Molecular Study on Stem Cell Differentiation into Endometrial Cell By Serum Endometriosis Inducing Factor

Thesis

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DEDICATION

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Hesham Mohamed Naguib Amer

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List of Abbreviations

AFS	American Fertility Society
AM	Amniotic membrane
AMV	Avian Myeloblastosis Virus
ASCs	Adult stem cells
ASCs	Adipose-derived stem cells
BM	Bone marrow
CA-125	Cancer antigen 125
cAMP	Cyclic adenosine monophosphate
cDNA	complementary Deoxyribonucleic acid
CO2	Carbon dioxide
COCPs	Combined oral contraceptive pills
CSCs	Cardiac stem cells
CT	Cycle threshold
ΔCT	Normalized cycle threshold
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
EIF	Endometriosis inducing factor

EpCAM	Epithelial Cell Adhesion Molecule
ESCs	Embryonic stem cells
ET	Embryo transfer
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GnRH	Gonadotropin-releasing hormone
HATs	Histone acetyltransferases
HESCs	Human embryonic stem cells
HSCs	Hematopoietic stem cells
HOXA5	Homeobox A5
ICM	Intra cellular mass
iPSCs	induced pluripotent stem cells
IVF	In vitro fertilization
Let 7	Lethal 7 gene
LUNA	Laparoscopic uterine nerve ablation
MAPCs	Multipotent adult progenitor cells
mRNA	messenger Ribonucleic acid
MRI	Magnetic resonance imaging
MSCs	Mesenchymal stem cells
MYC	Myelocytomatosis

NSAIDs	Non steroidal anti-inflammatory drugs
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
REC	Research Ethical Committee
RQ	Relative quantity
RNA	Ribonucleic acid
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase polymerase chain reaction
SCs	Stem cells
SCNT	Somatic cell nuclear transfer
SPARC	Secreted protein, acidic, cysteine-rich
TE	Tissue engineering
TGF-b	Transforming Growth Factor Beta
TVS	Transvaginal ultrasound
UCB	Umbilical cord blood
USA	United States of America
VEGF-A	Vascular endothelial growth factor A

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BACKGROUND

Endometriosis is one of the most frequent benign gynecological diseases characterized by the presence of endometrial glands and stroma outside the uterine cavity,most commonly implanted over visceral and peritoneal surfaces within the female pelvis ,but rarely also in the pericardium,pleura and even brain.It is usually associated with infertility,pelvic pain and dyspareunia.The incidence of the diseases is 7-10% of women world wide and 35-60% of women with pain and infertility**(Baldi et al., 2008)**.

Several theories have attempted to explain the development of such disease but the definitive cause is still unclear. The most commonly accepted mechanism for the development of peritoneal endometriotic lesions is the Sampson's theory claiming the adhesion and growth of endometrial fragments deposited into the peritoneal cavity via retrograde menstruation**(Signorile et al., 2009)**.This theory does not account for the existence of endometriosis in areas far removed from the pelvis; endometriosis is reported to occur in locations that do not communicate with the peritoneal cavity, such as the lung and brain, where retrograde menstruation cannot account for the presence of this tissue which suggests an alternative theory for etiology of endometriosis **(Du and Taylor,2007)**.

On the other hand, the coelomic metaplasia theory claims that formation of deep endometriosis is caused by metaplasia of the original coelomic membrane, perhaps induced by environmental factors. Other theories for the genesis of endometriosis include different mechanisms such as hematogenous metastasis, genetic predisposition or altered cellular immunity. Nevertheless, all these theories

remain speculative and no definitive evidences have been produced to demonstrate them. **(Signorile et al., 2009)**

Stem cells have the unique capacity not only to give rise to more stem cells (self-renewal) but also to generate differentiated progeny.They are present at all stages of development and probably exist in all multicellular organisms**(Weissman ,2002)**.

Although bone marrow (BM) has been the main source for the isolation of multipotent mesenchymal stem cells(MSCs), the harvest of BM is a highly invasive procedure and the number, differentiation potential, and maximal life span of MSCs from BM decline with increasing age. One alternative source is umbilical cord blood (UCB), which can be obtained without harm for the mother or the infant.**(Kern et al., 2006)**.UCB is obtained after full-term delivery of the newborn from a sample that would inevitably be discarded.**(Secco et al.,2007).**

The stem cell theory explains how endometriosis can be found remote from the peritoneal cavity, resists some treatments, and occasionally occurs even after hysterectomy. On this stem cell theory basis study has proved that an endometriosis inducing factor (EIF) does exit in the serum of women suffering from endometriosis by adding the serum of women with varying degrees of endometriosis to MSC for 4 weeks, the stem cells acquired endometrial morphology after cultivation. Whereas adding the serum of women without endometriosis could not lead to these morphological changes. Furthermore, the change of the stem cells status into endometrial like cells were confirmed by Annexin-1 expression.**(Rasheed et al.,2010)**.They postulated a triggering substance spark for that endometrotic transformation and they named this substance as "Endometriosis Inducing Factor, EIF ".

AIM OF THE WORK

To study the role of endometriosis inducing factor (EIF) on differentiation of cultured stem cells into endometrial cells using SPARC (secreted protein, acidic, cysteine-rich) and MYC (myelocytomatosis) genes expression .

PATIENTS AND METHODS

Study Design: This study is Case-Control Prospective study that acquired the approval of Bioethical Committee of the National Research Center.

Setting: The study will be carried out in collaboration between the Obstetrics and Gynecology Department, Ain Shams University and Reproductive Health Research Department, National Research Centre.

Patients: The study will be carried out on 130 infertile patients undergoing diagnostic laparoscopy for fertility work out. The study group will include 65 patients where laparoscopy shows endometriotic implants and the control group will include the other 65 patients where the diagnostic laparoscopy shows no endometriotic implants.

Inclusion criteria:

- Patients in the child bearing period.
- Infertile patients
- Patients diagnosed as having endometriosis by laparoscopy which is the gold standard for diagnosis

of endometriosis according to the American Fertility Society **(Johns Hopkins manual of Obstetrics & Gynecology,2007).**

Exclusion criteria:

- Patients with history of immunological diseases and also patients who received any type of hormonal therapy six months before the diagnostic laparoscopy.

PLAN OF WORK

- Blood samples will be collected from all patients (5ml on plane tube).
- Stem cells will be derived from human umbilical cord blood from full term deliveries after informed consent obtained from mothers according to the bio-ethical Committee of National Research center guidelines and Research Ethical Committee (REC) of Obstetrics & Gynecology Department, Ain Shams University.
- Stem cells will be co cultured with serum of women with endometriosis and also with serum of women without endometriosis then stem cells will be observed weekly for 10 weeks for possible differentiation into endometrial like cells using real time PCR for SPARC (Hs00277762_ml) and MYC (Hs00153408_ml) genes expression.
- Data will then be collected, tabulated and statistically analyzed.