

行政院國家科學委員會專題研究計劃成果報告

TGF- 因子對豬早期胚體外發育及胚內源性基因表現之影響

Effect of TGF- on the early development of porcine embryos and on the expression of embryonic genes assessed by *in vitro* culture

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一、中英文摘要：

本研究計畫旨在探討，豬發情配種後不同生理階段之輸卵管上皮細胞，其基因轉譯物對於豬胚早期發育之調控機制。供試豬隻於注射人類絨毛激性腺素 (hCG) 後 50~60、96 及 120 小時，應用外科手術截取其輸卵管，分別抽取其 Total RNA，並經進行反轉錄-聚合酵素連鎖反應 (DDRT-PCR) 測試，比較各不同生理時段之豬輸卵管上皮細胞之基因表現差異性；試驗結果證實以隨機引子 4 (arbitrary primer 4) 及錨定引子 3 (anchor primer 3)，可明確分辨豬輸卵管上皮細胞中 (96/120-1-R4A3) 基因表現有生理時間特異性；進一步經選殖、定序及 GCG 軟體比對後，確認該 (96/120-1-R4A3) 基因片段為豬轉型生長因子- (TGF-)。後續試驗針對豬胚體外培養系統中添加該轉型生長因子- 並探討其對胚源性基因表現之調控情形；結果發現添加 20ng/ml 的轉型生長因子-

條件下對於 4-細胞期之豬胚在進行共培養 3 小時後，再抽取其 Total RNA 進行基因差異表現之分析，證實豬胚添加有轉型生長因子- 被培養者，表現有一基因 TIG-I，係有別豬胚之在未添加生長因子被培養者。而該 TIG-I 基因進一步選殖、定序及 GCG 軟體比對後，證實其序列類似於小鼠早期胚之

mo25 基因 (89%) 者然，鑑於在小鼠之 mo25 基因序列中具有鈣離子結合區，顯示其功能涉及細胞訊息之傳導；處於 4-細胞期豬胚表現之 TIG-I，其作用是否亦類似於小鼠之 mo25 者然，誠值得進一步試驗詳加探討。

Abstract:

In the present study, timely expression of gene(s) in porcine oviduct epithelia was identified and the consequent effect of the specified ligand on expression of embryonic gene at early stages of development was elucidated. Total RNA extracted from the oviduct epithelia of gilts, fallotomized at 50-60, 96 and 120 h post the injection of human chorionic gonadotrophin (hCG), were subjected to analyses by mRNA differential display and by reverse transcription-polymerase chain reaction (DDRT-PCR), respectively. Results from sequencing analysis confirmed that the sequence of gene fragment of (96/120)-1-R4A3, expressed by porcine oviduct epithelia at 96 and 120 h post the hCG injection, was identical to that of gene encoding the porcine transforming growth factor- (TGF-). Moreover, by using an *in vitro* culture system, it was found that the

expression of TIG-I gene in early 4-cell of porcine embryos does appear to be TGF- dependent. The cDNA sequence of TIG-I gene was further confirmed to be homologous to that of mo25 cDNA found in mouse early embryos and the sequence also appeared to be having a Ca-binding domain located within the coding region. These results imply that TGF- gene timely expressed in the epithelia of porcine oviducts is essentially required for porcine early embryos to activate their signal transduction pathway.

二、緣由與目的：

處於早期發育階段之豬胚在體外被培養時，經常面臨無法發育超越 4-細胞期之難題；此一現象稱為豬胚之“培養障阻”。鑑於豬之受精卵自 1-細胞卵裂至 4-細胞階段係在其輸卵管內完成者，本研究室乃嘗試針對母豬在發情後不同生理階段，探討其輸卵管上皮細胞之蛋白質分泌模式及其基因之表現。初步結果證明，母豬自開始發情後第 1 日至第 3 日，其輸卵管上皮細胞之蛋白質分泌模式，確實頗有差異；且在該特定時段中，其輸卵管上皮細胞內有 13 個基因表現具時間特异性。其中細胞轉型因子- (transformation growth factor- ,TGF-)在輸卵管上皮細胞內表現之時間為 hCG 注射後 96 h (圖 1)，適與豬胚之 4-細胞發育障阻時間相吻合，本研究遂進一步深入探討 TGF- 與豬胚早期發育之關係。此等研究之完成，除有助於瞭解豬胚早期發育之分子調控外，對於未來建立豬胚之體外培養系統，及應用遺傳工程技術改善母豬生殖效率等，亦具有極大之助裨。

三、結果與討論：

有關生殖道上皮細胞所表現之各類生長因子，對於胚早期發育之重要性，可經由其在胚之體外培養系統添加與否，分別予以比較證明之。Paria *et al.*, (1990) 曾使用不同濃度之 EGF、IGF-I、TGF- 及 TGF- β 1 等，分別添加於簡單化學培養液中進行 2-細胞鼠胚體外試驗。結果證明，培養液中被添加有 2、4 或 10 ng/ml 之 EGF、TGF- 或 TGF- β 1 者，其發育至囊胚期之胚數比例均顯著高於對照組之未添加生長因子者 ($p < 0.001$)；惟此等結果皆僅止於細胞型態學之研究，實際探討分子細胞層次者尚屬少見 (Babalola and Schultz, 1995)。本試驗因此應用高靈敏性之 mRNA Differential Display (Liang and Pardee, 1992) 技術，探討早期豬胚與生長因子間之分子調控機制。

本試驗以隨機引子 H-AP 7 及 錨定引子 H-T11G 之配對，分析 4-細胞階段之豬胚經培養於添加生長因子 TGF- (20 ng/ml) 之 NCSU-23 培養液中，對其表現之 mRNA 是否可因 TGF- 添加與否而有差異。試驗結果證明，經使用上述引子組於 mRNA Differential Display 分析後，可有效確認一條具有特异性之基因片段，係用添加生長因子 TGF- 之添加而被表現者 (圖 2)。此具有 TGF- 特异性之基因片段，經應用各原先使用之相同引子組，進行各基因片段之第二次 PCR 擴增結果，更確認其長度約為 300 bp (圖 3)；進一步試驗將該基因片段分別構築於 TA vector 及 pGEM-T 質體上 (圖 3)，經選殖大量回收基因片段之質體 DNA 後，將經核酸限制酵素截切分析結果，確認其長度無誤外，並分別經定序 (圖 4) 及使用 GCG 軟體進行比對結果 (圖 4)，證明該經 TGF- 誘發之基因片段，其核甘酸序列與在小鼠早期胚表現之 mo25 基因 (Miyamoto *et al.*, 1993; Nozaki *et al.*, 1996) 之相似性高達 89%；此外，試驗更以巢式聚合酵素連鎖反應

(nested PCR)及原位雜交(*in situ* hybridization) (圖 5 及 6) ; 進行重複驗證 , 確認豬胚之

mo25 基因係在 4-細胞階段被 TGF- α 誘發而表現者。

四、參考文獻：

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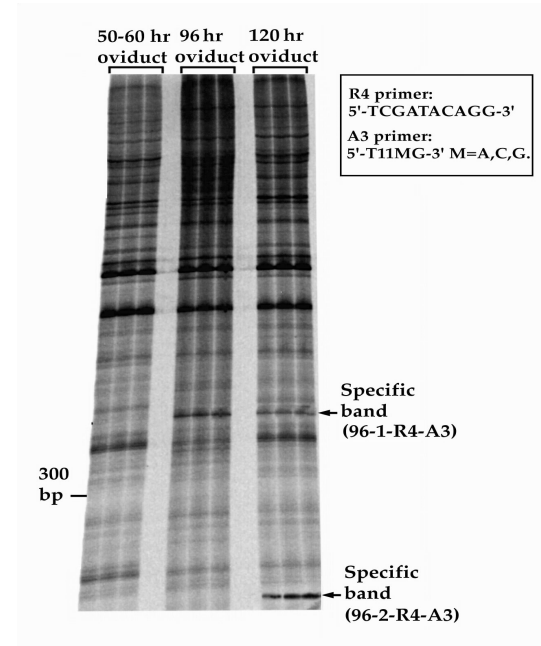


Fig. 1. Differential display of mRNAs from epithelial cells of pig oviducts using primer set of R4 and A3. Arrow indicate expressed specific gene fragment found after DDRT-PCR. Fig. 2. Differential Display of mRNAs from porcine early embryos using primer set of H-AP7 and H-T11G. Arrow indicates expressed specific gene fragment found after DDRT-PCR.

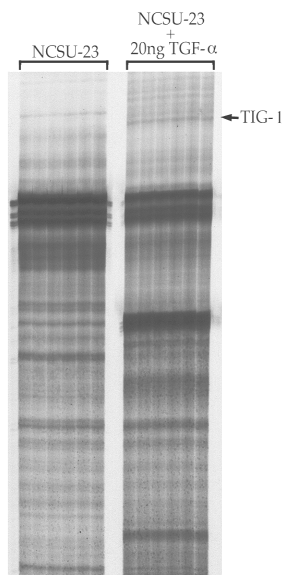


Fig. 3. Re-amplification of specific gene fragment was carried out with the primer set indentured to that used during the DDRT-PCR (A).

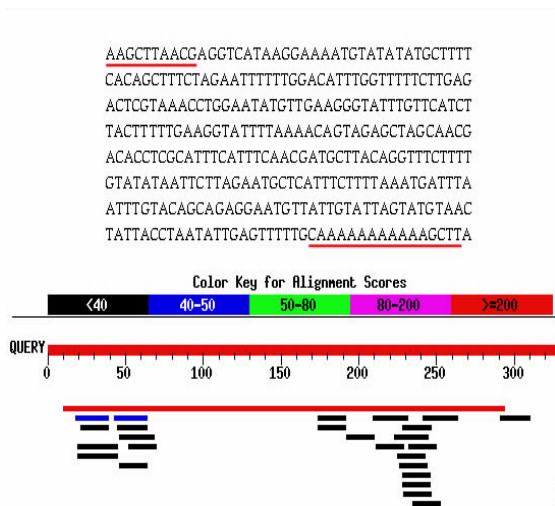


Fig. 4. Similarly analysis of specific gene fragment of TIG-I identified by differential mRNA display; comparisons were based on sequences listed in GCG gene bank.

preimplantation embryos by RT-PCR. RNA from 10 early 4-cell, 6 eight-cell, 5 16- ~ morular-cell, and 4 Blastocyst embryo was reverse transcribed with oligo-dT, and the RT products was amplified by 35-cycle of PCR with oligonucleotide primers for TIG-I (pMo25).

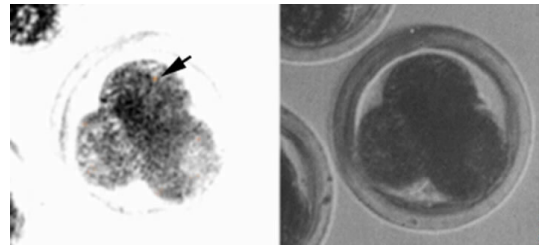


Fig.6. Distribution of pMo25 transcripts in early 4-cell porcine embryo with In situ hybridization. Arrow indicate expressed pmo25 mRNA. Early 4-cell embryos were treated with 20 ng/ml TGF-a for 3 hours.

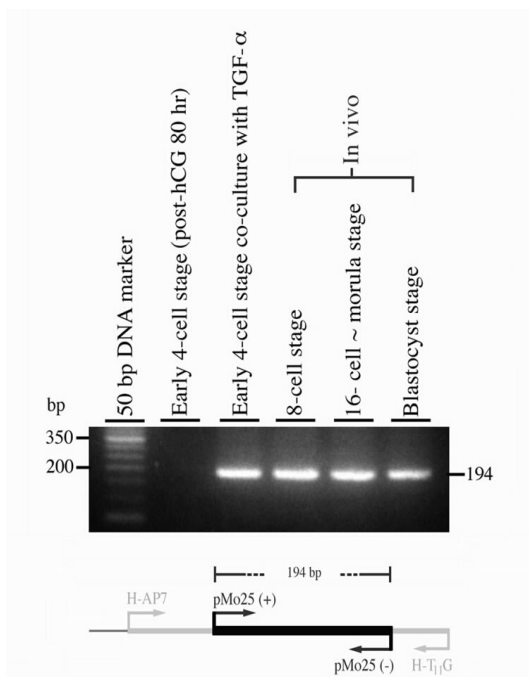


Fig.5. Detection of transcripts in RNA from