行政院國家科學委員會專題研究計畫成果報告

探討假懷孕及懷孕期間子宮蛻膜瘤形成之机制

Investigation of the mechanism of the developement of deciduomata during pseudopregnancy and pregnancy

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一、中文摘要

子宮蛻膜瘤是子宮內膜的基底細胞經由荷爾蒙（如 Estrogen 和 Progesterone）刺激誘發轉化形成(2)，它被認為在胚胎著床過程中扮演非常重要的角色。在我們的知識領域裡，子宮蛻膜瘤形成的機制了解仍然有限，可能與荷爾蒙（如 Progesterone、Estradiol、Histamine、TSH/LH、Relaxin…等）刺激 c-AMP 表現有關 (2)。然而這些荷爾蒙在其它細胞的作用與蛋白激酵素 C (Protein Kinase C; PKC) 活化有關(3,4)，但是 PKC 是否參與子宮蛻膜形成作用至今尚未被證明。目前我們初步研究結果顯示，蛻膜瘤組織形成期間與對照之子宮組織比較時，細胞質的 PKC 活性在統計上有顯著減少(5)，並且發現 PKC 異構體（α，δ，ζ，λ，ι）的表現與假孕鼠之子宮內膜增殖有密切關聯 (6,7)，推論 PKC 異構體的表現可能與調節蛻膜瘤組織的生長有關。子宮蛻膜瘤形成與 PKC 的關係雖是我們實驗室首先提出，但是其機制我們仍然一無所知，因此本計畫即針對這些問題進行廣泛的研究。利用大白鼠假懷孕及懷孕的動物模式，觀察抗組織銨(Anti-histamine)抑制子宮蛻膜瘤之形成，然後測定 PKC 異構體的表現。結果抗組織銨不僅影響蛻膜組織形成，而且子宮內層細胞內的 PKC 異構體（α和ζ）的蛋白含量都迅速下降，而下降的量有劑量依賴的情形。利用 PKC 抑制劑 H7 和 staurosporine 無法影響抗組織胺抑制蛻膜組織形成。因此我們認為抗組織銨導致 PKC 含量下降的因果是否與蛻膜織形成受到抑制有關需進一步探討。

Abstract

Deciduomata, which requires an adequate hormonal stimulation during the luteal phase of the cycle, is known to be come from the decidual transformation of stromal cells (2). It is thought to be very important for the implantation of the embryo. Our knowledge of the mechanisms of decidualization and its precise physiological role is still limited. Many reports indicated that the control and function of decidualization had been associated with the generation of of cAMP induced by hormones (including progesterone, estradiol , histamine, TSH/LH, Relaxin...et al.) (2). These hormones were also considered to cause PKC activation in other cellular systems (3,4). However, the role of PKC in the decidualization is still undefined. Our previous data showed that the activity of cytosolic PKC was significantly decreased in the deciduomata as comparing with that in the control uterine tissue (5), and that the various expression of PKC isoforms (α, δ, ζ, λ and ι) were observed in the trauma-induced decidualization (6,7). It is suggested that the various expression of PKC isoforms were involved in the modulation of the development of deciduomata. Although the correlation between the dcidualization and PKC expression was first published by our laboratory, the mechanism is still unclear. In this study, we use the same animal model to detect the effect of anti-histamine on the expression of PKC isoforms during decidualization in pseudopregnancy and pregnancy rats. The result showed that antihistamine not only inhibited the development of deciduomata but also depleted the level of PKC isoforms (α and ζ) in the endometrial cells. However, this phenomenon was not prevent by the PKC inhibitor (H7 and staurosporine). Thus, the inhibitory effect of decidualization and the PKC isoform expressions by antihistamine remain discussed.

二、緣由與目的

近年來不孕症比率有增加的趨勢。自 1965 年到 1982 年美國增率高達百分之 177，平均每六對夫婦中有一對是不孕，臺灣約是十比一 (8)。增加的原因可能如下：造成排卵不規則約佔 10%，子宮疾病約佔 6%，子宮頸病因約佔 5%，輸卵管阻塞的病例約佔 20%，著床時所需黃体荷爾蒙不足造成著床失敗約佔 5%，子宮內膜症約佔 20%，男性精子不良症約佔 35%和男性不明原因約佔 20%(以上的統計字之所以會超過 100%乃病因重複之故)。這些原因都可能與緊張的生活壓力和懷孕年齡移後有關，因此不孕的問題漸漸形成夫妻生活上的問題。

胚胎著床對於懷孕與否是一項非常重要的步驟，任何影響該步驟的因素都可能造成著床失敗懷孕不成，在臨床實驗報告顯示，人工受孕失敗的原因主要在胚胎著床失敗(1)，而著床失敗一直是人工受孕技術失敗的主要原因，儘管取卵率、受精率和胚胎分裂率都可突破 90%，但是著床成功的機會對每個胚胎而言卻只有 15-20%。目前對於這個問題，

有些可以利用荷爾蒙或胚胎助孵化術解決，有些在醫學上仍是盲點，所以解決的方法可能需要從其它方面著手，例如研究子宮蛻膜瘤形成之機制等相關問題。子宮蛻膜瘤是子宮內膜的基底細胞經由荷爾蒙(如Estrogen 和 Progesterone)刺激誘發轉化形成(2)，它被認為在胚胎著床過程中扮演非常重要的角色，如調控胚胎侵入作用(9), 而且也是提供胚胎生長的溫床，如供應胚胎營養(10)、分泌內泌素(11)和保護胚胎免於母体的免疫排斥作用 (12)。因為子宮內膜的接受度是否良好有賴於子宮蛻膜瘤形成正常，有良好的胚胎和足夠的荷爾蒙，也要有鍵全良好的子宮蛻膜瘤，胚胎才能順利著床發育，因此探討子宮蛻膜瘤形成的機制有助於解決著床失敗的問題。

子宮蛻膜瘤形成的好壞，在人工受孕過程中也常常用來判斷是否進行胚胎移殖手術。因此了解子宮蛻膜瘤形成的機制有肋我們作更好的辨別和了解胚胎與子宮內膜接合下最適當的環境。在我們的知識領域裡，子宮蛻膜瘤形成的機制了解仍然有限。子宮內膜基質細胞 (Stromal cell) 的蛻膜形成 (Decidulization) 已知與荷爾蒙有關，包括泌乳素(Prolactin; PRL) (13) 、女性素(Estradiol;E_2) 與助孕素(Progesterone; P) (2)，體外培養的模式亦證實助孕素促進基質細胞轉變為蛻膜細胞(Decidual cell) (14)。Tabanelli et al. (15)曾經應用體外培養人類子宮內膜基質細胞針對卵巢荷爾蒙 (Ovarian hormone) 促使蛻膜細胞形成有所探討。性腺刺激激素 (Gonadotropins)對於人類子宮內膜基質細胞的轉形作用為直接作用亦被指出 (16)。許多報告指出子宮蛻膜瘤形成與荷爾蒙如 Progesterone, Estradiol 、Histamine、TSH/LH、Relaxin…等刺激cAMP 表現有關(2)，Tang et al. (17)指出泌乳素及助孕素在子宮蛻膜形成之初步機轉為增加 cAMP 的含量。然而在其它細胞也被證實荷爾蒙(如Histamine, TSH/LH, Relaxin) 的作用與蛋白激酵素 C (Protein Kinase C; PKC) 活化有關 (3,4)，但是PKC 是否參與子宮蛻膜形成作用至今尚未被證明。

PKC是一種鈣離子和磷脂依賴之蛋白激酵素，在細胞內扮演訊息傳遞的角色。一種已知的細胞內傳遞訊息物質Diacylglycerol (DAG) ，即是增強鈣離子和磷脂活化PKC (18-20)。當細胞受到外在刺激(External Stimuli) 時，諸如生長因子(Growth Factor)、荷爾蒙 (Hormone)和神經傳遞物質(Neurotransmitter) ，DAG 由Phosphatidylinositol分解形成，PKC即被活化，並進行細胞內訊息傳遞的工作 (21)。此外PKC亦能誘發許多細胞反應，包括細胞增殖 (Cell Proliferation) 、分化(Differentiation)、基因表現 (Gene Expression) 和腫瘤促進形成作用(Tumor Promotion) (22)。

目前所知PKC12種異構體(α、β I、β II、γ、δ、ε、ζ、η、θ、λ、μ and ι) (23-26)， 在功能上和分佈上有其特殊性 (27-30)。如近期的研究報告顯示某些PKC異構體可能與組織再生 (Tissue Regeneration) 有關：部份肝臟切除後

肝細胞再生時，細胞內PKC呈現重新分配的現象，PKC從細胞質的部份轉移到細胞膜的部份(31-32)，細胞核內PKC α 含量減少，而PKC δ 則增加(33)；在Carbon Tetrrachloride誘發肝臟再生時，PKC α 呈現增加 (34)；在Folic Acid誘發腎臟再生時，則PKC α 呈現減少，但是PKC δ 和PKC ε 不變(35)。另外尚有報告顯示初級反應基因 (Primary Response Gene) 如c-fos、c-myc 、TIS1 、TIS8 和 TIS11 的活化也與肝臟和腎臟兩者之再生有關 (36-39)。

在子宮蛻膜形成過程中PKC所扮演的角色所知甚少，先前我們研究已證實PKC活性的變化在假孕鼠與懷孕鼠之子宮蛻膜瘤形成中發生(5)，子宮蛻膜形成之第5天，子宮蛻膜瘤組織與對照之子宮組織比較時，細胞質中 PKC (Cytosolic PKC) 活性在統計上有顯著減少，在微粒成份 (Particulate Fraction) 之PKC活性也有降低，但並無統計上之差異。顯示子宮蛻膜組織之生長可能與 細胞質的PKC活性減少有關。至於PKC異構體的改變，吾等亦已得知初步結果，12種PKC異構體中，尤其 PKC α，δ，ζ，λ，ι 與假孕鼠之子宮內膜增殖有密切關聯，PKCα減少，但PKCζ增加，確定PKC活性降低與PKCα減少有關 (6,7)，此結果與肝臟和腎臟再生結果相類似。因此推論PKC異構體的表現可能調節蛻膜瘤形成。

子宮蛻膜瘤形成與PKC的關係雖是我們實驗室首先提出，但是其機制我們仍然一無所知，因此本計畫即針對這些問題進行廣泛的研究。利用抗組織銨(Anti-histamine)抑制大白鼠子宮蛻膜瘤形成，然後測定 PKC 異構體之表現，以確定 PKC 活化之事實。

三、結果與討論

我們利用抗組織銨處理，發現不僅蛻膜組織形成受到抑制，而且子宮內層細胞內的PKC的蛋白含量都迅速下降，而下降的量有劑量依賴的情形。所以我們懷疑蛻膜組織形成受到抑制是否與PKC活化下降調節有關(activation and down-regulation)。然而當我們利用PKC抑制劑H7和staurosporine抑制PKC活化作用，結果得到相反的作用，即H7和staurosporine並不能抑制抗組織胺抑制蛻膜組織形成的效果。可見抗組織銨抑制蛻膜組織形成應與PKC活化下降調節無關，而且PKC含量下降的因果是否與蛻膜織形成受到抑制有關則有待進一步探討。

四、參考文獻

1. 游士弘、金玉堂、簡利婷、溫慧勤和王鈴琪 (1997) 人工生殖之醫學與法律(上)。當代醫學雜誌 24(7): 89-94.
2. Tang, B., Guller, S. and Gurpide, E. Mechanism of human endometrial stromal cells decidualization [Review]. Annals of the New York Academy of Sciences 734: 19-25,1994.
3. Tilly, B.C., L.G. Tertoolen, R. Remorie, A. Ladoux, I. Verlaan, S.W. deLaat, and W.H. (1990) Moolenaar. Histamine as a growth

factor and chemoattractant for human carcinoma and melanoma cells: action through Ca2(+)-mobilizing H1 receptors. J. Cell. Biol. 110: 1211-1215.

4. Niedel, J.E., L.J. Kuhn, and G.R. Vandenbark. (1983) Phorbol diester receptor copurifies with protein kinase C. Proc. Natl. Acad. Sci. USA 80: 36-40.
5. Shyu,J.C., Hsieh,Y.S., Chang,C.L., Tsai,C.C., Cheng,C.K. and Liu,J.Y. (1996) Chang in protein kinase C activity on day 5 of decidualization in pseudopregnant rat. Chinese Journal of Physiology, 40:107-112.
6. Shyu,J.C., Hsieh,Y.S., Chang,C.L., Tsai,C.C., Chang,A.C. Yang, L.C., Lin, M.T., Cheng, M.H. and Liu,J.Y. (1996) Localization of protein kinase Ca and ? during the decidualization in pseudopregnant rats. Chinese Journal of Physiology, 40:243-247.
7. Liu, J.Y., Shyu, J.C., Chang, C.L., Tsai,C.C., Chang,A.C., Yang, L.C., Lin,L.Y., and Hsieh, Y.S. (1998) Protein kinase C isoforms during the development of deciduomata in pseudopregnant rats. Life sci.(submit)
8. 蔡泳杰(1997) 治療不孕症最新情報。Mom Baby, 125: 132-137.
9. Pijnenborg, R., Dixon, G., Robertson, W.B., Brosens, I. (1980) Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy. Placenta, 1:3.
10. Kearns, M., Lala, P.K. (1983) Life history of decidual cells: a review. Am. J. Reprod. Immunol. Mivrobiol., 3: 78.
11. Maslar, I.A., Kaplan, B.M., Luciano, A.A., Riddick, D.H. (1980) Prolactin production by the endometrium of early human pregnancy. J. Clin. Endocrinol. Metab., 51: 78.
12. Golander, A., Zakuth, V., Shechter, Y., Spirer, Z. (1988) Suppression of lymphocyte reactivity in vitro by a soluble factor secretion by explants of human decidua. Eur. J. Immunol., 11: 849.
13. Daly,D.H., Maslar,I.A. and Riddick,D.H. Prolactin production during in vitro decidualization of proliferative endometrium. Am.J.Obstet.Gynecol. 145:672-678, 1982.
14. Sananes, N., Weiller, S., Baulieu, E.E., and Goascogne C. (1978) In vitro decidualization of rat endometrial cells. Endocrinology 103: 86-95.
15. Tabanelli, S., Tang, B., and Gurpide, E. (1992) In vitro decidualization of human endometrial stromal cells. J Steroid Biochem Mol Biol 42: 337-344.
16. Tang, B., and Gurpide, E. (1993) Direct effect of gonadotropins on decidualization of human endometrial stromal cells. J Steroid Biochem Mol Biol 47: 115-121.
17. Tang, B., Guller, S., and Gurpide, E. (1993) Mechanisms involved in the decidualization of human endometrial stromal cell. Acta Eur

Fertil 24: 221-223.

18. Nishizuka, Y. (1984) The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature, 308: 693-698.
19. Berridge, M.J. (1987) Inositol triphosphate and diacylglycerol: two interacting second messengers. Annu. Rev. Biochem., 56:159-193.
20. Ganong, B.R., Loomis, C.R., Hannun, Y. A., and Bell, R.M. (1986) Specificity and mechanism of protein kianse C activation by sn-1,2-diacylglycerols. Proc. Natl. Acad. Sci. USA, 83: 1184-1188.
21. Rozengurt, E. (1989) Signal transduction pathways in mitogenesis. Brit. Med. Bull., 45: 515-528.
22. Nishizuka, Y. (1988) The molecular heterogeneity of protein kinase C and its implication for cellular regulation. Nature, 334: 661-665.
23. Koide, H., Ogita, K., Kikkawa, U. and Nishizuka, Y. (1992) Isolation and characterizatonof the ◦ subspecies of protein kinase C from rat brain. Proc. Natl. Acad. Sci. USA., 89: 1149-1153.
24. Wetsel, W.C., Khan, W.A., Merchenthaler, I., Rivera, H., Halpern, A.E., Phung, H.M., Negro-Vilar, A. and Hannum, Y.A. (1992) Tissue and cellular distribution of the extended family of protein kinase C isoenzymes. J. Cell Biol., 117: 121-133.
25. Selbie, L.A., Schmitz-Peiffer, A., Sheng, Y. and Biden, T.J. (1993) Molecular cloning and characterization of PKC ◦ , and atypical isoform of protein kinase C derived from insulin-secreting cells. J. Biol. Chem., 268: 24296-24302.
26. Dekker, L.V. and Parker, P.J. (1994) Protein kinase C-a question of specificity. Trends. Biochem. Sci., 19: 73-77.
27. Huang, K.P., Huang, F.L., Nakabayashi, H. and Yoshida, Y. (1988) Biochemical characterization of rat brain protein Kinase. C isozymes. J. Biol. Chem., 263:14839-14845.
28. Hidaka, H., Tanaka, T., Onoda, K., Hagiwara, M., Watanabe, W., Ohta, H., Ito, Y., Tsurudomw, M. and Yoshida, T. (1988) Cell type-specific expression of protein kinase C isozymes in the rabbit cerebellum. J. Biol. Chem., 263: 4523-3426.
29. Hocevar, B.A. and Fields, A.P. (1991) Selective translocation of ◦ II-protein kinase C to the nucleus of human promyelocytic (HL60) leukemia cells. J. Biol. Chem., 266: 28-33.
30. Liyanage, M., Frith, D., Livneh, E. and Stabel, S. (1992) Protein C group B members PKC- ◦ , - ◦ , - ◦ and PKC-L(◦). Comparison of properties of recombinant proteins in vitro and in vivo. Biochem. J., 283: 781-787.
31. Okamoto, Y., Nishimura, K., Nakayama, M., Nakagawa, M. and Nakano, H. (1988) Protein kinase C in the regenerating rat liver.

Biochem. Biophys. Res. Commun., 151: 1144-1149.

32. Rush, J.S., Klein, J., Fanti, P., Bhat, N.R. and Waechter, C.J. (1992) Direct assay of membrane-associated protein kinase C activity in lymphocytes B in the presence of Brij-58. Anal. Biochem., 207: 304-310.
33. Alessenko, A., Khan, W.A., Wetsel, W.C. and Hannun, Y.A. (1992)
34. Sasaki, Y., Hayashi, N., Ito, T., Fusamoto, H., Sato, N. and Kamada, T. (1989) Heterogeneous activation of protein kinase during rat liver regeneration induced by carbon tetrachloride administration. FEBS Lett., 254: 59-65.
35. Dong, L., Stevens, J.L., Fabbro, D. and Jaken, S. (1993) Regulation of protein kinase C isozymes in kidney regeneration. Cancer Res., 53: 4542-4549.
36. Kondo, T., Inui, H., Konishi, F. and Inagami, T. (1992) Phospholipase D mimics platelet-derived growth factor as a competence factor in vascular smoothe muscle cells. J. Biol. Chem., 267: 23609-23616.
37. Carpenter, G. (1992) Receptor tyrosine kinase substrates-src homology domains and signal transduction. FASEB J., 6: 3283-3289.
38. Towley, B. D., Chadwick, L.J., Grantham, J.J. and Calvet, J. P. (1989) Sequently protooncogene expression in regenerating kidney following acute renal injury. J. Biol. Chem., 264: 8389-8393.
39. Kujubu, D.A., Norman, j.T., Herschman, H.R. and Fine, L.G. (1991) Primary response gene expression in renal hypertrophy and hyperplasia: evidence for different growth initiation processes. Am. J. Physiol., 260: F823-F827.