行政院國家科學委員會專題研究計畫　成果報告

氧化性壓力對子宮內膜細胞生長之影響

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計畫主持人：高淑慧

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中文摘要

現代人的生活壓力逐年升高，根據資料顯示婦女罹患子宮內膜異位症的發生率愈來愈高，其發生率約佔婦女人口的 10%。此疾病常造成患者在生理上、心理上、生活上造成許多痛苦，成為相當令人困擾的問題。依據研究顯示，子宮內膜異位症常為女性不孕的主要族群，大約50%的不孕婦女患有此一疾病。所謂子宮內膜異位症是子宮內膜(endometrium)生長於子宮內腹腔以外的部位。此種異位性內膜上皮細胞具有可侵入性(invasion)及轉移性(metastasis)的特性。迄今，對於子宮異位性內膜上皮細胞侵入性及轉移性的機轉及成因尚未清楚。許多學者認為子宮內膜異位症與氧化壓力(oxidative stress)有關，在異位的組織受到發炎反應影響而有較高含量的氧化傷害(oxidative damage)，而使 oxidatively modified complexes 增加。此外，在免疫系統上，經由 cytokine 及 chemokine 活化 macrophage 也會造成發炎反應及增加氧化性傷害。在我們先前的研究證明在子宮內膜異位症患者的病灶處的檢體中，檢測出高含量的氧化性傷害物質(如 8-OH-dG 及脂質過氧化產物(lipoperoxide)及粒線體基因突變的堆積(mitochondrial DNA mutation)。經由實驗結果，我們推測氧化性傷害於子宮異位性內膜上皮細胞引發(initiation)及進展(progression)扮演重要的角色。本計畫中，我們將子宮內膜異位上皮細胞及基質細胞(stroma cell)加以分離，分別探討這兩者細胞於氧化性傷害(如 H_2O_2 and CCCP)處理後的分別變化。並將此兩中細胞共同培養及分離培養，此兩種細胞間的互相影響，並釐清其扮演的腳色。並且尋找可減低侵入性及轉移性的因子及治療方法。冀望經由對此疾病的治病機轉的了解以其對此疾病的診斷方法及治療有更進一步的進展。

關鍵字：子宮內膜異位症、侵入性、轉移性、共同培養、粒線體 DNA 突變、氧化性傷害

Abstract

Endometriosis, one of frequent diseases in gynecology, is a considerable threat to the physical, psychological and social integrity of women. More, up to 50% of infertile patients have this disease. The etiology and pathogenesis of this important disease is poorly understood, which is defined as the ectopic location of the endometrium-like glandular epithelium and stroma ouside the uterine cavity. Clinical observations and *in vitro* experiment imply that endometriotic cells are invasive and able to metastasize. To date, however, little is know about the mechanisms of invasion and metastasis in endometriosis. It still remains an open question as what extent the peritoneal environment influences the establishment and/or progression of endometriosis. As a result of such stress, a sterile, inflammatory reaction with the secretion of growth factors, cytokines, and chemokines is generated, which is deleterious especially to successful reproduction. In our preliminary data, the significantly higher amounts of oxidative damages were detected in endometriotic lesions than in controlled normal endometrium such as the mitochondrial DNA rearrangement, 8-OH-normal endometrium such as the mitochondrial DNA rearrangement, 8-OH- deoxyguanosine (8-OH-dG), and lipoperoxide contents (TBA reacted compounds). Our central hypothesis proposes that oxidative damages might be anticipated in the initiation or progression of endometriosis. In this study, we propose that such a pro-oxidant environment promotes growth of ectopic endometrium. In the future, in order to elucidate the oxidative stress promotes growth of ectopic endometrium, in the first, we must establish the primary culture of endometrial epithelial and stromal cell from eutopic endometrium, as co-culture model *in vitro*. In the study, we established the primary co-culture model of endometrial cells from the patients with endometriosis. Epithelial cells and stromal cells were separated and co-cultured. In order to clarify the cross talk between two types of cells, we traced on the regulation of cell growth by treating with oxidants. Only by understanding the mechanisms involved in the pathogenesis of endometriosis we can develop the basis for new diagnostic and therapeutic approaches.

Key words: Endometriosis, invasion, metastasis, mtDNA mutation, oxidative damage

研究計畫之背景及目的

子宮內膜異位症(Endometriosis)是一種具有細胞侵入性的良性婦科疾病。根據組織學上的特性，子宮內膜異位症被定義為是子宮內膜(endometrium)及內膜腺體和基質細胞生長於腹腔內子宮以外的部位。此種異位性內膜上皮細胞具有可侵入性(invasion)及轉移性(metastasis)的特性。根據資料顯示婦女罹患子宮內膜異位症的發生率愈來愈高，其發生率約佔婦女人口的15%-50%，並且影響婦女的生殖能力(1)。此種異位性內膜細胞與子宮內的內膜細胞相似，會隨著婦女生理週期而有週期性的增生及剝落，常造成異位處產生局部性的發炎反應。這些過程常引發患病婦女月經困難，性生活障礙(dyspareunia)，骨盆腔疼痛，月經性血尿(catamenial hematuria)，或其他受影響的器官所產生的症狀。此病症常為女性不孕的主要族群，約50%的不孕婦女患有此一疾病(2)。

迄今，對於子宮內膜異位症的病理形成機轉尚未清楚。由近年來的研究指出，月經溢流物中含有許多因子，而這些因子可以造成骨盆腔內間皮細胞(mesothelial cells)發生改變，產生了新的粘著處(adhesion site)，使異位的內膜細胞可以發生粘著及附著生長(3)。當黏附分子(adhesion molecules)(4)、matrix metalloproteinase(5)及plasminogen activator(6)過度表現時，可增加異位之內膜細胞的著附生長，並由異位細胞引發而造成局部細胞外間質(extracellular matrix)的破壞。當異位的子宮內膜細胞產生粘著後，於粘著處開始增生及逐漸地侵入粘著處附近組織如骨盆腔。有許多因子可以引發異位內膜細胞的著附生長處的血管形成，促使異位細胞可以繼續生長。這些因子如cytokines(7-9)及生長因子(growth factors(10)，如transforming growth factor、interleukin-8、interleukin-1、tumor necrosis factor、interferon-γ(11)以及 vascular growth factor(12))被認為與引發子宮內膜異位細胞的著附生長、細胞增生及血管新生成有關。此外，子宮內膜異位症中所引起的吞噬細胞(marcrophages)的選召(recruitment)及活化的增加，這被認為與此疾病的發展有重要相關性(13)。

子宮內膜異位症是屬於多因子性疾病並伴隨骨盆腔的發炎反應。許多學者認為子宮

內膜異位症與氧化壓力(oxidative stress)有關(14)。並且氧化壓力可以增加異位內膜細胞的著附生長，及引發的局部細胞外間質(extracellular matrix)的破壞。有許多證據顯示氧化壓力確實與子宮內膜異位症有關，並提出許多假說來解釋氧化壓力引發子宮內膜異位症形成(如圖一所示)。如氧化損壞的紅血球(15)、凋亡的子宮內膜細胞和未分解完成的內膜組織(16)常可以黏附於骨盆腔，並產生訊息使吞噬細胞的產生選召及活化。但是於罹患子宮內膜異位症的婦女常見到其對於這些訊息刺激無法產生適當的macrophage scavenger receptor反應。而這些骨盆腔內的活化吞噬細胞可以產生氧化壓力，常造成脂質過氧化物、崩解的氧化產物、氧化的low-density lipoprotein (17)、apoprotein (18)以及其他相互作用而成的蛋白質形成。另一方面,由對抗氧化壓力所形成的自體抗體(autoantibody)於罹患子宮內膜異位症的婦女中有顯著性增加的現象(19)。由此氧化壓力所形成的發炎及伴隨growth factors、cytokines、chemokines的產生常危害女性的生殖能力。此外，暴露於環境毒物 (如戴奧辛等)及重金屬的環境中亦會引發高濃度的氧化性自由基的產生，並且破壞氧化劑和抗氧化劑的平衡。而高濃度氧化性自由基的產生常與子宮內膜異位症的形成有關，進而降低受孕的能力。本研究中，我們推測氧化性傷害於子宮內膜上皮細胞異位性引發(initiation)及進展(progression)扮演重要的角色。由初步結果顯示，我們在子宮內膜異位症患者的病灶處的檢體中，檢測出高含量的氧化性傷害物質,如粒線體基因重組的堆積(mitochondrial DNA rearrangement)、8-OH-dG 及脂質過氧化產物(lipoperoxide, TBA-reacted 化合物)。尤其於巧克力囊腫(chocolate cyst)檢測出遠高於正常值5倍的氧化性傷害如8-OH-dG及脂質過氧化產物。並且於病灶處標本中檢測出新的粒線體基因斷損突變 (5755 bp斷損)的堆積。此外，我們針對病患的*GSTM1*的基因加以檢測，我們發現於null-type *GSTM1*的病患中有較高比例的子宮內膜異位症罹患率。並且於null-type *GSTM1*的病患中有較高含量的氧化性傷害物質堆積。本研究中，我們推測氧化性環境可以增進子宮異位性內膜細胞的生長。人類的子宮內膜是由子宮內膜上皮細胞及內膜腺體覆蓋於基質上所組成。而子宮內膜上皮細胞及基質細胞皆具有增生的能力。為了進一步證明氧化性壓力引發子宮內膜細胞異位性的生長，本計畫已初步建立子宮內膜上皮細胞

及基質細胞的體外初期培養。並針對於體外初期培養的內膜細胞加以氧化劑處理(如H_2O_2

及2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD))，並且檢測內膜細胞的生長模式、上皮細胞侵入性及轉移性的變化。並且測定細胞中interleukin-6及TNF-α 等cytokines的改變，以及氧化性壓力相關性基因及戴奧辛相關的transactivating genes表現的變化。另一方面，對於體外初期培養的內膜細胞中入抗氧化劑如Vit E 及mifepristone加以分析。此外，我們將子宮內膜異位上皮細胞及基質細胞(stromal cell)加以分離，分別探討這兩者細胞於氧化性傷害(如H_2O_2 and TCDD)處理後的分別變化。並將此兩中細胞共同培養及分離培養，此兩種細胞間的互相影響，並釐清其扮演的角色。並且尋找可降低細胞侵入性及轉移性的因子及治療方法。冀望經由對此疾病的治病機轉的了解以其對此疾病的診斷方法及治療有更進一步的進展。

實驗成果

(1) Higher content of lipoperoxides in the endometriotic tissues

In this study, we detected the lipoperoxides (as malondialdehyde) in the endometrium, myoma, adenoma, ovary, and chocolate cyst from women with endometriosis or normal individuals. The content of lipoperoxides was 1.50±0.25 (n=10), 4.48±0.44 (n=10), 2.61±0.66 (n= 7), 1.85±0.11 (n= 6), 2.80±0.72 (n=12), 2.68±0.44 (n=10), 3.34(n= 1), 1.27 (n= 1), 2.61±0.43 (n=11), 19.84±2.50 (n=10) in the normal endometrium, chocolate cyst, endometrium, myometrium, myoma, ovary, peritoneal fluid, placenta, decidual tissue, and villi, respectively. The higher contents of lipoperoxides were detected in the tissues from the eutpotic and the ectopic endometriosis. There were approximately four to five folds of lipoperoxides in the chocolate cyst.

(2) Higher amount of 8-OH-dG in the endometriotic tissues

The amounts of oxidized DNA base (e.g. 8-OH-dG) were detected by using HPLC-ECD in the endometrium, myoma, adenoma, ovary, and chocolate cyst from women with endometriosis or normal individuals. The content of 8-OH-dG was 0.17±0.07 ($x10^{-3}$%, n=5), 0.56±0.15 ($x10^{-3}$%, n=6), 1.21±0.10 ($x10^{-3}$%, n=10), 0.72±0.17 ($x10^{-3}$%, n=4), 0.55±0.18 ($x10^{-3}$%, n=5), 0.66±0.30 ($x10^{-3}$%, n=9), 0.62±0.05 ($x10^{-3}$%, n=9), 0.73 ($x10^{-3}$%, n=1), 0.31 ($x10^{-3}$%, n= 1), 0.65±0.19($x10^{-3}$%, n=9), 0.88±0.50($x10^{-3}$%, n=10) in the normal endometrium, adenomyoma, chocolate cyst, endometrium, myometrium, myoma, ovary, peritoneal fluid, lacenta, decidual tissue, and villi, respectively. The higher contents of 8-OH-dG were detected in the tissues from the eutpotic and the ectopic endometriosis. There were approximately four to five folds of lipoperoxides in the chocolate cyst.

(3) Accumulation of large scale deletion and DNA rearrangement of mtDNA in the endometriotic tissues

The accumulations of mtDNA rearrangements are shown in aged tissues, degenerated diseases, and several types of cancer in the humans. In our study, we detected mtDNA mutation in these tissues such as endometrium, myoma, adenoma, ovary, and chocolate cyst from women with endometriosis or normal individuals. Using primer-pair L8150-H14020, three types of PCR products were generated. The 5871 bp fragment was produced from the wild type mtDNA, the 894 bp fragment was from the 4977 bp deleted mtDNA, and the near 600 bp fragment was from 5270 bp mtDNA deletion. A scheme illustrating the strategy for the determination of multiple mtDNA deletion of various human tissues by the long-range PCR techniques (Fig.2) Lower panel, Lane 1 to 3 were examined from of ovaries, myometrium, and endometrium from 3 individuals with endometriosis, respectively. Lane M is the 100 bp DNA ladder and lane M' is 10 Kb DNA ladder in Fig 2.

(3) 4,977 bp deletion and a novel deletion of mtDNA in the endometriotic tissues

We applied primer-shift PCR to ensure the existence of 4,977 bp deletion and DNA sequencing to identify the novel 5,755 bp mtDNA deletion found in the endometriotic tissue. The primer-shift PCR products were amplified from the mtDNA with specific 4977 bp deletion in the ovaries with endometriosis. The lengths of the PCR products were according to the designed primer pairs. In Fig 3, Lane 1 to 3 indicate the PCR products of 894 bp (L8150-H14020), 793 bp (L8251-H14020), and 423 bp (L8251-H13650) amplified from 4977 bp deleted mtDNA. Furthermore, we sequenced the generated PCR products. In Fig. 4, a schematic illustration of the nucleotide sequence flanking the junction sites at the 5'-end of the novel 5755 bp deletion on the heavy strand of mtDNA in the endometriotic tissue. It revealed a 3-nucleotide indirect repeat (5'-CTT-3') located in the junction sites at nucleotide position (np) 8062-8064 or np 13819-13821 (5' to 3') on the heavy strand of mtDNA.

(4) Increased stabilization of HIF-1α in the endometriotic tissues

Cell were incubated with 100nM TCDD at 37℃for various time period, and then immunodetected with HIF-α specific antibody as described in Methods. Equal loading in each lane was demonstrated by the similar intensities of α-tubulin. The mean densitometry data from independent experiments were normalized to the result obtain in cells in the absence of TCDD (control). Increased HIF-α accumulation were found in the TCDD-treated cells.(Fig 4).

(4) Oxidative damages on the primary co-cultured cells derived from the endometriotic tissues

In order to underlying the effect(s) on the endometrial cells by oxidative stress, we established the primary co-cultured cells derived from the endometriotic cells *in vitro*. In this study we used two types of reagents, one was hydrogen peroxides and the other was TCDD (dioxin). Moreover, we traced the oxidative biomarkers (such as lipid peroxides and oxidized DNA and the expression of DDH in the differentially treated cells. There were approximately 6.5-fold increase of oxidized DNA, 8-OH-dG, in TCDD-treated cells. 5.8-fold increased of lipid peroxides, MDA, in TCDD-treated cells.

DDH was used as tracing marker of disease developing and progression. The ability of DDH to generate ROS during the oxidation of PAH *trans*-dihydrodiols (proximate carcinogens) may have important implications for tumor initiation and promotion. In our data, the differential expression of DDH was detected in the different types of endometriotic tissues. The highly expressed *DDH* mRNA was detected in the endometrial cell line and differentially expressed in adenomyosis, not in normal endometrium (Fig. 8). The 961 bp was generated from the cDNA of *DDH* and the 475 bp was from *β-actin*. Differential expression of DDH was detected in the different types of endometriotic tissues. The highly expressed DDH was detected in the endometrial cell line (lane 1) and differentially expressed in adenomyosis (lane 2, 3, 4,5, and 9). In addition, the culture cells were treated with H_2O_2 and TCDD. The

dose-dependent mRNA expression was shown in the treated culture cells. (A) The different dose of H_2O_2 from 0, 50, 100, 250 and 500 μM (Panel A, lane 1 to lane 5) was applied in treating cells. (B) Dose and time dependence expression of DDH was also detected in the treated cells with TCDD from 0 nM for 1 hr, 100 nM for 1 hr, 500nm for 1 hr, 100 for 3 hr, 500nm for 5 hr (Panel B, lane 1 to lane 5) of the treated cells.

(5) Induced generation of reactive oxygen species in TCDD-treated endometrial cells

Cells were incubated with 100nM TCDD at 37℃for various time periods. Reactive oxygen species were measured by flow cytometry as described in *Methods*. Increased mean fluorescent intensities (MFI) were identified in the TCDD-treated cells. (Fig. 9)

Table 1 The contents of 8-OH-dG and lipoperoxides were examined in this study.

Type of tisssue	8-OH-dG/dG ($x10^{-3}$%)	Lipoperoxide content (pmole/μg protein)
Normal	0.17±0.07 (n= 5)	0.18±0.02 (n=10)
Adenomyoma	0.56±0.15 (n= 6)	0.25±0.01 (n=6).
Chocolate cyst	1.21±0.10 (n=10)	1.14±0.68 (n=10)
Endometrium	0.72±0.17 (n= 4)	0.40±0.31 (n= 7)
Myometrium	0.55±0.18 (n= 5)	0.38±0.20 (n= 6)
Myoma	0.66±0.30 (n= 9)	0.42±0.23 (n=10)
Ovary	0.62±0.05 (n= 9)	0.68±0.44 (n=10)
Peritoneal fluid	0.73 (n= 1)	2.34 (n= 1)

Table 2 Various tissues collected from 46 women with or without endometriosis. MtDNA mutation with 4977 bp deletion and 5335 bp deletion were examined in each sample.

Type of tisssue	Endometriosis (endometriosis/tissue no.)	4977 bp mtDNA deletion	5335 bp mtDNA deletion
Adenomyoma	8/8	2/8	2/8
Chocolate cyst	12/12	4/12	7/12
Endometrium	4/8	2/8	1/8
Myometrium	3/8	1/8	0/8
Myoma	11/17	4/17	4/17
Ovary	2/3	1/3	0/3
Peritoneal fluid	1/1	1/1	0/1

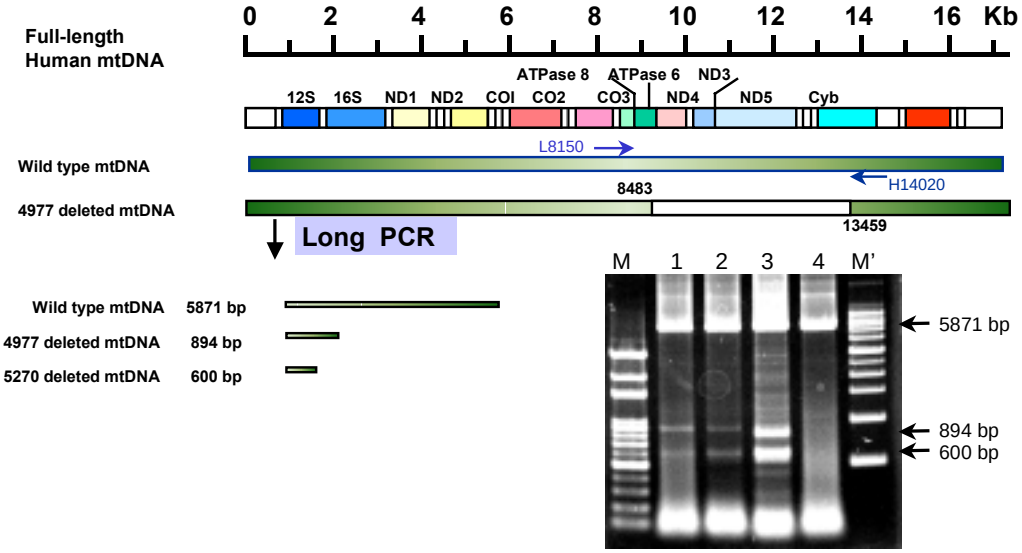


Fig1 Upper panel. A scheme illustrating the strategy for the determination of multiple mtDNA deletion of various human tissues by the long-range PCR techniques. Using primer-pair L8150-H14020, three types of PCR products were generated. The 5871 bp fragment was produced from the wild type mtDNA, the 894 bp fragment was from the 4977 bp deleted mtDNA, and the near 600 bp fragment was from 5270 bp mtDNA deletion. Lower panel, electrophorectogram of the PCR products amplified from mtDNA with specific deletions in women tissues with or without endometriosis. Lane 1 to 3 were examined from of ovaries, myometrium, and endometrium from 3 individuals with endometriosis, respectively. Lane M is the 100 bp DNA ladder and lane M' is 10 Kb DNA ladder.

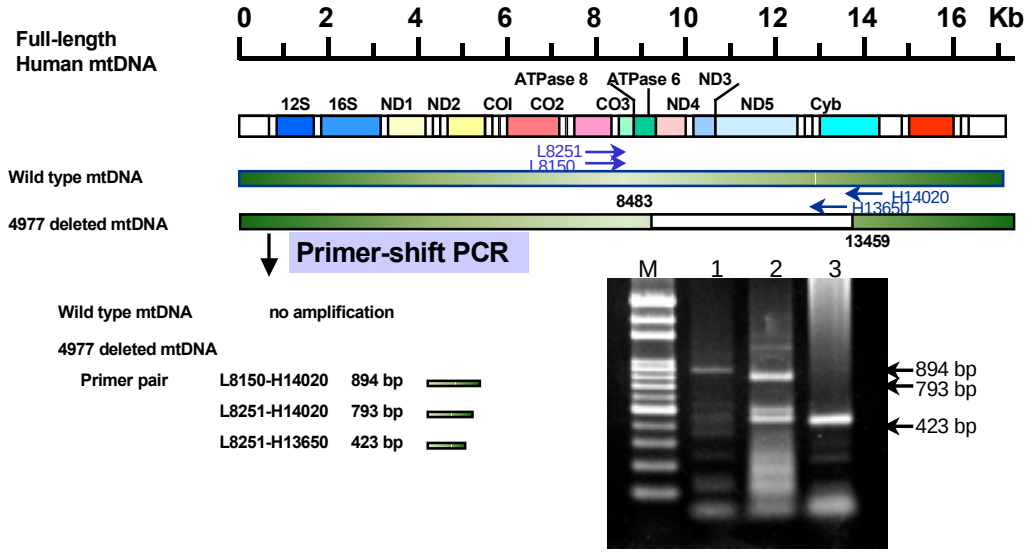


Fig. 2 Primer shift and electrophorectogram of the PCR products amplified from the mtDNA with specific 4977 bp deletion in the ovaries with endometriosis. Upper panel, by using different primer pairs, the PCR products were amplified from the 4977 bp deleted mtDNA. Lane 1 to 3 indicate the PCR products of 894 bp (L8150-H14020), 793 bp (L8251-H14020), and 423 bp (L8251-H13650) amplified from 4977 bp deleted mtDNA. Lane M is the 100 bp DNA ladder size marker.

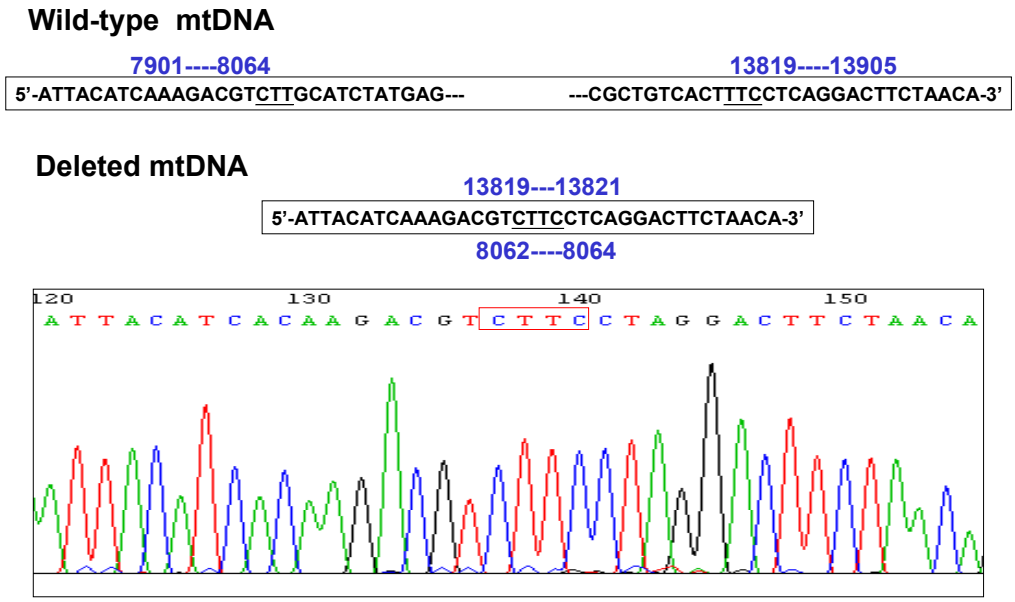


Fig 3 A schematic illustration of the nucleotide sequence flanking the junction sites at the 5'-end of the novel 5755 bp deletion on the heavy strand of mtDNA in human sperm. It revealed a 3-nucleotide indirect repeat (5'-CTT-3') located in the junction sites at nucleotide position (np) 8062-8064 or np 13819-13821 (5' to 3') on the heavy strand of mtDNA.

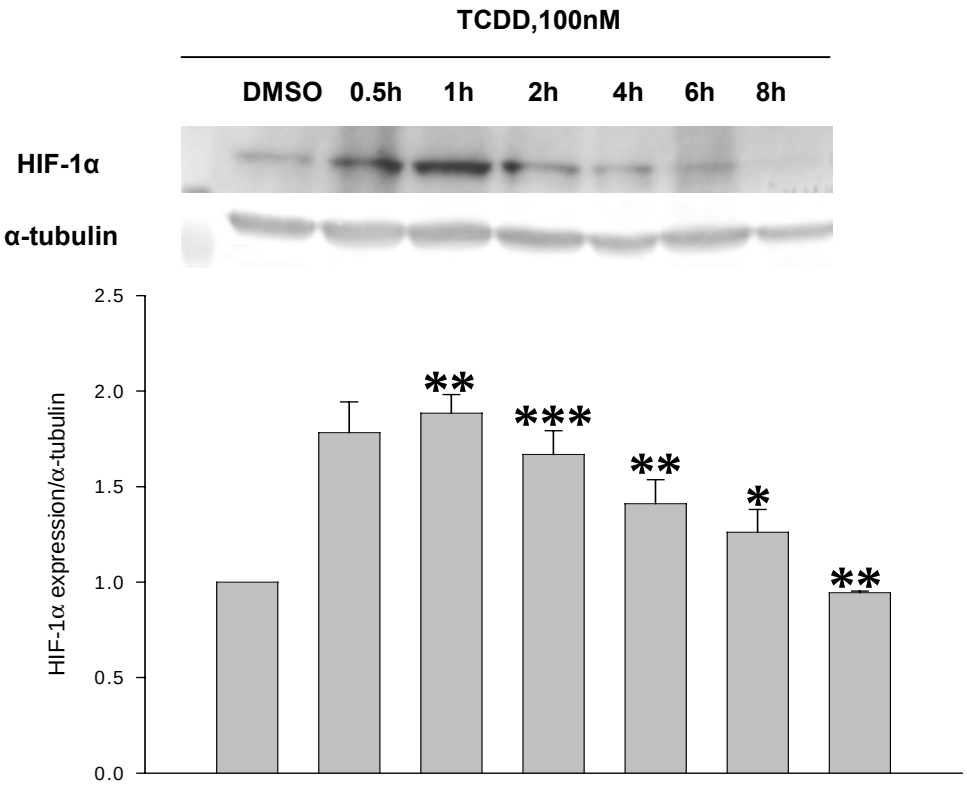


Figure 4.

Time-dependent effect of dioxin on HIF-1α expression in endometrial cells.

Cell were incubated with 100nM TCDD at 37°Cfor various time period, and then immunodetected with HIF-α specific antibody as described in *Methods*. Equal loading in each lane was demonstrated by the similar intensities of α-tubulin.The mean densitometry data from independent experiments werenormalized to the result obtain in cells in the absence of TCDD(control).Plots are mean ± S.E. values(n=3);*,p＜0.05 compared with the control;,p＜0.01 compared with the control;***,p＜0.001 compared with the control.**

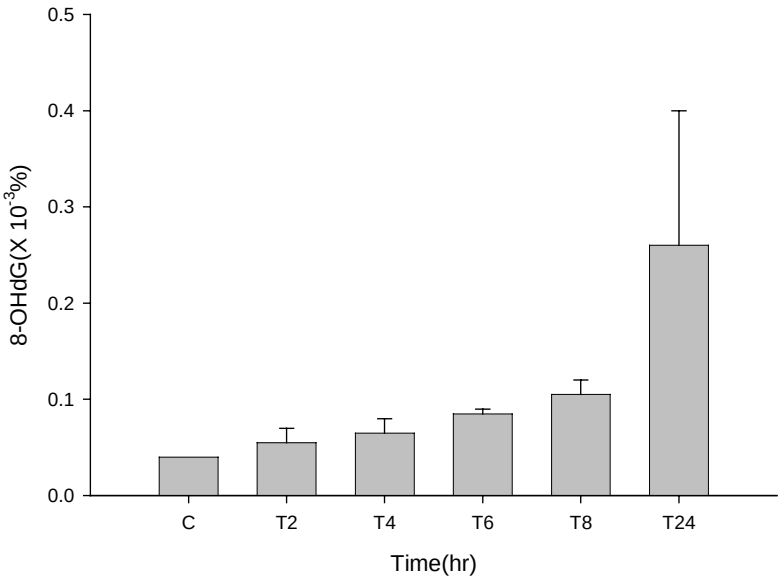


Figure 5.

Effect of TCDD on DNA oxidation in endometrial cell.

Cells were incubated with 100nM TCDD at 37°Cfor various time period. Oxidized DNA were measured by HPLC as described in *Methods*.

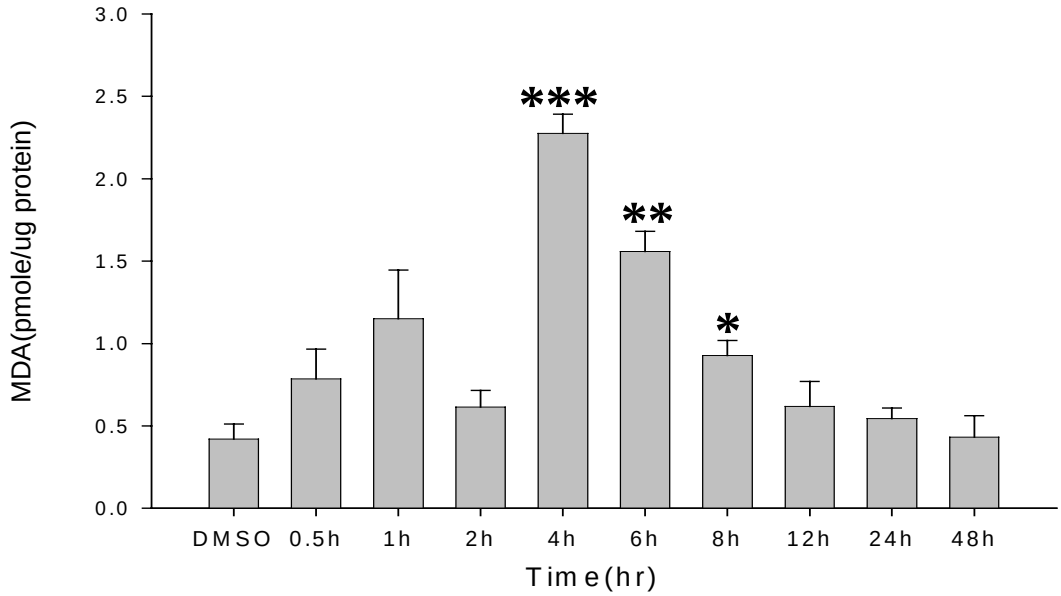


Figure 6.

Effect of TCDD on lipid peroxids in endometrial cell.

Cells were incubated with 100nM TCDD at 37°Cfor various time period. lipid peroxides were measured by HPLC as described in *Methods*.

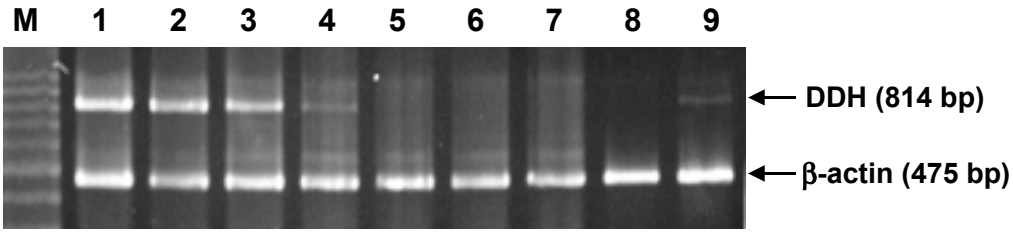


Fig 7 Electrophorectogram of the RT-PCR products amplified from the dihydrodiol dehydrogenase (DDH) mRNA in the endometriotic tissues. The 961 bp was generated from the cDNA of DDH and the 475 bp was from β-actin. Differential expression of DDH was detected in the different types of endometriotic tissues. The highly expressed DDH was detected in the endometrial cell line (lane 1) and differentially expressed in adenomyosis (lane 2, 3, 4,5, and 9).

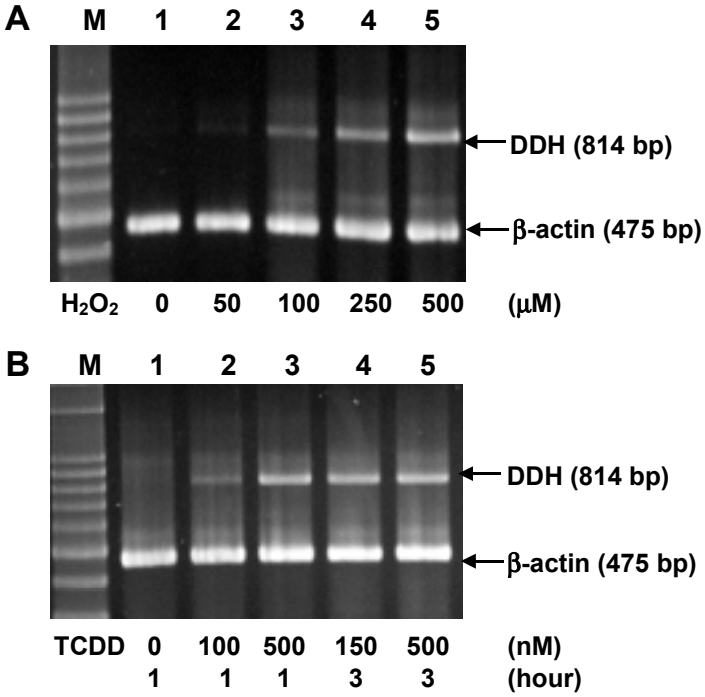
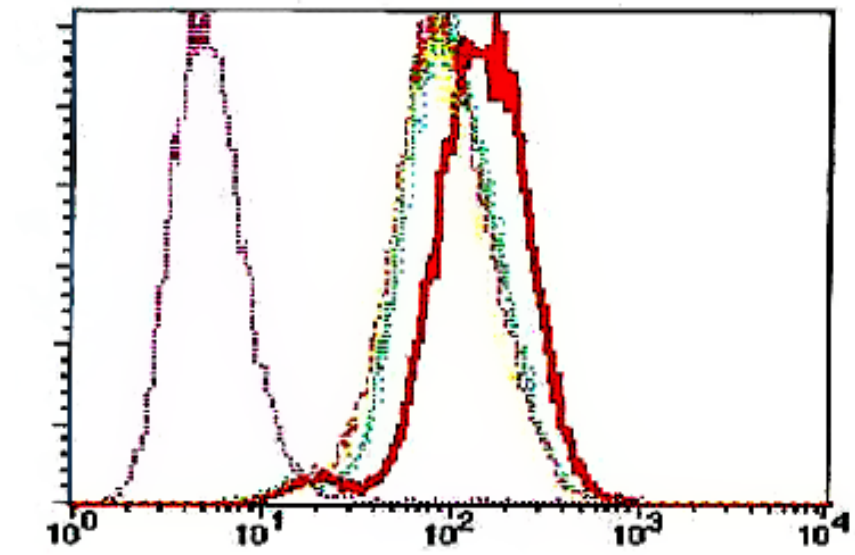


Fig 8 Electrophorectogram of the RT-PCR products amplified from the dihydrodiol dehydrogenase (DDH) mRNA in the endometriotic culture cells from two individuals with adenomyosis. The primary cell culture of endometriotic epithelium cells from the individual with adenomyosis was established. The culture cells were treated with H_2O_2 and TCDD. The dose-dependent mRNA expression was shown in the treated culture cells. (A) The different dose of H_2O_2 from 0, 50, 100, 250 and 500 贡M (Panel A, lane 1 to lane 5) was applied in treating cells. (B) Dose and time dependence expression of DDH was also detected in the treated cells with TCDD from 0 nM for 1 hr, 100 for 1 hr, 500nm for 1 hr, 100 for 3 hr, 500nm for 5 hr (Panel B, lane 1 to lane 5) of the treated cells.

Shift of ROS generation



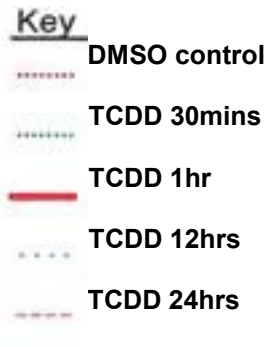


Figure 9.

Effect of TCDD on reactive oxygen species production in endometrial cell. Cell were incubated with 100nM TCDD at 37°Cfor various time period. Reactive oxygen species were mearsure by flow cytometry as described in *Methods*.

Discussion

Endometriosis is a frequent disorder that commonly presents with infertility and pelvic pain, and affects younger women of childbearing age. However, despite a growing number of reports on endometriosis, the pathophysiology of this disease remains poorly understood. Although the precise etiology of endometriosis is unclear, it is generally considered to involve multiple genetic, environmental, immunological, angiogenic and endocrine processes.

Recent studies have suggested that menstrual effluent contains factors that induce alterations in the morphology of the peritoneal mesothelium,[3] which may create adhesion sites for endometrial cells. Attachment of endometrial cells appears to be enhanced by induction of adhesion molecules[10] and overexpression of matrix metalloproteinases[11] and plasminogen activators,[12] which ensure local destruction of the extracellular matrix in endometriosis. After adhesion, endometrial cells proliferate and gradually invade the peritoneal tissue. Some factors induce vascularization of endometriotic implants, allowing their further development. Cytokines[13-15] and growth factors,[16] such as transforming growth factor-㸱, interleukin-8, interleukin-1, tumor necrosis factor, interferon-㱗,[17] and vascular growth factor,[18] have been implicated as inducers of attachment, proliferation, and neovascularization.

Oxidative stress has been proposed as a potential factor involved in the pathogenesis of the disease.[8,19] This disease is characterized by the increased presence of activated macrophages, erythrocyte destruction, iron deposition,[20] and associated increases in growth-promoting activities and the production of inflammatory cytokines. In this study, significantly higher amounts of oxidative damage were detected in endometriotic lesions than in controlled normal endometrium such as the mitochondrial DNA (mtDNA) rearrangement, 8-OH-deoxyguanosine (8-OH-dG), and lipoperoxide contents. In the future, we will explore the identification of the molecular pathway and factors of reactive oxygen species (ROS) generation and eradication. A better understanding of the mechanisms of ROS detoxification and further investigation of their effect on the peritoneal environment are essential to obtaining new insights into this disease and eventually developing new diagnostic and therapeutic strategies.