行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □期中進度報告

雄性激素接受器和其作用的蛋白質在攝護腺腫瘤的角色

計畫類別：■ 個別型計畫　□ 整合型計畫
計畫編號：NSC 96－ 2314 － B －038 － 007 －
執行期間： 96 年 02 月 01 日至 96 年 07 月 31 日

計畫主持人：張育嘉
共同主持人：
計畫參與人員：

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

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

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：台北醫學大學

中 華 民 國 96 年 10 月 30 日

中文摘要：

雄性激素接受器(androgen receptor)的訊息傳遞路徑，在攝護腺腫瘤(prostate cancer)生成及發展的過程中扮演著關鍵性的角色。雄性激素接受器的做用蛋白質應該在攝護腺腫瘤的生長過程中扮演著重要的角色。在此計畫中，我們利用 yeast two hybrid system 的方法找出雄性激素接受器的作用蛋白質，在研究的結果中，發現 transgelin 能藉由與雄性激素接受器之作用蛋白質 ARA54 結合來影響或抑制 ARA54 對於雄性激素接受器的促進訊息傳遞的功能。阻斷此訊息傳遞的路徑，利用對於作用蛋白質的調控來影響雄性激素接受器的訊息路徑，這個研究結果，應該在攝護腺癌的治療上開啟另一個方向。不過，還需要更多的研究，來了解其中的作用機轉，或是否其他的作用蛋白質，也有相類似的作用，都值得更深入地做研究。

英文摘要：

The androgen receptor (AR) requires coregulators for its optimal function. However, whether AR coregulators further need interacting protein(s) for their proper function remains unclear. Here we describe transgelin as the first ARA54-associated negative modulator for AR. Transgelin suppressed ARA54-enhanced AR function in ARA54-positive but not in ARA54-negative cells. Transgelin suppressed AR transactivation via interruption of ARA54 homodimerization and AR-ARA54 heterodimerization. Stable transfection of transgelin in LNCaP cells suppressed AR-mediated cell growth and PSA expression, while this suppressive effect was abolished by the addition of ARA54-siRNA. They also demonstrate that AR coregulators, like ARA54, may have dual *in vivo* roles to both function as a coactivator directly and as a mediator for its interacting protein's influence on the AR function indirectly. The success of this proposal will not only demonstrate that proper AR function also needs modulators of AR coregulators, but also provides us the opportunity to target and modify AR function without targeting the AR directly, which can reduce many potential side effects as the AR has many other important *in vivo* functions. However, the detail mechanism still is unclear. More studies are necessary to further dissect that.

INTRODUCTION

Prostate cancer is the most frequently diagnosed malignancy in aging males, and each year about 31,500 men in the United States lose their lives because of this malignancy (1). Androgen and the androgen receptor (AR) play pivotal roles in the progression of prostate cancer. Dissecting the precise molecular mechanism of how AR signaling is regulated and how it contributes to the prostate cancer progression may, therefore, greatly help in battling this disease. AR is a ligand-dependent transcription factor that belongs to the superfamily of nuclear receptors (NR) (2, 3). The proper function of AR requires coregulators for its optimal signaling (4, 5). Several AR coregulators, including the CREB-binding protein (CBP), SRC-1, ARA54, ARA55 , ARA67/PAT1, ARA70, hRad9, and PTEN have been identified and their potential pathophysiological roles in the prostate cancer progression have been studied (6-20). ARA54 enhances AR function in a ligand-dependent manner and co-expression of ARA54 with other AR

coactivators like SRC-1 or ARA70 additively enhances AR function (10). Our previous study showed that a dominant-negative mutant of ARA54 suppresses the AR-mediated LNCaP cell growth and the expression of AR target gene prostate-specific antigen (PSA) (21). The detailed mechanisms of the AR transcriptional machinery still remain to be further elucidated. It is unknown whether these AR coregulators need interacting proteins to modulate their coregulator activity. Recently, Shields *et al.* characterized transgelin as a gene whose expression was abolished by Ras and loss of transgelin expression may represent an early event for the tumor progression in breast and colon cancers (22). Here we describe the identification of transgelin as a potential prostate cancer suppressor via inhibition of ARA54-enhanced AR transactivation and prostate cancer cell growth.

AIMS

Studies of how Tgln and hnRNP A1 (via interaction with ARA54) influence AR transactivation /target genes and AR-mediated prostate cancer cell growth and/or apoptosis.

Results and Discussion

Identification of transgelin as an ARA54 interacting protein--Full-length ARA54 was used as bait to screen its associate proteins from the human prostate cDNA library using the CLONTECH matchmaker yeast two-hybrid system. Figure 1A and 1B shows ARA54 interacted with transgelin in 3T3-L1 and COS-1 mammalian cells in the presence or absence of DHT (lane 5). In contrast, transgelin showed little interaction with AR (lane 6). To further confirm that, we demonstarted that endogenous transgelin in LNCaP cells could be co-immunoprecipitated with endogenous ARA54 using an anti-ARA54 antibody (Figure 1C).

Transgelin suppressed the ARA54-enhanced AR transactivation in mammalian cells--

In 3T3-L1, which is an ARA54-negative cell line (see Figure 3A), addition of ARA54 can further enhance AR transactivation in the presence of 10 nM DHT (Figure 2A, lanes 5 and 9 vs. 2). This ARA54-enhanced optimal AR transactivation was suppressed after addition of transgelin (Figure 2A, lanes 6, 7, and 8 vs. 5, lanes 10, 11, and 12 vs. 9). As shown in Figure 2B, lanes 13 and 14 vs. 12, addition of transgelin could effectively suppress the ARA54-enhanced mtART877A transactivation induced by E2 or HF in COS-1 cells. We further tested the effect of transgelin on the AR transactivation enhanced by other AR coregulators such as the ARA70, ARA55, SRC-1, supervillin, gelsolin, and CBP. As shown in Figure 2C, transgelin showed little effect on the AR transactivation enhanced by these coregulators except for ARA54. These data suggested that transgelin might function as a relatively specific modulator for ARA54.

ARA54 is essential for mediating transgelin's suppressive effect-- Figure 3A showed the detection of endogenous ARA54 using western blot in LNCaP but not in 3T3-L1 cells. We found that transgelin could significantly repress the AR transactivation in the ARA54-positive LNCaP cells (Figure 3B) but had only a marginal effect in the ARA54-negative 3T3-L1 cells (Figure 3C). The suppressive effect of transgelin was abolished after the silencing of endogenous ARA54 (Figure 3D and 3E, upper panel).

Transgelin blocked the interaction between AR and ARA54 as well as the ARA54

homodimerization-- We applied mammalian two-hybrid assay to test if transgelin has any effect on the interaction between AR and ARA54 as well as ARA54 homodimerization. In Figure 4A, co-transfection of pcDNA3-Flag-transgelin significantly suppressed the homodimerization interaction between pM-ARA54 and pVP16-ARA54 (lanes 3, 4, and 5 vs. 2). Androgen dependent interaction between ARA54 and AR was also suppressed by the addition of transgelin in a dose-dependent manner (lanes 8, 9, and 10 vs. 7). As control, the addition of transgelin showed little influence on the interaction between pVP16-T and pM-53 (lanes 11-14), indicating the effect of transgelin is not the result of squelching effect on transcription. These results suggested transgelin might be able to suppress ARA54-enhanced AR transactivation via interruption of interaction between ARA54 and AR as well as ARA54 homodimerization.

Small interfering RNA (siRNA) suppression of endogenous transgelin enhanced AR transactivation in LNCaP cells-- As shown in Figure 5A and 5B, transgelin expression was suppressed by more than 60% as demonstrated in real-time RT-PCR and western blot assay. As shown in Figure 5C, AR transactivation was further enhanced by 2-4 folds when the endogenous transgelin was suppressed. These results suggest that the silencing of endogenous transgelin via its siRNA may reduce transgelin's suppressive effect on AR function, which further confirmed the suppressor role of transgelin on the AR transactivation.

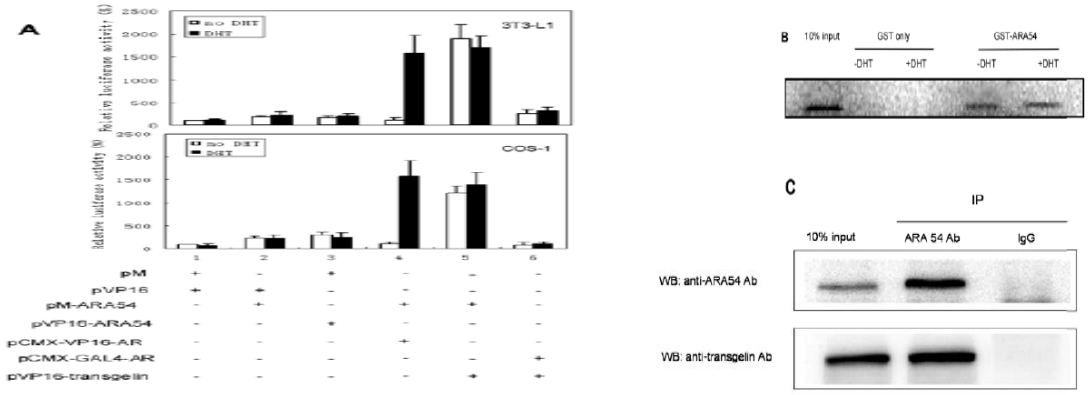

Fig.1

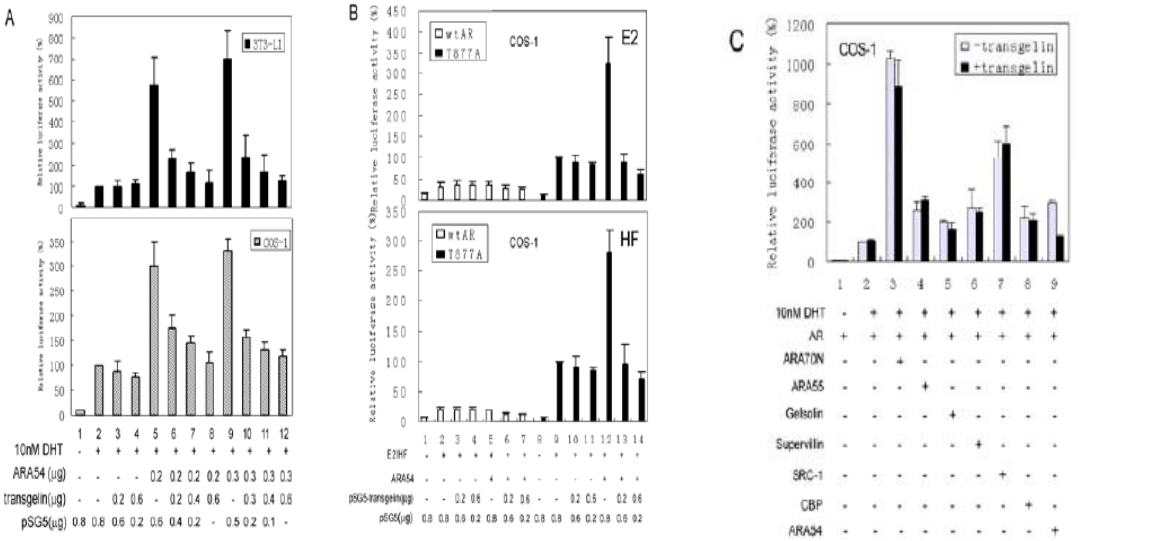

Fig.2

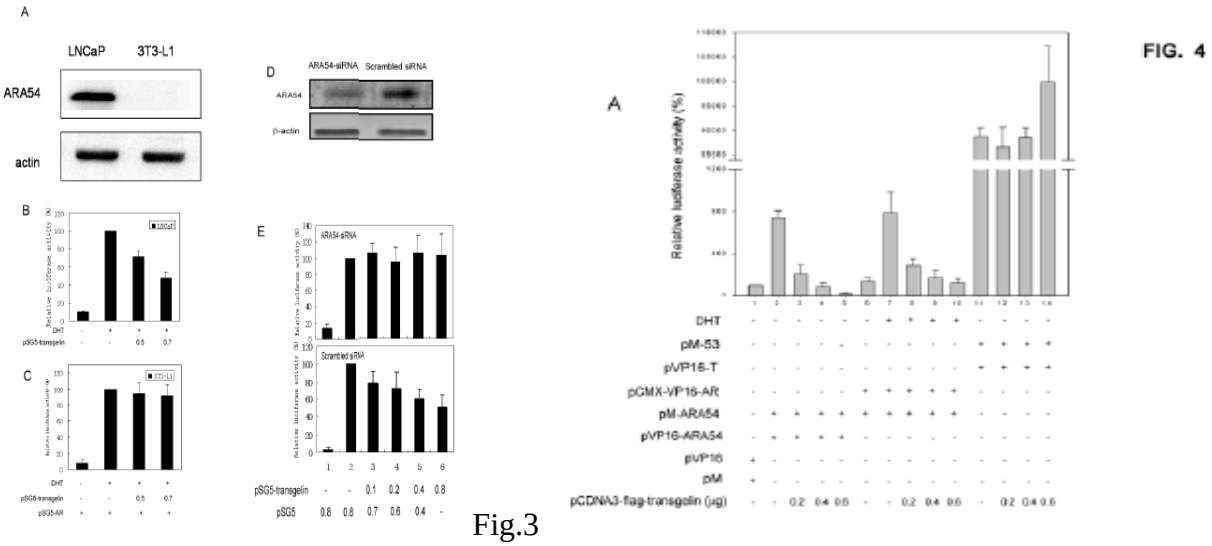

Fig.3

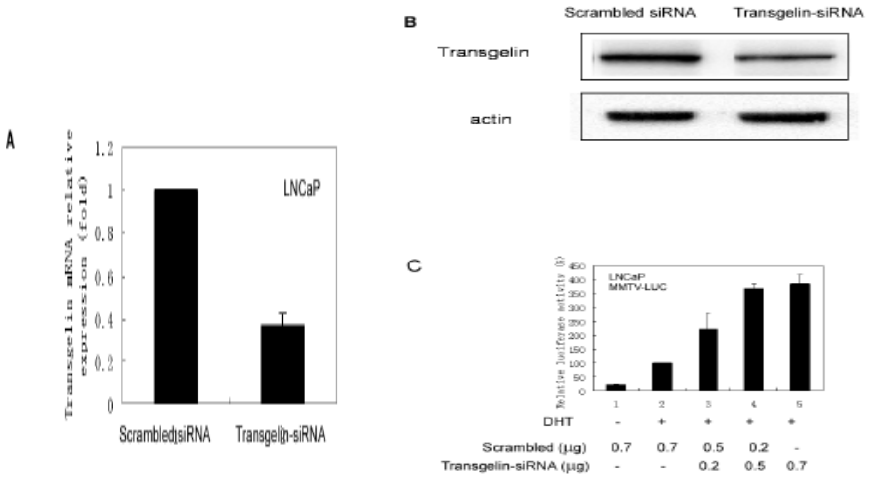

Fig.5

References

1. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ 2004 Cancer statistics, 2004. CA Cancer J Clin 54:8-29
2. Chang CS, Kokontis J, Liao ST 1988 Molecular cloning of human and rat complementary DNA encoding androgen receptors. Science 240:324-6
3. Chang CS, Kokontis J, Liao ST 1988 Structural analysis of complementary DNA and amino acid sequences of human and rat androgen receptors. Proc Natl Acad Sci U S A 85:7211-5
4. Sampson ER, Yeh SY, Chang HC, Tsai MY, Wang X, Ting HJ, Chang C 2001 Identification and characterization of androgen receptor associated coregulators in prostate cancer cells. J Biol Regul Homeost Agents 15:123-9
5. McKenna NJ, Lanz RB, O'Malley BW 1999 Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321-44
6. Hsiao PW, Chang C 1999 Isolation and characterization of ARA160 as the first androgen receptor N-terminal-associated coactivator in human prostate cells. J Biol Chem 274:22373-9
7. Fujimoto N, Yeh S, Kang HY, Inui S, Chang HC, Mizokami A, Chang C 1999 Cloning and characterization of androgen receptor coactivator, ARA55, in human prostate. J Biol Chem 274:8316-21
8. Yeh S, Chang C 1996 Cloning and characterization of a specific coactivator, ARA70, for the

androgen receptor in human prostate cells. Proc Natl Acad Sci U S A 93:5517-21
9. Wang X, Yeh S, Wu G, Hsu CL, Wang L, Chiang T, Yang Y, Guo Y, Chang C 2001 Identification and characterization of a novel androgen receptor coregulator ARA267-alpha in prostate cancer cells. J Biol Chem 276:40417-23
10. Kang HY, Yeh S, Fujimoto N, Chang C 1999 Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. J Biol Chem 274:8570-6
11. Zhang Y, Yang Y, Yeh S, Chang C 2004 ARA67/PAT1 functions as a repressor to suppress androgen receptor transactivation. Mol Cell Biol 24:1044-57
12. Wang L, Hsu CL, Ni J, Wang PH, Yeh S, Keng P, Chang C 2004 Human checkpoint protein hRad9 functions as a negative coregulator to repress androgen receptor transactivation in prostate cancer cells. Mol Cell Biol 24:2202-13
13. Lin HK, Hu YC, Lee DK, Chang C 2004 Regulation of androgen receptor signaling by PTEN (phosphatase and tensin homolog deleted on chromosome 10) tumor suppressor through distinct mechanisms in prostate cancer cells. Mol Endocrinol 18:2409-23
14. Heinlein CA, Chang C 2002 Androgen receptor (AR) coregulators: an overview. Endocr Rev 23:175-200
15. Aarnisalo P, Palvimo JJ, Janne OA 1998 CREB-binding protein in androgen receptor-mediated signaling. Proc Natl Acad Sci U S A 95:2122-7
16. Fronsdal K, Engedal N, Slagsvold T, Saatcioglu F 1998 CREB binding protein is a coactivator for the androgen receptor and mediates cross-talk with AP-1. J Biol Chem 273:31853-9
17. Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, Juguilon H, Montminy M, Evans RM 1996 Role of CBP/P300 in nuclear receptor signalling. Nature 383:99-103
18. Onate SA, Tsai SY, Tsai MJ, O'Malley BW 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354-7
19. Rowan BG, Weigel NL, O'Malley BW 2000 Phosphorylation of steroid receptor coactivator-1. Identification of the phosphorylation sites and phosphorylation through the mitogen-activated protein kinase pathway. J Biol Chem 275:4475-83
20. Alen P, Claessens F, Verhoeven G, Rombauts W, Peeters B 1999 The androgen receptor amino-terminal domain plays a key role in p160 coactivator-stimulated gene transcription. Mol Cell Biol 19:6085-97
21. Miyamoto H, Rahman M, Takatera H, Kang HY, Yeh S, Chang HC, Nishimura K, Fujimoto N, Chang C 2002 A dominant-negative mutant of androgen receptor coregulator ARA54 inhibits androgen receptor-mediated prostate cancer growth. J Biol Chem 277:4609-17
22. Shields JM, Rogers-Graham K, Der CJ 2002 Loss of transgelin in breast and colon tumors and in RIE-1 cells by Ras deregulation of gene expression through Raf-independent pathways. J Biol Chem 277:9790-9