal bias could be minimized.

For future research, it would be particularly relevant to observe how these genes may demonstrate an altered expression in adenomyosis patients as well. This could enable identification of a subgroup of patients at risk of adenomyosis, which appears to be more strongly associated with endometrial cancer than endometriosis alone (Kok et al., 2015).

5 CONCLUSIONS AND FUTURE DIRECTIONS

This thesis addresses the need for attention to the clinical impact of endometriosis, focusing on the involvement of endometrial stem cells in the development of EAC. Endometriosis is a multifactorial complex disease without a fully understood aetiology.

We provided new information regarding pathogenesis, because for the first time, a set of molecules relevant to endometrial adhesion and attachment could be demonstrated in the endometrium of women with and without endometriosis (Study I).

Our further molecular studies showed how endometrial mesenchymal stem cells, in a subset of patients, presented an up-regulation of cancer promoting genes such as PTEN, TP53, K-ras, TGF-alfa-1, SNAI1, SOX2, and NANOG, and a down-regulation of the genes important for apoptosis, BCL-2. Based upon the biological significance of the deregulated genes, we could identify a subset of patients as high-risk. In this group, a MSC invasion was observed in a chemo-sensitivity assay in 3D-tumour microenvironment (Study III).
Confirmed across different molecular techniques, we believe that these findings contribute to a better understanding of the mechanisms involved in endometriosis-associated cancer and we now plan to carry out an in vivo study to further strengthen our results.

Regarding the link to endometrial cancer development, we found in the global gene expression array 11 dis-regulated genes relevant for endometrial malignant transformation in both glands and the stromal compartment (Study IV). This is significant because available evidence of endometriosis-associated cancer is restricted and frequently contradictory mainly due to old studies with limited sample size.

The molecular links responsible for malignant transformation in endometriosis patients are still not fully understood and we lack sufficient criteria to identify at-risk patients.
As endometriosis is a common disease and malignant transformation is rare, it's extremely important to expand research in this field to avoid the risk of overtreatment.
Current knowledge does not provide any consensus on screening and risk-reducing prophylactic surgery. Therefore, efforts must be made to achieve better understanding of the molecular links to enable identification of patients according to their future risk of malignancy.

We also showed for the first time how an epigenetic mechanism such as DNA-methylation is engaged in alternating the fertility-regulating gene HOXA10 gene in different types and stage of endometriosis (Study II). The field of epigenetics is expanding and more extensive surveys of epigenetic modifications in endometriosis are required to better categorize the epigenetic modification and the extent/type of endometriosis disease.

Epigenetic knowledge could open up an attractive field of diagnostic tools and lead to novel therapeutic approaches as well as risk factor recognition. New epigenetic treatment approaches could also overcome the side effects and limits of current, short-term medical and surgical treatment strategies.

6 ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and gratitude to all the pepole that have been involved in my research, in particular I would like to thank

Kristina Gemzell-Danielsson, my main supervisor. Thank you for being the one you are, so amazingly generous with your knowledge, encouragement and time (even when you don't have any). Your energy and enthusiasm is truly unique.
Thank you for believing in me and in this project.

Lalit Parameswaran Grace Kumar- my co-supervisor. Thank you for providing all your precious expertise in the field and thank you for your endless patience. It has been a pleasure working with you and your collaborators - thanks for making me always feeling so welcome in the lab.

Eva Broberg- Thanks for all your coordination (from sample collection to after-work when I am in town!). Thanks for the lovely time (and swim) at your archipelago paradise.

Lena Elffors-Söderlund for her precious contribution in sample collection coordination at WHO-Collaborating center.

Sakthi Ponandai-Srinivasan and *Nageswara Boggavarapu*, my co-Authors and co-workers. Thank you for being so generous in teaching me your techniques and for helping me with the time consuming experiments. It has been so nice and fun working with you, wish you both good luck with the rest of your PhD projects.

Catharina Karlsson – thank you for your flexibility and administrative support- in particular during the latest months!

Berit Ståby, Birgitta Byström and *Eva Andersson,* thank you for always being so kind and nice these years teaching me from very scratch everything I didn't know in the lab!

Thanks *Annette Aronsson*, *Ingrid Sääv* and *Helena Kopp Kallner*, - for always being there for that "after-work whenever it happens". It has been so nice to have you around for sharing experiences and good laughs.

Thanks *Lena Marions* for the nice time together at Sesam and in Florence!

Susanne Sjöström and *Elin Larsson*, always nice and inspiring to eat lunch with you at the 5th floor! Thanks also *Cecilia Berger*- so nice to, from old KI, end up in the same research group! Thanks for contributiong in precious sample collection!

Thanks to the 9th floor group at ALB, for good co-work in the lab and for always opening the door when I pop up without badge every once in a while!

Kevin Sunde Oppegaard and *Christian Fiala*- grateful to have got to know you- brave men struggling for women's right!

Mauro Marchionni, thanks for believing in me from start. Thanks for inspiring me to chose the field of Gynecology.

Massimiliano Fambrini, co-Author and collegue, thanks for introducing me to scientific writing.

Cecilia Bussani, co-Author, thank you for sharing your expertise in the field of Pyrosequencing.

Stefania Capassoni – the very best nurse and friend you can ever desire- without you this thesis wouldn't have been concluded, thanks for all your generous support.

My Italian collegues that during the years have given me support in being friends always interested in my research work; *Dr. E. Martini, A. Galli*, *A Mattei, E Vanzi, M Franchini* and *L Cianferoni* – grazie!

Nina Di Claudio– my sister in law – I admire your strength and positive attitude, you are a lovely aunty and your three girls are simply wonderful.

Elisabetta Busi – my Italian soul-mate. You are a role model for all of us, your strength and sharpness is unique, you are unique.

Karin Dakhoo Forssberg, even though much time might pass, it always seems like yesterday we had our Peach Teas in Barneswille – thank you for your friendship all these years!

Annika Hjort, I am so grateful to your patience- for giving me endless chances in our long distance friendship! You are so generous and kind, thanks for being here today!

Monica Sångberg Moen and *Stefano Crivelli* –thank you for being special friends to me, for your unique sense of humor and capacity to distinguish important from not important.

Fam. Mautner – la "Famiglia". From the times as teenager daughter in the cellar commuting to Italy up to today- you were, are and will always be the best!!

Emma Medin, what would life be without your constant presence and engagement even though the distance that keeps us apart! Love you!

Jenny and Tom- my loving sister and my brother in law (BC)- thank you for always being the ones you are, simply the best. True best friends and family, I am so lucky to have you!!

My father *Håkan Andersson* and his wife *Britt-Marie Sandin-Andersson*; thanks for all encouraging support during these years…(without your nice little central apartment this commuting has been possibile)! Your positivity has really helped me on this challenging road, thanks! And thanks pappa, for always being an inspiring role model, as an exceptional doctor and father.

My mother *Birgitta Moberger* and her partner *Bosse Lindström*; thanks for always believing in the happy ending of this story. During the most difficult moments you have never doubted in me, I am truly grateful for that. And thanks mamma for transmitting your love for Italy and languages when I was a small girl… and now, some years later there are two small girls that more than everything else love their holiday with the grannies in Sweden!

Ulla Moberger, my grandmother. For being a role model during my youth, I miss you.

Rosina and *Nazario Di Claudio*– my in-laws. Nothing would have been possible without you. Your constant love and limitless support is truly extraordinary. I am deeply and truly thankful, you mean a lot to me and we learn from you every day.

Paolo Di Claudio. Words would never be enough for my gratitude. Thank you for always being present, positive and ready to face whatever challenge. Thank you for everything you have taught me during the years, thank you for being the best husband and father in this world!

Louise and Ellen, thank you for making every single day of my life the most wonderful. You are the light in my eyes.

7 REFERENCES

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. 2003. “Prospective Identification of Tumorigenic Breast Cancer Cells.” Proc Natl Acad Sci U S A.100(7):3983–88.

Albertsen HM, Chettier R, Farrington P, Ward K. 2013. “Genome-Wide Association Study Link Novel Loci to Endometriosis.” PLoS ONE, 8(3): e58257.

Amemiya S, Sekizawa A, Otsuka J, Tachikawa T, Saito H, Okai T. 2004. “Malignant Transformation of Endometriosis and Genetic Alterations of K-Ras and Microsatellite Instability.” Int J of Gynecol Obstet, 86(3):371–76.

Aris A. 2010. “Endometriosis-Associated Ovarian Cancer: A Ten-Year Cohort Study of Women Living in the Estrie Region of Quebec, Canada.” J Ovarian Res, 3:2.

Ayhan A, Mao TL, Seckin T, Wu CH, Guan B, Ogawa H, Futagami M, Mizukami H, Yokoyama Y, Kurman RJ, Shih IeM. 2012. “Loss of ARID1A Expression Is an Early Molecular Event in Tumor Progression from Ovarian Endometriotic Cyst to Clear Cell and Endometrioid Carcinoma.” Int J Gynecol Cancer, 22(8):1310–15.

Baba A, Yamazoe S, Dogru M, Ogawa M, Takamatsu K, Miyauchi J. 2016. “Clear Cell Adenocarcinoma Arising from Adenomyotic Cyst: A Case Report and Literature Review.” J Obstet Gynaecol Res, Feb;42(2):217-23.

Ballard K, Lowton K, Wright J. 2006. "What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis." Fertil Steril, 86(5):1296–1301.

Bapat SA. 2010. “Human Ovarian Cancer Stem Cells.” Reproduction, 140(1):33–41.

Béliard, Aude, Jacques Donnez, Michelle Nisolle, and Jean Michel Foidart. 1997. “Localization of Laminin, Fibronectin, E-Cadherin, and Integrins in Endometrium and Endometriosis.” Fertil Steril, 67(2):266–72.

Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, Weinberg RA. 2008. “An Embryonic Stem Cell-like Gene Expression Signature in Poorly Differentiated Aggressive Human Tumors.” Nat genet, 40(5):499–507.

Brosens I, Brosens J, Benagiano G. 2013. “Neonatal Uterine Bleeding as Antecedent of Pelvic Endometriosis.” Hum Reprod, 28(11):2893–97.

Brosens I, Brosens JJ, Benagiano G. 2012. “The Eutopic Endometrium in Endometriosis: Are the Changes of Clinical Significance?” Reprod BioMed Online, 24(5):496–502.

Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, Martin R, Utsunomiya H,

Thung S, Gurates B, Tamura M, Langoi D, Deb S. 2005. “Regulation of Aromatase Expression in Estrogen-Responsive Breast and Uterine Disease: From Bench to Treatment.” Pharmacol Rev, 57(3):359–83.

Bulun SE. 2009. "Endometriosis". N Engl J Med, Jan 15;360(3):268-79.

Bungum HF, Vestergaard C, Knudsen UB. 2014. “Endometriosis and Type 1 Allergies/immediate Type Hypersensitivity: A Systematic Review.” Eur J Obstet Gynecol Reprod Biol, 179:209–15.

Burney RO, Talbi S, Hamilton AE, Vo KC, Nyegaard M, Nezhat CR, Lessey BA,

Giudice LC 2007. “Gene Expression Analysis of Endometrium Reveals Progesterone Resistance and Candidate Susceptibility Genes in Women with Endometriosis.” Endocrinology, 148(8):3814–26.

Burney RO. 2013. “The Genetics and Biochemistry of Endometriosis.” Curr Opin Obstet Gynecol, 25(4):280–86.

Burney RO and Giudice LC. 2012. “Pathogenesis and Pathophysiology of Endometriosis.” Fertil Steril, 98(3):511–19.

Catasús L, Bussaglia E, Rodrguez I, Gallardo A, Pons C, Irving JA, Prat J. 2004. “Molecular Genetic Alterations in Endometrioid Carcinomas of the Ovary: Similar Frequency of Beta-Catenin Abnormalities but Lower Rate of Microsatellite Instability and PTEN Alterations than in Uterine Endometrioid Carcinomas.” Hum Pathol, 35(11):1360–68.

Chan RW, Ng EH, Yeung WS.. 2011. “Identification of Cells with Colony-Forming Activity, Self-Renewal Capacity, and Multipotency in Ovarian Endometriosis.” American J Pathol, 178(6):2832–44.

Chen X, Horiuchi A, Kikuchi N, Osada R, Yoshida J, Shiozawa T, Konishi I. 2007. “Hedgehog Signal Pathway Is Activated in Ovarian Carcinomas, Correlating with Cell Proliferation: It’s Inhibition Leads to Growth Suppression and Apoptosis.” Cancer Science, 98(1):68–76.

Cheng L, Sung MT, Cossu-Rocca P, Jones TD, MacLennan GT, De Jong J,

Lopez-Beltran A, Montironi R, Looijenga LH. 2007. “OCT4 : Biological Functions and Clinical Applications as a Marker of Germ Cell Neoplasia.” J Pathol, Jan;211(1):1-9.

Cho, KR and Ie-Ming S. 2009. “Ovarian Cancer.” Annu Rev Pathol, 4:287–313.

Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. 2005. “Prospective Identification of Tumorigenic Prostate Cancer Stem Cells.” Cancer Res, 65(23):10946–51.

Curley, Michael D. et al. 2009. “CD133 Expression Defines a Tumor Initiating Cell Population in Primary Human Ovarian Cancer.” Stem cells (Dayton, Ohio) 27(12):2875–83.

D' Hooghe, T and Hummelshoj L. 2006. “Multi-Disciplinary Centres / Networks of Excellence for Endometriosis Management and Research : A Proposal.” Hum Reprod, 21(11):2743–48.

Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, Shelton AA, Parmiani G, Castelli C, Clarke MF. 2007. “Phenotypic Characterization of Human Colorectal Cancer Stem Cells.” Proc Natl Acad Sci U S A, 104(24):10158–63.

Dang H, Ding W, Emerson D, Rountree CB. 2011. “Snail1 Induces Epithelial-to-Mesenchymal Transition and Tumor Initiating Stem Cell Characteristics.” BMC cancer, 11(1):396.

Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, Li C, Wang LP, Roby KF, Orsulic S, Connolly DC, Zhang Y, Montone K, Bützow R, Coukos G, Zhang L. 2010. “Distinct Expression Levels and Patterns of Stem Cell Marker, Aldehyde Dehydrogenase Isoform 1 (ALDH1), in Human Epithelial Cancers.” PloS One, 5(4):e10277.

Devoogdt, N. 2004. “Secretory Leukocyte Protease Inhibitor in Cancer Development.” Ann N Y Acad Sci, 1028(1):380–89.

De Ziegler D, Borghese B, Chapron C. 2010. “Endometriosis and Infertility: Pathophysiology and Management.” Lancet, 376(9742):730–38.

Donnez O, Orellana R, Van Kerk O, Dehoux JP, Donnez J, Dolmans MM. 2015. “Invasion Process of Induced Deep Nodular Endometriosis in an Experimental Baboon Model: Similarities with Collective Cell Migration?” Fertil Steril, 104(2):491–97.

Du H, Taylor HS. 2015. "The Role of Hox Genes in Female Reproductive Tract Development, Adult Function, and Fertility". Cold Spring Harb Perspect Med, Nov 9;6(1)

Eisenberg VH, Zolti M, Soriano D. 2012. “Is There an Association between Autoimmunity and Endometriosis?” Autoimmun Rev, 11(11):806–14.

Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C, De Maria R 2008. “Identification and Expansion of the Tumorigenic Lung Cancer Stem Cell Population.” Cell Death Differ, 15(3):504–14.

Falconer H, Sundqvist J, Gemzell-Danielsson K, von Schoultz B, D'Hooghe TM,Fried G. 2009. “IVF Outcome in Women with Endometriosis in Relation to Tumour Necrosis Factor and Anti-Mullerian Hormone.” Reprod Biomed Online 18(4):582–88.

Fambrini M, Sorbi F, Bussani C, Cioni R, Sisti G, Andersson KL. 2013. “Hypermethylation of HOXA10 Gene in Mid-Luteal Endometrium from Women with Ovarian Endometriomas.” Acta Obstet Gynecol Scand, 92(11):1331–34.

Filippi I, Carrarelli P, Luisi S, Batteux F, Chapron C, Naldini A, Petraglia F. 2015. “Different Expression of Hypoxic and Angiogenic Factors in Human Endometriotic Lesions.” Reprod Sci, Apr;23(4):492-7.

Friedl P and Gilmour D. 2009. “Collective Cell Migration in Morphogenesis, Regeneration and Cancer.” Nat Rev Mol Cell Biol, 10(7):445–57.

Gadducci A, Lanfredini N, Tana R. 2014. “Novel Insights on the Malignant Transformation of Endometriosis into Ovarian Carcinoma.” Gynecol Endocrinol, 30(9):612–17.

Gargett CE. 2007. “Uterine Stem Cells: What Is the Evidence?” Hum Reprod Update 13(1):87–101.

Gargett CE. 2006. “Identification and Characterisation of Human Endometrial Stem/progenitor Cells.” Aust N Z J Obstet Gynaecol, 46(3):250–53.

Gargett CE, Schwab KE, Brosens JJ, Puttemans P, Benagiano G, Brosens I. 2014. “Potential Role of Endometrial Stem/progenitor Cells in the Pathogenesis of Early-Onset Endometriosis.” Mol Hum Reprod, 20(7):591–98.

Gargett CE and Masuda H. 2010. “Adult Stem Cells in the Endometrium.” Mol Hum Reprod, 16(11):818–34.

Gargett CE., Kjiana E. Schwab KE, Deane JA. 2016. “Endometrial Stem / Progenitor Cells : The First 10 Years.” Hum Reprod Update, Mar;22(2):137-63.

Garrido N, Pellicer A, Remohí J, Simón C. 2003. "Uterine and ovarian function in endometriosis" Semin Reprod Med, May;21(2):183-92

Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G 2007. “ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome.” Cell stem cell, 1(5):555–67.

Giudice LC and Kao LC. 2004. “Endometriosis.” Lancet, Nov 13-19;364(9447):1789-99.

Giudice LC. 2010. “Endometriosis.” N Engl J Med, Jun 24;362(25):2389-98.

Goel HL and Mercurio AM. 2013. “VEGF Targets the Tumour Cell.” Nat Rev Cancer, 13(12):871–82.

Goud PT, Goud AP, Joshi N, Puscheck E, Diamond MP, Abu-Soud HM. “Dynamics of Nitric Oxide, Altered Follicular Microenvironment, and Oocyte Quality in Women with Endometriosis.” Fertil Steril, 102(1):151–59.

Gumbiner BM. 2005. “Regulation of Cadherin-Mediated Adhesion in Morphogenesis.” Nat Rev Mol Cell Biol, 6(8):622–34.

Guo SW. 2009. “Epigenetics of Endometriosis.” Mol Hum Reprod, 15(10):587–607.

Gupta S, Goldberg JM, Aziz N, Goldberg E, Krajcir N, Agarwal A. 2008. “Pathogenic Mechanisms in Endometriosis-Associated Infertility.” Fertil Steril, 90(2):247–57.

Gupta S, Agarwal A, Krajcir N, Alvarez J. 2006. “Role of Oxidative Stress in Endometriosis.” Reprod Biomed Online, 13(1):126–34.

Götte M(1), Wolf M, Staebler A, Buchweitz O, Kelsch R, Schüring AN, Kiesel L. 2008. "Increased expression of the adult stem cell marker Musashi-1 in endometriosis and endometrial carcinoma". J Pathol, Jul;215(3):317-29.

Hadfield RM., Mardon HJ, Barlow DH, Kennedy SH. 1997. “Endometriosis in Monozygotic Twins.” Fertill Steril, 68(5):941–42.

Hapangama DK, Turner MA, Drury J, Heathcote L, Afshar Y, Mavrogianis PA, Fazleabas AT. Aberrant expression of regulators of cell-fate found in eutopic endometrium is found in matched ectopic endometrium among women and in a baboon model of endometriosis. Hum Reprod. 2010 Nov;25(11):2840-50.

Hapangama DK, Raju RS, Valentijn AJ, Barraclough D, Hart A, Turner MA, Platt-Higgins A, Barraclough R, Rudland PS. Aberrant expression of metastasis-inducing proteins in ectopic and matched eutopic endometrium of women with endometriosis: implications for the pathogenesis of endometriosis. Hum Reprod. 2012 Feb;27(2):394-407.

Harris HR, Costenbader KH, Mu F, Kvaskoff M, Malspeis S, Karlson EW, Missmer SA. 2015. “Endometriosis and the Risks of Systemic Lupus Erythematosus and Rheumatoid Arthritis in the Nurses ’ Health Study II.” Ann Rheum Dis. Aug 3:207704.

Heidemann LN., Hartwell D, Heidemann CH, Jochumsen KM. 2014. “The Relation between Endometriosis and Ovarian Cancer - A Review.” Acta Obstet Gynecol Scand 93(1):20–31.

Herreros-Villanueva M(1), Zhang JS, Koenig A, Abel EV, Smyrk TC, Bamlet WR, de Narvajas AA, Gomez TS, Simeone DM, Bujanda L, Billadeau DD. 2013. “SOX2 Promotes Dedifferentiation and Imparts Stem Cell-like Features to Pancreatic Cancer Cells.” Oncogenesis, Aug 5;2:e61.

Holoch KJ and Lessey BA. 2010. "Endometriosis and Infertility". Clin Obstet Gynecol, Jun;53(2):429-38.

Huvila J, Brandt A, Rojas CR, Pasanen S, Talve L, Hirsimäki P, Fey V, Kytömäki L, Saukko P, Carpén O, Soini JT, Grénman S, Auranen A. 2009. “Gene Expression Profiling of Endometrial Adenocarcinomas Reveals Increased Apolipoprotein E Expression in Poorly Differentiated Tumors.” Int J Gynecol Cancer, 19(7):1226–31.

Jess T, Frisch M, Jørgensen KT, Pedersen BV, Nielsen NM. 2012. “Increased Risk of Inflammatory Bowel Disease in Women with Endometriosis: A Nationwide Danish Cohort Study.” Gut 61(9):1279–83.

Kao AP, Wang KH, Chang CC, Lee JN, Long CY, Chen HS, Tsai CF, Hsieh TH, Tsai EM 2011. “Comparative Study of Human Eutopic and Ectopic Endometrial Mesenchymal Stem Cells and the Development of an in Vivo Endometriotic Invasion Model.” Fertil Steril, 95(4):1308–15.

Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, Giudice LC 2003. “Expression Profiling of Endometrium from Women with Endometriosis Reveals Candidate Genes for Disease. Based Implantation Failure and Infertility.” Endocrinology 144(7):2870–81.

Kato N, Sasou S, Motoyama T 2006. “Expression of Hepatocyte Nuclear Factor-1beta (HNF-1beta) in Clear Cell Tumors and Endometriosis of the Ovary.” Mod Pathol, 19(1):83–89.

Kim KH, Choi JS, Kim JM, Choi YL, Shin YK, Lee HC, Seong IO, Kim BK, Chae SW, Kim SH 2009. “Enhanced CD24 Expression in Endometrial Carcinoma and Its Expression Pattern in Normal and Hyperplastic Endometrium.” Histol Histopathol, 24(3):309–16.

Kobayashi H(1), Sumimoto K, Kitanaka T, Yamada Y, Sado T, Sakata M, Yoshida S, Kawaguchi R, Kanayama S, Shigetomi H, Haruta S, Tsuji Y, Ueda S, Terao T. 2008. “Ovarian Endometrioma — Risks Factors of Ovarian Cancer Development.” Eur J Obstet Gynecol Reprod Biol, 138:187–93.

Koike N, Tsunemi T, Uekuri C, Akasaka J, Ito F, Shigemitsu A, Kobayashi H 2013. “Pathogenesis and Malignant Transformation of Adenomyosis (Review).” Oncol Rep, (7):861–67.

Kok VC, Tsai HJ, Su CF, Lee CK. 2015. “The Risks for Ovarian, Endometrial, Breast, Colorectal, and Other Cancers in Women With Newly Diagnosed Endometriosis or Adenomyosis.” Int J Gynecol Cancer, 25(6):968–76.

Koshiba H, Hosokawa K, Kubo A, Tokumitsu N, Watanabe A, Honjo H.2009. “Junctional Adhesion Molecule [corrected] expression in Human Endometrial Carcinoma..” Int J Gynecol Cancer, 19(2):208–13.

Kryczek I, Liu S, Roh M, Vatan L, Szeliga W, Wei S, Banerjee M, Mao Y, Kotarski J, Wicha MS, Liu R, Zou W2012. “Expression of Aldehyde Dehydrogenase and CD133 Defines Ovarian Cancer Stem Cells.” Int J Cancer, 130(1):29–39.

Kusumbe AP, Mali AM, Bapat SA. 2009. “CD133-Expressing Stem Cells Associated with Ovarian Metastases Establish an Endothelial Hierarchy and Contribute to Tumor Vasculature.” Stem cells, 27(3):498–508.

Kvaskoff M, Mu F, Terry KL, Harris HR, Poole EM, Farland L, Missmer SA. "Endometriosis: A High-Risk Population for Major Chronic Diseases?" Hum Reprod Update, 21(4):500–516.

Kvaskoff M, Mesrine S, Fournier A, Boutron-Ruault MC, Clavel-Chapelon F. 2007. "Personal History of Endometriosis and Risk of Cutaneous Melanoma in a Large Prospective Cohort of French Women." Arch Intern Med, 167(19):2061–65.

Kyama CM, Debrock S, Mwenda JM, D'Hooghe TM. 2003. "Potential Involvement of the Immune System in the Development of Endometriosis." Reprod Biol Endocrinol, Dec 2;1:123.

Lalitkumar S, Boggavarapu NR, Menezes J, Dimitriadis E, Zhang JG, Nicola NA, Gemzell-Danielsson K, Lalitkumar LP. "Polyethylene Glycated Leukemia Inhibitory Factor Antagonist Inhibits Human Blastocyst Implantation and Triggers Apoptosis by down-Regulating Embryonic AKT." Fertil Steril, 100(4):1160–69.

Lamouille S, Xu J, Derynck R. 2014. "Molecular Mechanisms of Epithelial-Mesenchymal Transition." Nat Rev Mol Cell Biol, 15(3):178–96.

Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994 Feb17;367(6464):645-8.

Lebovic DI, Bentzien F, Chao VA, Garrett EN, Meng YG, Taylor RN 2000. "Induction of an Angiogenic Phenotype in Endometriotic Stromal Cell Cultures by Interleukin-1beta." Mol Hum Reprod, 6(3):269–75.

Leone Roberti Maggiore U, Ferrero S, Mangili G, Bergamini A, Inversetti A, Giorgione V, Viganò P, Candiani M. 2016. "A Systematic Review on Endometriosis during Pregnancy : Diagnosis , Misdiagnosis , Complications and Outcomes." Hum Reprod Update, 22(1):70–103.

Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. 1989. "Vascular Endothelial Growth Factor Is a Secreted Angiogenic Mitogen." Science, 246(4935):1306–9.

Lessey BA, Lebovic DI, Taylor RN. 2013 "Eutopic endometrium in women with endometriosis: ground zero for the study of implantation defects". Semin Reprod Med, Mar;31(2):109-24.

Lessey BA, Young SL. 2014. "Homeostasis imbalance in the endometrium of women with implantation defects: the role of estrogen and progesterone". Semin Reprod Med, Sep;32(5):365-75.

Leyendecker G, Herbertz M, Kunz G, Mall G. 2002. "Endometriosis Results from the Dislocation of Basal Endometrium." Hum Reprod, 17(10):2725–36.

Liakakos T,Thomakos N, Fine PM, Dervenis C, Young RL. 2001. " Peritoneal adhesions: etiology, pathophysiology, and clinical significance. Recent advances in prevention and management" Dig Surg, 18(4):260-73.

Liang D, Ma Y, Liu J, Trope CG, Holm R, Nesland JM, Suo Z. 2012. "The Hypoxic Microenvironment Upgrades Stem-like Properties of Ovarian Cancer Cells." BMC cancer, 12(1):201.

Liu T, Cheng W, Lai D, Huang Y, Guo L 2010. "Characterization of Primary Ovarian Cancer Cells in Different Culture Systems." Oncol Rep, May;23(5):1277-84.

Lleo A, Invernizzi P, Gao B, Podda M, Gershwin ME. 2010. "Definition of Human Autoimmunity - Autoantibodies versus Autoimmune Disease." Autoimmun Rev, 9(5):A259–66.

Lu Z, Zhang W, Jiang S, Zou J, Li Y. 2014. "Effect of Oxygen Tensions on the Proliferation and Angiogenesis of Endometriosis Heterograft in Severe Combined Immunodeficiency Mice." Fertil Steril, 101(2):568–76.

Lucidi RS, Witz CA, Chrisco M, Binkley PA, Shain SA, Schenken RS. 2005. "A Novel in Vitro Model of the Early Endometriotic Lesion Demonstrates That Attachment of Endometrial Cells to Mesothelial Cells Is Dependent on the Source of Endometrial Cells." Fertil Steril, 84(1):16–21.

Ma I, Allan AL. 2011. "The Role of Human Aldehyde Dehydrogenase in Normal and Cancer Stem Cells." Stem cell Rev, 7(2):292–306.

Macer ML and Taylor HS. 2012. "Endometriosis and Infertility. A Review of the Pathogenesis and Treatment of Endometriosis-Associated Infertility." Obstet Gynecol Clin of North Am, 39(4):535–49.

Matsuura K, Ohtake H, Katabuchi H, Okamura H. 1999. "Coelomic Metaplasia Theory of Endometriosis : Evidence from in Vivo Studies and an in Vitro Experimental." Gynecol Obstet Invest, 47(suppl 1):18–22.

McConechy MK, Ding J, Senz J, Yang W, Melnyk N, Tone AA, Prentice LM, Wiegand KC, McAlpine JN, Shah SP, Lee CH, Goodfellow PJ, Gilks CB, Huntsman DG. 2014. "Ovarian and Endometrial Endometrioid Carcinomas Have Distinct CTNNB1 and PTEN Mutation Profiles." Mod Pathol, 27(1):128–34.

Melin A, Sparen P, Bergqvist A. 2007. "The Risk of Cancer and the Role of Parity among Women with Endometriosis." Hum Reprod, 22(11):3021–26.

Melin A, Sparen P, Persson I, Bergqvist A. 2006. "Endometriosis and the Risk of Cancer with Special Emphasis on Ovarian Cancer." Hum Reprod, 21(5):1237–42.

Meuleman C, Vandenabeele B, Fieuws S, Spiessens C, Timmerman D, D'Hooghe T. 2009. "High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners". Fertil Steril, Jul;92(1):68-74

Mirantes C, Espinosa I, Ferrer I, Dolcet X, Prat J, Matias-Guiu X. 2013. "Epithelial-to-Mesenchymal Transition and Stem Cells in Endometrial Cancer." Hum Pathol, 44(10):1973–81.

Moradi M, Parker M, Sneddon A, Lopez V, Ellwood D. 2014. "Impact of Endometriosis on Women's Lives: A Qualitative Study." BMC Women's Health, 14(1):123.

Nair AS, Nair HB, Lucidi RS, Kirchner AJ, Schenken RS, Tekmal RR, Witz CA. 2008. "Modeling the Early Endometriotic Lesion: Mesothelium-Endometrial Cell Co-Culture Increases Endometrial Invasion and Alters Mesothelial and Endometrial Gene Transcription." Fertil Steril, 90(4):1487–95.

Nasu K, Kawano Y, Kai K, Aoyagi Y, Abe W, Okamoto M, Narahara H. 2014 "Aberrant histone modification in endometriosis". Front Biosci, Jun 1;19:1202-14.

Naqvi H, Ilagan Y, Krikun G, Taylor HS. 2014. "Altered Genome-Wide Methylation in Endometriosis." Reprod Sciences, 21(10):1237–43.

Nezhat F, Cohen C, Rahaman J, Gretz H, Cole P, Kalir T. 2002. "Comparative Immunohistochemical Studies of Bcl-2 and p53 Proteins in Benign and Malignant Ovarian Endometriotic Cysts." Cancer, 94(11):2935–40.

Nielsen NM, Jørgensen KT, Pedersen BV, Rostgaard K, Frisch M. 2011. "The Co-Occurrence of Endometriosis with Multiple Sclerosis, Systemic Lupus Erythematosus and Sjogren Syndrome." Hum Reprod, 26(6):1555–59.

Nnoaham KE, Hummelshoj L, Webster P. 2011. "Impact of Endometriosis on Quality of Life and Work Productivity : A Multicenter Study across Ten Countries." Fertil Steril, 96(2):366–73.

Nothnick WB. 2001. "Treating Endometriosis as an Autoimmune Disease." Fertil Steril, 76(2):223–31.

Nyholt DR, Low SK, Anderson CA, Painter JN, Uno S, Morris AP, MacGregor S, Gordon SD, Henders AK, Martin NG, Attia J, Holliday EG, McEvoy M, Scott RJ, Kennedy SH, Treloar SA, Missmer SA, Adachi S, Tanaka K, Nakamura Y, Zondervan KT, Zembutsu H, Montgomery GW 2012. "Genome-Wide Association Meta-Analysis Identifies New Endometriosis Risk Loci." Nat Genet, 44(12):1355–59.

Noyes RW, Hertig AT, Rock J. 1975. Dating the endometrial biopsy. Am J Obstet Gynecol, May;122(2):262-3.

O'Brien CA, Pollett A, Gallinger S, Dick JE. 2007. “A Human Colon Cancer Cell Capable of Initiating Tumour Growth in Immunodeficient Mice.” Nature, 445(7123):106–10.

Painter JN, Anderson CA, Nyholt DR, Macgregor S, Lin J, Lee SH, Lambert A, Zhao ZZ, Roseman F, Guo Q, Gordon SD, Wallace L, Henders AK, Visscher PM, Kraft P, Martin NG, Morris AP, Treloar SA, Kennedy SH, Missmer SA, Montgomery GW, Zondervan KT 2011. “Genome-Wide Association Study Identifies a Locus at 7p15.2 Associated with Endometriosis.” Nat Genet, 43(1):51–54.

Pardal R, Clarke MF, Morrison SJ. 2003. “Applying the Principles of Stem-Cell Biology to Cancer.” Nat Rev Cancer, 3(12):895–902.

Pearce CL, Templeman C, Rossing MA, Lee A, Near AM, Webb PM, Nagle CM, Doherty JA, Cushing-Haugen KL, Wicklund KG, Chang-Claude J, Hein R, Lurie G, Wilkens LR, Carney ME, Goodman MT, Moysich K, Kjaer SK, Hogdall E, Jensen A, Goode EL, Fridley BL, Larson MC, Schildkraut JM, Palmieri RT, Cramer DW, Terry KL, Vitonis AF, Titus LJ, Ziogas A, Brewster W, Anton-Culver H, Gentry-Maharaj A, Ramus SJ, Anderson AR, Brueggmann D, Fasching PA, Gayther SA, Huntsman DG, Menon U, Ness RB, Pike MC, Risch H, Wu AH, Berchuck A; Ovarian Cancer Association Consortium. 2012. “Association between Endometriosis and Risk of Histological Subtypes of Ovarian Cancer: A Pooled Analysis of Case-Control Studies.” Lancet Oncol, 13(4):385–94.

Peng S, Maihle NJ, Huang Y. 2010. “Pluripotency Factors Lin28 and Oct4 Identify a Sub-Population of Stem Cell-like Cells in Ovarian Cancer.” Oncogene, 29(14):2153–59.

Plaks V, Kong N, Werb Z. 2015. “The Cancer Stem Cell Niche: How Essential Is the Niche in Regulating Stemness of Tumor Cells?” Cell Stem Cell, 16(3):225–38.

Podzielinski I, Saunders BA, Kimbler KD, Branscum AJ, Fung ET, DePriest PD, van Nagell JR, Ueland FR, Baron AT. 2013. “Apolipoprotein Concentrations Are Elevated in Malignant Ovarian Cyst Fluids Suggesting That Lipoprotein Metabolism Is Dysregulated in Epithelial Ovarian Cancer.” Cancer Invest, 31(4):258–72.

Pollacco J, Sacco K, Portelli M, Schembri-Wismayer P, Calleja-Agius J. 2012. “Molecular Links between Endometriosis and Cancer.” Gynecol Endocrinol, 28(8):577–81.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Rahmioglu N, Missmer SA, Montgomery GW, Zondervan KT. 2012. “Insights into Assessing the Genetics of Endometriosis.” Curr Obstet Gynecol Rep, 1(3):124–37.

Randall GW, Gantt PA, Poe-Zeigler RL, Bergmann CA, Noel ME, Strawbridge WR, Richardson-Cox B, Hereford JR, Reiff RH. 2007. “Serum Antiendometrial Antibodies and Diagnosis of Endometriosis.” Am J Reprod Immunol 58(4):374–82.

Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R.. 2007. “Identification and Expansion of Human Colon-Cancer-Initiating Cells.” Nature, 445(7123):111–15.

Rizzino A. 2009. “Sox2 and Oct-3 / 4 : A Versatile Pair of Master Regulators That Orchestrate the Self-Renewal and Pluripotency of Embryonic Stem Cells.” Wiley Interdiscip Rev Syst Biol Med, Sep-Oct;1(2):228-36.

Saintz de la Cuesta R, Izquierdo M, Cañamero M, Granizo JJ, Manzarbeitia F. "Increased prevalence of p53 overexpression from typical endometriosis to atypical endometriosis and ovarian cancer associated with endometriosis". Eur J Obstet Gynecol Reprod Biol, Mar 15;113(1):87-93

Sampson, JA. 1927. “Peritoneal Endometriosis due to the Menstrual Dissemination of Endometrial Tissue into the Peritoneal Cavity.” Am J Obstet Gynecol, 14:422–69.

Sapkota Y, Fassbender A, Bowdler L, Fung JN, Peterse D, O D, Montgomery GW, Nyholt DR, D'Hooghe TM. 2015. “Independent Replication and Meta-Analysis for Endometriosis Risk Loci.” Twin Res Hum Genet, 18(05):518–25.

Sasson IE and Taylor HS. 2008. “Stem Cells and the Pathogenesis of Endometriosis.” Ann N Y Acad Sci, 1127:106–15.

Sato N, Tsunoda H, Nishida M, Morishita Y, Takimoto Y, Kubo T, Noguchi M. 2000. “Loss of Heterozygosity on 10q23 . 3 and Mutation of the Tumor Suppressor Gene PTEN in Benign Endometrial Cyst of the Ovary : Possible Sequence Progression from Benign Endometrial Cyst to Endometrioid Carcinoma and Clear Cell Carcinoma of the Ovary Loss of.” Cancer Res, Dec 15;60(24):7052-6.

Schmittgen TD and Livak KJ. 2008. “Analyzing Real-Time PCR Data by the Comparative CT Method.” Nature Protoc, 3(6):1101–8.

Seshadri S and Sunkara SK. 2014. “Natural Killer Cells in Female Infertility and Recurrent Miscarriage: A Systematic Review and Meta-Analysis.” Hum Reprod Update, 20(3):429–38.

Shah MM, Zerlin M, Li BY, Herzog TJ, Kitajewski JK, Wright JD. 2013. “The Role of Notch and Gamma-Secretase Inhibition in an Ovarian Cancer Model.” Anticancer Res, 33(3):801–8.

Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, Brodszky V, Canis M, Colombo GL, DeLeire T, Falcone T, Graham B, Halis G, Horne A, Kanj O, Kjer JJ, Kristensen J, Lebovic D, Mueller M, Vigano P, Wullschleger M, D'Hooghe T. 2012. “The Burden of Endometriosis: Costs and Quality of Life of Women with Endometriosis and Treated in Referral Centres.” Hum Reprod, 27(5):1292–99.

Simpson JL and Bischoff FZ. 2002. “Heritability and Molecular Genetic Studies of Endometriosis.” Ann N Y Acad Sci, 955(1):239–51; discussion 293–95, 396–406.

Sinaii N, Cleary SD, Ballweg ML, Nieman LK, Stratton P. 2002. “High Rates of Autoimmune and Endocrine Disorders, Fibromyalgia, Chronic Fatigue Syndrome and Atopic Diseases among Women with Endometriosis: A Survey Analysis.” Hum Reprod, 17(10):2715–24.

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB. 2003. “Identification of a Cancer Stem Cell in Human Brain Tumors" Cancer Res, Sep 15;63(18):5821-8.

Somigliana E, Benaglia L, Paffoni A, Busnelli A, Vigano P, Vercellini P. 2014. “Risks of Conservative Management in Women with Ovarian Endometriomas Undergoing IVF.” Hum Reprod Update, 21(4):486–99.

Sourial S, Tempest N, Hapangama DK. Theories on the pathogenesis of endometriosis. Int J Reprod Med. 2014;2014:179515.

Staal AH, van der Zanden M, Nap AW. 2016. “Diagnostic Delay of Endometriosis in the Netherlands.” Gynecol Obstet Invest, 8–11.

Stephansson O, Falconer H, Ludvigsson JF. 2011. “Risk of Endometriosis in 11 000 Women with Celiac Disease.” Hum Reprod, Oct;26(10):2896-901.

Taylor H, Olive D, Arici A. 1999. “HOXA10 Gene Expression Is Altered in the Endometrium of Patients with Endometriosis.” Hum Reprod, May;14(5):1328-31.

Therneau TM and Ballman K. "What Does PLIER Really Do?" Cancer Inform, 2008; 6: 423–431.

Thiruchelvam U, Wingfield M, O’Farrelly C. 2015. “Natural Killer Cells: Key Players in Endometriosis.” Am J Reprod Immunol, Oct;74(4):291-301.

Uno S, Zembutsu H, Hirasawa A, Takahashi A, Kubo M, Akahane T, Aoki D, Kamatani N, Hirata K, Nakamura Y. 2010. “A Genome-Wide Association Study Identifies Genetic Variants in the CDKN2BAS Locus Associated with Endometriosis in Japanese.” Nat Genet 42(8):707–10.

Wang ML, Chiou SH, Wu CW. 2013. “Targeting Cancer Stem Cells: Emerging Role of Nanog Transcription Factor.” Onco Targets Ther, Sep 4;6:1207-20.

Weijenborg PT, Greeven A, Dekker FW, Peters AA, Ter Kuile MM. 2007. “Clinical Course of Chronic Pelvic Pain in Women.” Pain, Nov; 132 Suppl :S117–23.

Wu Y, Halverson G, Basir Z, Strawn E, Yan P, Guo SW 2005. “Aberrant Methylation at HOXA10 May Be Responsible for Its Aberrant Expression in the Endometrium of Patients with Endometriosis.” Am J of Obstet Gynecol, 193(2):371–80.

Wu Y, Strawn E, Basir Z, Halverson G, Guo SW. 2007. “Aberrant Expression of Deoxyribonucleic Acid Methyltransferases DNMT1, DNMT3A, and DNMT3B in Women with Endometriosis.” Fertil Steril, 87(1):24–32.

Yang ZF, Ho DW, Ng MN, Lau CK, Yu WC, Ngai P, Chu PW, Lam CT, Poon RT, Fan ST. 2008. “Significance of CD90+ Cancer Stem Cells in Human Liver Cancer.” Cancer Cell 13(2):153–66.

Ye J, Wu D, Wu P, Chen Z Huang J. 2014. “The Cancer Stem Cell Niche: Cross Talk between Cancer Stem Cells and Their Microenvironment.” Tumour Biol, 35(5):3945–51.

Zafrakas M, Grimbizis G, Timologou A, Tarlatzis BC. 2014. “Endometriosis and Ovarian Cancer Risk: A Systematic Review of Epidemiological Studies.” Front Surg, 1(May):14.

Zohn IE, Li Y, Skolnik EY, Anderson KV, Han J, Niswander L. 2006. “p38 and a p38-Interacting Protein Are Critical for Downregulation of E-Cadherin during Mouse Gastrulation.” Cell, 125(5):957–69.

Zucchetto A, Serraino D, Polesel J, Negri E, De Paoli A, Dal Maso L, Montella M, La Vecchia C, Franceschi S, Talamini R. 2009. Hormone-related factors and gynecological conditions in relation to endometrial cancer risk. Eur J Cancer Prev, Aug;18(4):316-2.