Circulating microRNAs in endometriosis

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ABSTRACT

Endometriosis is defined as the presence of endometrial tissue outside the uterus and is the leading cause of disability in reproductive-age women. The pathogenesis of endometriosis remains unclear and no blood test is available for early diagnosis of the disease. MicroRNAs (miRNAs) are endogenous small ribonucleic acids (RNAs) that have important gene-regulatory roles via posttranscriptional suppression of target genes. The biological importance of miRNAs, initially demonstrated in cancer, has more recently been confirmed in other diseases. In light of the sustained presence of miRNAs in the circulation and given the emerging evidence on aberrant miRNA expression in endometriotic tissue, we hypothesized that endometriosis is associated with unique plasma miRNA signatures that have diagnostic potential and possibly contribute to disease pathogenesis.

In order to test this hypothesis, we established a procedure to measure plasma miRNAs and created a tissue bank of prospectively collected blood and eutopic endometrium samples. Next, by using qRT-PCR-based arrays we screened the plasma of a small set of women (n = 16) with or without endometriosis for miRNA content at 3 different phases of the menstrual cycle. We demonstrated that plasma miRNAs do not fluctuate across the cycle and identified 12 plasma miRNAs that are differentially abundant in endometriosis. In silico functional analyses revealed that these miRNAs and their predicted targets have functional relevance in endometriosis, being involved in molecular pathways known to be associated with the disease.

Using a microarray methodology, we profiled miRNAs in eutopic endometrium from women with and without endometriosis. We demonstrated no correlation between dysregulated miRNAs in endometrium and plasma, suggesting that the differentially abundant circulating miRNAs are not released from the endometrium. Mir-551a and mir-148a* were significantly dysregulated in the endometrium from women with endometriosis, and thus are putative diagnostic markers and therapeutic targets. We also identified differences in miRNA expression between endometriosis-free women with and without pelvic pain, suggesting that pelvic pain might independently modify the endometrial miRNA profile.

Finally, we assessed the value of plasma miRNAs as molecular markers for endometriosis in a prospective diagnostic study in a larger cohort (n = 68) of symptomatic women by using singleplex qRT-PCR. The diagnostic accuracy of circulating miRNAs for patients with

endometriosis was assessed with a predictive algorithm incorporating miRNA expression levels and clinical parameters. A model that included mir-155, mir-574-3p, mir-133a and mir-30c, history of infertility and previous miscarriages demonstrated an accuracy of 84.1% with 93.5% sensitivity, 58.8% specificity and AUC = 0.831.

This thesis presents potential novel biomarkers for early detection of endometriosis, laying the ground work for future efforts to develop blood-based biomarkers for this disease. An accurate non-invasive test would reduce the need for a surgical diagnosis, would be more accessible to women, and is likely to lead to an earlier diagnosis and treatment of endometriosis. Our results need to be confirmed in larger independent patient groups in different populations. In addition, this work raises the possibility that plasma miRNAs may provoke some of the adverse health epiphenomenon associated with endometriosis, which potentially could be altered by therapeutic manipulations of endometriosis-associated plasma miRNAs. Future studies and broader miRNA profiling may elucidate a relationship between miRNAs, endometriotic disease and its severity.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma at any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australian Digital Thesis Program and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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PUBLICATIONS ARISING FROM THIS THESIS

Non-invasive tests for the diagnosis of endometriosis. Protocol for the Cochrane review.

Nisenblat V, Farquhar C, Akoum A, Fraser I, Bossuyt PMM, Hull ML.

The Cochrane library, January 2012

Plasma miRNA profiles in women with and without endometriosis – additional considerations towards development of a miRNA-based blood test.

Nisenblat V, Print C, Evans S, Ohlsson-Teague EMC, Robertson S, Hull ML.

Submitted for review, Human Reproduction, January 2013.

Role of miRNAs in endometrial disease in association with reproductive disorders and prospects for circulating miRNAs in diagnosis of endometrial function.

Nisenblat V, Hull ML.

RBM Online, in press 2013.

COMMERCIAL POTENTIAL ARISING FROM THIS THESIS

Patent

Method for identifying endometriosis.

Key contributors: Nisenblat V, Print C, Robertson SA, Ohlsson Teague EMC, Hull ML.

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Funding for further work

This thesis served a basis for successful grant application for further work to develop a validated prototype blood test for diagnosis of endometriosis.

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ABSTRACTS ARISING FROM THIS THESIS

Menstrual cycle variations in plasma microRNA expression profiles

Ohlsson Teague EMC, Nisenblat V, Robertson SA, Hull ML

Poster presentation. Presented at SRB conference on 23-26.08.2009, Adelaide, Australia.

A unique plasma microRNA expression profile is identified in women with endometriosis

Nisenblat V, Print CG, Evans S, Ohlsson Teague EMC, Robertson SA, Hull ML.

Poster presentation. Presented at the 11th World Congress of Endometriosis on 4-7.09.2011, Montpelier, France.

Plasma miRNAs as non-invasive biomarkers for endometriosis

Nisenblat V, Robertson SA, Evans SF, Hull ML.

Poster presentation. Presented at the ASRM annual meeting on 22-26.10.2012, San Diego, USA.

Circulating microRNAs as potential biomarkers for endometriosis.

Nisenblat V, Wang Z, Robertson SA, Evans SF, Hull ML.

Oral presentation. Presented at Fertility Society of Australia (FSA) conference 28-31.10.12, Auckland, NZ.

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"…I'm just a normal woman except that I have a horrifying disease called endometriosis. Every month my cycles seem to get worse, making work difficult. I don't enjoy calling in sick and being perceived as being unreliable. I suffer from migraines daily, along with fatigue and pelvis/ back pains … and I never feel well. I feel like its one thing after another and I'm tired of seeing so many doctors. I'm taking 8 pills a night and I'm only 19. When does it end!!! This has to be one of the most frustrating conditions in the world. Peoples reaction seems to be suck it up, it won't kill you be thankful for that. But what kind of life is this?" **Sarah**

"… It took me 3 years for a doctor to finally listen to me. I begged doctors to listen to my symptoms....they all assumed I just wanted the pain-relief pills. I hate depending on anything. I can't do the hormones anymore. I want my life back..." **Hannah**

"My family doesn't quite understand and I've lost almost all of my friends due to the fact that I can't stay out for longer than a couple of hours without feeling drained and ill. My boyfriend of 3 years thinks that I use it as an excuse to not be more active and it's starting to tear us apart. I don't know what to do and I feel completely alone and hopeless at this point. I've lost all hope in the chance that I'll become better ..." **Fiona**

"… The pain has made me jealous of so many things. Jealous of people who don't have pain, jealous of people who have been able to easily start families, jealous of people who have never known what it feels like to have to make the choices I do. Like should I go out tonight or stay home? Should I take these pain killers and feel better but damage my insides even more? Should I eat this now and feel like crap for days after? Sometimes I think jealousy is the worst side effect of this disorder". **Tracey**

(Quotations taken from women diagnosed with endometriosis, 2010-2012)

ABBREVIATIONS

3’-UTR	3’-untranslated regions
AE	amplification efficiency
Ago	argonaute proteins
AID	activation-induced cytidine deaminase
AML	acute myeloid leukaemia
ANOVA	analysis of variance
Anti -2HSG	2 Heremans-Schmidt glycoprotein
ANXA 1	annexin 1
AUC	area under the ROC curve
Bax	BCL2-associated X protein
BCL-2	B-cell lymphoma protein2
B-H	Benjamini-Hochberg method
Bic	B cell integration cluster gene
C. Elegans	Caenorhabditis elegans
Ca-125	cancer antigen 125
CASP	caspase, apoptosis related cysteine peptidase
CCND1	cyclin D1
CDC42	targets cell division cycle 42 protein
CDKN	cyclin-dependent kinase inhibitor
cDNA	complementary DNA
cEBP	CCAAT enhancer binding protein
CK1a	Cysteine kinase 1 alpha
CLL	chronic lymphocytic leukaemia
cMaf	musculoaponeurotic fibrosarcoma oncogene homolog
COL	collagen-matrix proteins
COX	cyclooxygenase
Cq	quantification cycle
CREBBP	CREB binding protein
Ct	cycles to threshold
CTNNB1	β-catenin
CV	coefficient of variance
CXCL	chemokine (C-X-C motif) ligand
CYP19A1	cytochrome P450, family 19, subfamily A, polypeptide
CYR61	cysteine-rich, heparin-binding protein
DNA	deoxyribonucleic acid
DNMT	DNA-(cytosine-5-methyltransferase
DUSP5	dual specificity phosphatase 5
E2	oestradiol
E2F8	E2F transcription factor 8
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ER	oestrogen receptor
ERBB	v-erb-erythroblastic leukaemia viral oncogene homolog
ERK	extracellular signal-regulated kinase
ESCs	endometrial stromal cells
FADD	Fas associated death domain protein
FC	fold change
FDR	false discovery rate
FGF	fibroblast growth factor
FOXO3	fork head box O3 gene

FSH	follicle-stimulating hormone
GBM	glioblastoma multiforme
GBS	glial cells missing binding site
GEO	gene expression omnibus
GM-CSF	granulocyte macrophage colony-stimulating factor
GNRH	gonadotropin-releasing hormone
HCC	hepatocellular carcinoma
HDL	high-density lipoprotein
HESF	human endometrial stromal fibroblasts
HGF	hepatocyte growth factor
HIF-1α	hypoxia inducible transcription factor -1 alpha
HLA	human leukocyte antigen
ICAM	intercellular adhesion molecule
IFN	interferon
IGF	insulin-like growth factor
IgG	immunoglobulin G
IKB	ingenuity knowledge base
IKKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta
IKKέ	IkB kinase epsilon
IL	interleukin
ILGF	insulin-like growth factor
INF	Interferon
IPA	ingenuity systems pathway analysis
IRS-1	insulin receptor substrate-1
JNK	c-Jun N-terminal kinase
KRAS	Kirsten ras oncogene homolog
LDL	low density lipoprotein
LH	luteinizing hormone
LIF	leukaemia inhibitory factor
LIMMA	linear models for microarray analysis
LNA	locked nucleic acid
LOWESS	locally weighted regression and smoothing scatterplots
LPS	Lipopolysaccharide
MAPK	mitogen-activated protein kinase
MAQC	microarray quality control
MCAP	monocyte chemoattractant protein
MCL1	myeloid leukaemia cell differentiation protein
MESDC1	mesoderm development candidate 1
MET- proto-oncogene	MNNG HOS Transforming gene
MGMT	methyl guanine methyl transferase
MIF	migration inhibitory factor
MIP	macrophage inflammatory protein
MIP	macrophage inflammatory protein
MIQE	minimum information for publication of qRT-PCR experiments
miRNA	micro RNA
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MSC	mesenchymal stem cells
MSH	melanocyte-stimulating hormone
MSP	miRNAs-specific reverse transcription primer
MTMMP	membrane type matrix metalloproteinase
MVB	multivesicular bodies

NFkB	nuclear factor kappaB
ng	nanograms
NK cells	natural killer cells
NP1	nucleoplasmin 1
NPM1	nucleophosmin
NRT	no reverse transcriptase
nSMase2	sphingomyelinase 2
NTC	no template controls
OD	optical density
P4	Progesterone
PAE	percentile amplification efficiency
PAGE	polyacrylamide gel electrophoresis
PCA	principal components analysis
PCOS	polycystic ovary syndrome
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PG	prostaglandin
pg	picograms
PGP 9.5	protein gene product 9.5
PP14	serum placental protein/ glycodelin A
PPARα	peroxisome proliferator-activated receptor, alpha
PR	progesterone receptor
pre-miRNA	precursor miRNA
pri-miRNA	primary miRNA
PRL-3	phosphatase regenerating liver-3 gene
PTEN	phosphatase and tensin homolog
qRT-PCR	quantitative reverse transcriptase PCR
QUADAS	quality assessment of diagnostic accuracy studies included in systematic reviews
r_s	Spearman correlation coefficient
rASRM	revised American Society of Reproductive Medicine classification
REST	RE1-silencing transcription factor
RHOA	Ras homolog gene family, member A
RIN	RNA integrity number
RIPK1	receptor interacting serine–threonine kinase1
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
ROC	receiver-operating characteristics
RQI	RNA quality index
RXRα	retinoid X receptor, alpha
SD	standard deviation
sEcadherin	soluble E-cadherin
SEM	standard error of mean
sEselectin	soluble E-selectin
SF	steroidogenetic factor
SHIP-1	Src homology 2-containing inositol phosphatase-1
sICAM	soluble intercellular adhesion molecules
SMAD	Sma- and Mad-related protein
SMRT	single-molecule real-time sequencing
SMS	single-molecule sequencing
SNR	signal-to-noise ratio
SOCS	suppression of suppressor of cytokine signalling
Sox17	sex determining region Y-related HMG box-17

SQSTM1	Sequestome1 gene
StAR	steroidogenic acute regulatory protein
STARD	standards for reporting of diagnostic accuracy
STAT	signal transducer and activator of transcription
sVCAM	soluble vascular cell adhesion molecule
TCF4	transcription factor 4
TCL1	T cell leukaemia/lymphoma 1
TDP-43	TAR-DNA-binding protein-43
TGF	transforming growth factor
Th	T helper cells
TIMP	tissue inhibitors of MMP
TLDA	TaqMan Low Density miRNA array
TLR	Toll-like receptor
TNF	tumour necrosis factor
TP-53	tumour protein p-53
TP-53INP1	TP-53-induced nuclear protein 1
TR4 [NR2C2]	nuclear receptor subfamily 2 group C member 2
TSP-1	thrombospondin 1 angiogenic protein
UPS	ubiquitin proteasome system
US	ultrasound
VEGF	vascular endothelial growth factor
VENTX	VENT homeobox
VIP	vasoactive intestinal peptide
VSN	variance stabilizing normalization
XIAP	X-linked inhibitor of apoptosis protein
ZFP36	zinc finger protein 36