行政院國家科學委員會專題研究計畫 成果報告

利用整合性的蛋白質體學技術解析活化之雌激素接受器貝它對產生經痛之作用機轉
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 98-2629-B-006-001-
執 行 期 間 ：98 年 08 月 01 日至 99 年 10 月 31 日
執 行 單 位 ：國立成功大學生理學科暨研究所

計 畫 主 持 人：蔡美玲

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報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 99 年 12 月 29 日

行政院國家科學委員會補助專題研究計畫 v 成 果 報 告
□期中進度報告

利用整合性的蛋白質體學技術解析活化之雌激素接受器貝它對產生經痛之作用機轉

計畫類別：v 個別型計畫　□整合型計畫

計畫編號：NSC 98-2629-B-006 -001 –

執行期間：　98 年 08 月 01 日至 99 年 07 月 31 日

執行機構及系所：國立成功大學 生理所

計畫主持人：蔡美玲

共同主持人：

計畫參與人員：劉瑩一，吳佩郁，王禹城

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢

□涉及專利或其他智慧財產權，□一年■二年後可公開查詢

執行單位：國立成功大學

中　華　民　國　99 年 12 月 28 日

中文摘要

懷孕的末期，子宮重塑(包括子宮腔室的變大則是與子宮壁變薄和子宮收縮力下降)是為了適應懷孕過程中胚胎的持續發育。同時，雌激素在懷孕末期也達到最大濃度。因此，本研究之目的乃結合蛋白質體與生物資訊學之技術以系統生物學之角度探討懷孕子宮重塑的機轉。第一個實驗目標是利用懷孕第十八天(G18)大鼠子宮為實驗模式，蛋白質體技術分析得知蛋白 iNOS 及發炎相關之蛋白存在。以西方墨點法和功能性分析確定其結果。

Abstract

Uterine quiescence occurs with the progression of pregnancy. To explore a mechanism responsible for uterine quiescence, the first objective of this study was to establish uterine proteome of late-gestation rats. Functional annotation showed 4 proteins related to inflammation and 6 proteins related to integrin-associated network. Based on the expression profiles of ED1 protein (a M1 macrophage marker) and iNOS protein in this study, we hypothesized that interleukin 1-β (IL-1β) reduces the abundance of iNOS and the frequency of uterine oscillation in post-implantation uteri. Analysis of uterine oscillation by muscle bath apparatus showed a higher contraction frequency of uteri in the first half of pregnancy than in the second half. Western blot and RT-PCR analyses indicated greater amounts of iNOS protein and lower levels of IL-1β mRNA in the first half of pregnancy than in the second half. The reduced expression of iNOS in mid-gestation uteri by a 24-hr treatment with IL-1β (0.1 ng/ml) *in vitro* confirmed the inverse correlation between IL-1β mRNA and iNOS protein ($y = -10.316x + 1094.2$, $R^2 = 0.6261$, $P<0.05$). Since cotreatment of L-NAME and IL-1β enhanced the relaxation response of mid-gestation uterus to L-arginine, our data suggest that the decrease of iNOS by IL-1β during post-implantation may, in part, contribute to the induction of uterine quiescence.

Introduction

Embryo implantation triggers decidual formation and lead to wall thickening. During post-implantation period, decidual tissues are gradually degraded and uterine wall becomes thinner (Welsh & Enders 1985). In addition to implantation-induced morphological remodeling, continuous growth of the fetus also contributes to functional remodeling of uterine smooth muscle. The remodeled uteri then become quiescent in the second half of pregnancy with the reduction in the contraction frequency of uterine oscillation (Kuriyama & Suzuki 1976). However, the molecular mechanism of implantation-induced uterine quiescence is still unclear.

Conventionally, two-dimensional gel electrophoresis with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) reveals large numbers of uterine proteins (Zhang *et al.* 2006). Because of the nature of this process, sample loss during in-gel digestion limits the discovery of proteins with low abundance. To solve the problem, non-gel-based two-dimensional separation coupled with trypsin digestion is developed (Zhang *et al*. 2006, Chen *et al.* 2008, Motoyama & Yates. 2008).

Materials and methods

Use and Care of Animals

Male (aged 9-14 weeks) and female (aged 8-12 weeks) Wistar rats (200-350 g) were housed at the Animal Center of National Cheng Kung University Medical College at 24 ± 1˚C under a 14:10 light/dark cycle (lights on

at 05:00). All experimental and surgical procedures were performed in accordance with the NIH Guidelines for the Care and Use of Experimental Animals and were approved by the National Cheng Kung University Animal Care and Use Committee.

Full-term gestation for Wistar rats is about 22 days. Embryo implantation in rats is on day 4.5. The day when a virginal plug was observed after mating was designated day 0 (G0) of gestation. Pregnant rats at each of the following time points were sacrificed: G7 (early gestation) and G10 (early mid-gestation) in the first half of pregnancy and G14 (mid-gestation), G18 (late gestation), and G21 (just before term) in the second half of pregnancy. Antimesometrial uteri were isolated, cut into uterine strips (1 mm wide, 10 mm long, and 1 mm thick), and kept in physiological saline solution (PSS) composed of (in mM): 116 NaCl, 4.6 KCl, 1.16 NaH_2PO_4, 1.16 $MgSO_4$, 21.9 $NaHCO_3$, 1.8 $CaCl_2$, 11.6 dextrose, and 0.03 Ca-Na-EDTA.

Measurement of Uterine Contractions

After mounted in an organ bath at a constant passive force of 1.0 g, uterine strips contracted spontaneously. The average force of spontaneous contraction in 10 min was defined as the sum of the forces from each contraction/relaxation cycle divided by the frequency (number of contraction/relaxation cycles in 10 min). The average force was then normalized to the KCl (60 mM)-induced maximal force and expressed as a percentage.

Preparation of Uterine Homogenates

Uterine strips were pulverized in liquid nitrogen and mixed with lysis buffer (in mM); 20 Tris, 150 NaCl, 1 EDTA, 1 PMSF and 1% NP40. After centrifugation (Microfuge R, Beckman, CA) at 7500 g at 4˚C for 20 min, the protein content in the supernatant was measured by the Lowry assay. Bovine serum albumin (BSA) was used as the standard.

Non-gel-based Two-dimensional Separation

An aliquot of 200 μg from a pooled homogenate of 8 rats was denatured in 8 M urea, alkylated by iodoacetamide (20 mM, Fluka, Buchs, Switzerland), loaded into a 125-μL cartridge packed with C18 beads (5 μm; Vydac, Hesperia, CA, USA), and then eluted into five fractions using buffers containing 30, 40, 50, 60 and 95% acetonitrile (Phillipsburg, NJ) in 0.1% trifluoric acid. Each eluent in 50 μL ammonium bicarbonate buffer (100 mM, pH 7.8-8.3) was digested by chymotrypsin (Shimakyu, Osaka, Japan) at 37 ˚C overnight. The resulting digest was loaded into a 125-μL cartridge packed with strong cation exchange beads (5 μm; Vydac, Hesperia, CA) and eluted with formic acid (50 mM) buffer containing (in mM) 50, 70, 100, 150, and 1000 NaCl sequentially. Twenty five fractions were collected and subjected to MALDI-TOF/MS.

Proteomic Analysis by MALDI-TOF/MS

The MS data were obtained using a MALDI-TOF spectrometer equipped with a 337-nm N_2 laser (Micromass, Manchester, UK). To identify proteins, the raw data acquired from each eluted fraction were transferred to mowse.txt files and submitted to the search engine of Peptide Mass Fingerprint using NCBInr in Mascot database (http://www.matrixscience.com). The following criteria were used: *Rattus* species; a 100 ppm mass error tolerance; one missed cleavage site; cysteine residues alkylated with iodoacetic acid as a fixed modification; but no restrictions on p*I*, or protein molecular weight. The proteins with Mascot scores >57 and *P*

<0.05 were regarded as identified. Only those proteins identified in two separate experiments are listed in Table 1.

Uterine Explant Culture

Uterine strips of G10 rats were placed in the 35-mm culture dishes contained: 3 ml RPMI-1640 medium (Gibco BRL No.11835-030; Rockville, Maryland), 0.1% gentamicin (50 μM/ml), 2% normal male rat serum, and various treatments for 24 hr at 37˚C in 5% CO_2. The treated strips were then collected for the measurement of uterine contraction or Western blots.

Western Blot Analysis

Uterine homogenates (100 μg protein/lane) were loaded onto each lane of a 7% SDS polyacrylamide gel. Protein markers were loaded in the first lane. The gel containing proteins was transferred onto a polyvinyldifluoride membrane (NEN, MA). The membrane was then treated with primary antibodies followed by secondary antibodies. Subsequently, chemiluminescence reagent (ECL, NEN, MA) was added to the membrane. The membrane was then exposed to light-imaging film (Kodak, Boston) to visualize the bands. The protein abundance of β-actin was used as an internal control. Densitometry with a computerized image analyzer was used to quantify the staining intensity (GelDoc-IT System, UVP, USA). Monoclonal antibodies to β-actin, iNOS (Transduction Lab, MA), Kv4.3 (Alomone Labs, Israel), and PI-3 kinase γ, ED1, and fibronectin (Santa Cruz, CA) were used.

Reverse Transcription and Polymerase Chain Reaction (RT-PCR)

Total RNA from uterine tissues was extracted by using an RNeasy™ kit (QUAGEN, Germany). After mixing with MMLV-Reverse Transcriptase (Amersham, USA), the cDNA for IL-1β and β-actin in a PCR solution was put onto the thermal cycler (Omini Gene, Hybaid, UK). The PCR solution was composed of 10×PCR buffer, 5 μL (Amersham, USA); specific primer (forward and reverse primer; 100 μM) 0.4 μL; dNTP mixture (25 μM) 0.4 μL; $MgCl_2$ buffer (25 mM) 2 μL; DNA-dependent polymerase (Taq, Amersham, USA; 5U/μL) 0.3 μL. The cycling parameters for PCR were: denaturation at 92 ˚C for 45 sec, annealing of IL-1β at 58˚C for 45 sec, and extension at 72 ˚C for 60 sec (elongation of primed DNA). A total of 35 cycles was performed and followed by a 10-min extension at 72 ˚C. The primers used for IL-1β and β-actin are shown below. After agrose electrophoresis and ethidium bromide staining (0.5 μg/ml), the PCR products and a 100 bp DNA ladder (Gibco BRL, USA) were detected by a UV Gel scanner (GVILBER LOURMAT) at 320 nm.

Statistical Analysis

All data are expressed as mean±SE (standard error). Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by pairwise comparisons in the means model (SYSTAT 10.2, SPSS Science, CA). Pearson's regression analysis was conducted using the coefficient of determination (R^2). In all cases, a *P* value <0.05 was considered statistically significant.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Results and Discussion

As shown in Fig. 1, G7 uteri in the first half of pregnancy exhibited a higher contraction frequency and lower contraction force than G18 in the second half of pregnancy. To reveal the proteins involved in uterine quiescence, the G18 uterus with lower contraction frequency was used for proteomic analysis. Thirty eight proteins repeatedly identified in two analyses with P <0.05 were listed in Table 1. Western blot analysis confirmed the presence of 2 proteins (fibronectin and glyceraldehyde-3-phosphate dehydrogenase) identified with relatively lower score in Table 1 and 3 proteins (PI-3 kinase γ with GI number 12621140, iNOS with GI number 299367, and Kv4.3 potassium channel auxiliary subunit with GI number 45445271) identified once and not listed in Table 1. Compared with G7 uteri, G18 uteri expressed lower levels of fibronectin and Kv4.3 (P<0.05, Fig. 1). Western blot analysis confirmed the reliability of the uterine proteome established in our study. Since the increased expression of Kv4.3 contributes to the elevation of pacemaker activity in dopaminergic neurons (Liss *et al.* 2001), our result that late-gestation uteri contained lower levels of Kv4.3 and lower contraction frequency is consistent with the reports mentioned previously (Kuriyama & Suzuki 1976, Liss *et al.* 2001).

When compared with the report of Chen et al. (2008), fewer proteins were identified in this study. However, in terms of relative number of protein species, more membrane proteins (such as marker proteins) were discovered as shown in Table 1. Due to the presence of inflammation-related proteins, such as CD163 (a surface marker of M2 macrophages) and interleukin 12 receptor (a marker protein of Th1 lymphocytes), and PPAR-α interacting complex protein 285 (Martinez *et al.* 2008, Kyaw et al. 1998, Crisafulli & Cuzzocrea 2009), we further searched current studies of macrophages in pregnant uteri. Today, two phenotypes of macrophages are characterized: M1 and M2. M1 macrophages express higher levels of iNOS and lower levels of arginase than M2. The release of proinflammatory cytokines (such as interleukin-1) from M1 macrophages induces lymphocyte differentiation to Th1 cells and exerts bacteria-killing effects; the release of anti-inflammatory cytokines (such as interleukin-10) from M2 macrophages induces lymphocyte differentiation to Th2 and exerts immunosuppressive effects (Martinez *et al.* 2008). Discovery of CD163 in our proteomic result agree with current reports that uteri from post-implantation to late-gestation period contain M2 macrophages (Gustafsson *et al.* 2008, Oliveira & Hansen 2008).

Although specific markers for M1 macrophages were not identified, interleukin 12 receptor (a marker protein of Th1 lymphocytes) was found in our study. It is known that Th1 cytokines (such as IFNγ) cause monocyte infiltration and induce phenotypic conversion of monocytes to M1 macrophages with the increased expression of iNOS (Modolell *et al.* 1995) and interleukin-1β (Masters *et al.* 2010). After infiltration from circulation to tissues, monocytes become $ED1^+$macrophages. Since $ED1^+$ macrophages (a M1 macrophage) are found in non-pregnant uteri (Mara Suburo *et*

al. 1995) and a marker protein of Th1 lymphocytes was identified in our study, it was plausible to analyze the expression profile of $ED1^+$ macrophages, interleukin-1β mRNA, and iNOS during pregnancy. Of the pregnant uteri examined, those in the first half of pregnancy (G7 and G10) expressed greater levels of iNOS but lower levels of ED1 and IL-1β mRNA ($P<0.05$, Fig. 2) than those in the second half. In the first half of pregnancy, G10 uteri expressed higher levels of iNOS and ED1 than G7 uteri. For those in the second half of pregnancy, G18 uteri expressed higher levels of iNOS but relatively lower levels of ED1 and IL-1β mRNA than G14 or G21 uteri. The relative expression of IL-1β mRNA was inversely correlated with that of iNOS protein ($y = -10.316x + 1094.2$, $R^2 = 0.6261$, $P<0.05$).

Although similar profiles of uterine iNOS in the second half of pregnancy have been reported (Arthur *et al.* 2008, Bansal *et al.* 1997), this is the first report to show an inverse relation of iNOS with ED1 or IL-1β. The findings raise the question of why the uteri expressing higher levels of iNOS had lower levels of ED1 and IL-1β mRNA. In past decades, it has been reported that in activated human T lymphocytes, prolonged incubation with nitric oxide for 24 hr selectively suppresses the expression of IFNγ (Roozendaal *et al.* 1999). Continuous elevation of nitric oxide suppresses Th1 lymphocyte conversion and enhances Th2-mediated responses (Daniel *et al.* 2006). Since Th2-type cytokines (IL-4, IL-10) are the dominant cytokines during pregnancy (Piccinni 2010) and iNOS mainly appears in the decidual tissues of early-gestation uteri (Yoshiki *et al*. 2000), future studies may further explore whether implantation-induced iNOS in the first half of pregnancy enhances the production of nitric oxide, triggers the release of Th2-type cytokines, and suppresses the expression of ED1 and IL-1β.

It was of importance to note a higher level of IL-1β mRNA in the second half of pregnant uteri where iNOS protein abundance and contraction frequency were lower ($P<0.05$, Figs. 1 and 2). The inverse correlation between IL-1β mRNA and iNOS protein in pregnant uteri ($y = -10.316x + 1094.2$, $R^2 = 0.6261$, $P<0.05$) allowed us to hypothesize that prolonged exposure of mid-gestation uteri to IL-1β suppressed the expression of iNOS and then reduced contraction frequency. Accordingly, G10 uteri which contained the greatest amount of iNOS were used to examine the effect of IL-1β on the expression of iNOS and contraction frequency of uterine oscillations. After a 24-hr incubation, IL-1β at 0.1 and 1 ng/mL lowered the protein abundance of iNOS ($P<0.05$, Fig. 3A and 3B). A 24-hr treatment with IL-1β at 0.1 ng/mL or L-NAME at 1 mg/mL (competitive inhibitor of NOS) lowered the frequency of carbachol (1 μM)-induced oscillations ($P<0.05$, Fig. 3C) but did not influence uterine responses to L-arginine-induced relaxation. Combination of both IL-1β and L-NAME enhanced the uterine response to L-arginine-induced relaxation with the reduction of contraction frequency ($P<0.05$, Fig. 3D).

It has been accepted that L-arginine (a substrate for NOS) effectively induces muscle relaxation in late-gestation uteri but not in early-gestation uteri (Izumi *et al.* 1993). Our result clearly showed that mid-gestation uteri elicit relaxation response to L-arginine as the uteri in late-gestation uteri only when the uteri were co-treated with IL-1β and L-NAME. Due to the presence of iNOS in early-gestation uteri but not in late-gestation

uteri, the importance of iNOS on uterine contraction has been proposed in the past decade (Arthur *et al.* 2008, Bansal *et al.* 1997). However, this is the first evidence to show that cotreatment with IL-1β and L-NAME lowered iNOS activity and enhanced the response of mid-gestation uteri to L-arginine-induced relaxation. The data suggest that the existence of iNOS in early gestation attenuate the relaxation response of uteri to L-arginine.

The stimulatory effect of pro-inflammatory cytokines (such as INFγ and IL-1β) on iNOS expression has been well documented (MacMicking *et al.* 1997), while only two studies report their inhibitory effect. When macrophages are repeatedly exposed to LPS, INFγ no longer induces the expression of iNOS (De Boer *et al.* 2001). In pregnant rats on G20, intravenous infusion with IL-1β for 24 hr reduces the expression of iNOS (Mitchell *et al.* 2005). Consistent with these two reports, our study confirms that prolonged exposure to IL-1β lowers the protein abundance of iNOS in pregnant uteri. This contradiction of IL-1β on the expression of iNOS may be due to the fact that under the conditions of both gestation and prolonged exposure to LPS or proinflammatory cytokines, endogenous iNOS has been elevated. Additional stimulation on iNOS may trigger an auto-inhibitory machinery to limit iNOS expression. In summary, combination of chymotrypsin digestion, non-gel-based separation, and MALDI-TOF/MS allows us to reveal more hydrophobic proteins which have not been reported before (Zhang et al. 2006, Chen et al. 2008, Motoyama & Yates. 2008). A proteome-driven hypothesis provides an alternative approach to solve biological issues. Our novel finding of prolonged exposure to interleukin-1β on the decrease of both iNOS expression and uterine contraction suggests that the decrease of iNOS during post-implantation plays a role in uterine quiescence.

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國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：99年12月28日

計畫編號	NSC 98-2629-B-006 -001 –		
計畫名稱	利用整合性的蛋白質體學技術解析活化之雌激素接受器貝它對產生經痛之作用機轉		
出國人員姓名	蔡美玲	服務機構及職稱	國立成功大學 生理所
會議時間	2010 年 9 月 28 日至 2010 年 10 月 2 日	會議地點	義大利羅馬
會議名稱	(中文)第八屆世界職業衛生學術大會-2010 (英文)The 8th IOHA International Scientific Conference – Rome 2010		
發表論文題目	(中文)超細微碳顆粒導致之心肺毒性作用 (英文) Cardiopulmonary effects induced by chronic exposure to ultrafine particles in mice		

一、參加會議經過

The Conference IOHA 2010 - Health, Work and Social Responsibility was held in the Urbaniana University, Rome, from 28 September to 2nd October 2010. This conference, which integrated occupational hygiene, environment, health and safety, had more than a thousand national and international professionals from over 50 countries. Participants included researchers and professionals, involved in occupational health and safety, from the most prestigious universities and institutions, organizations and professional associations.

During this conference 11 keynotes lectures, 25 workshops, 263 oral communications and 233 posters presented innovative studies, exclusive projects and valuable know-how in order to make work more productive and more compatible with health and quality of life. Several professional meetings and other scientific activities also occurred during the conference.

二、與會心得

Dr Jukka Takala, EU-OSHA in charge in The European Agency for Safety and Health at Work (EU-OSHA) gave a review on the latest information on traditional, new and emerging risks, indicators, trends, and recent strategies to make workplaces and countries safe, healthy, competitive and productive. As he mentioned, to date, various programs and strategies have reduced the work-related negative outcomes at various levels identified and analyzed. Work-related illnesses in particular are linked to ageing. Globally there are 2.3 million deaths annually for reasons attributed to work, and out of those, 167 000 in the EU27. Long term health aspects and related occupational hygiene problems will need much more attention than earlier. We should be aware of “new” health problems at work, such as nanomaterial-induced health problems and develop prevention methods. Legislation and enforcement, information on the existing state of problems and capacities (profile) offer knowledge of solutions and good practices, communication and promotion to increase awareness, and collaboration and networking for exchange of good practice.

Among many lectures, I would like to mention the following at the Opening Session: “Nanotechnology and nanomaterials in occupational setting ” by Dr. Paul A Schutte from Center for disease control and prevention, National Institution for occupational safety and health. He emphasized the importance of developing a prepared mind to face Nanotechnology which provides beneficial properties and also causes potential hazard. The workers in the areas of research lab, scale-up recycle, disposal may have greater risks. All nanomaterials at the atomic scale form different

shape and size. Therefore, their toxicity cannot be simply summarized as a simple mechanism. The future challenges of nanotoxicity are nanomaterial-induced hazard identification and exposure assessment. In addition, since limited studies in nanotoxicology have been reported, rapid development in engineered nanomaterials may create several uncertainties. Therefore, an international development agenda should further promote the awareness of occupational hygiene on new materials such as nanoparticles and be more visibility of workers' health problems in general, in order to motivate decision makers to take stronger action in this respect.

三、考察參觀活動(無是項活動者略)

四、建議

I was impressed with all the presentations I managed to attend. In particular, there are several panel discussions in wrap-up sections at the end of the conference. These sections provide great opportunities to participants for further discussion or reflection on several issues. In the future, we should develop more personal contact during international conferences held in Taiwan.

五、攜回資料名稱及內容

Proceeding of the 8th International Scientific Conference

六、其他

國科會補助計畫衍生研發成果推廣資料表

日期:2010/12/28

國科會補助計畫	計畫名稱: 利用整合性的蛋白質體學技術解析活化之雌激素接受器貝它對產生經痛之作用機轉
	計畫主持人: 蔡美玲
	計畫編號: 98-2629-B-006-001- 學門領域: 生理

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：蔡美玲	計畫編號：98-2629-B-006-001-
計畫名稱：利用整合性的蛋白質體學技術解析活化之雌激素接受器貝它對產生經痛之作用機轉	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	2	0	100%	人次	12000x12= 144000 NT
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	0	100%		10. Yu CH, Chen SH Chen, Wang CL, Ma WL, Tsai ML. Proteomic analysis reveals a role of iNOS in functional remodeling of rat uteri during post-implantation (submitted to Reproductive Science), 2010.
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		

		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等,請以文字敘述填列。)		無					

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 ■未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

The results has been arranged into a manuscript which has been submitted.

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

This is a one-year project. We have completed proteomic analysis of the uteri. Based on our proteomic proteomic results, we further validated the proteins identified and conducted a hypothesis driven studies. This study allows us to explore the new mechanism of activated ERbeta on uterine remodeling. If our hypothesis is supported, induction of uterine remodeling during menstruation could be potential mechanisms to induce Primary Dysmenorrheal.