行政院國家科學委員會專題研究計畫　期中進度報告

第二型血管張力素受器對皮質醛酮分泌和腎上腺細胞自毀的影響以及機制之探討(1/2)

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中文摘要

第二型血管張力素是體內 renin-angiotensin-aldosterone system (RAA system)非常重要的一種賀爾蒙,目前已有多種不同的受器被發現,其中第一類受器(angiotensin II receptor type 1, ATR1)與高血壓之關係,是最被大家所熟知的。目前對 ATR2 的一般功能瞭解如抑制細胞生長,促進細胞凋亡,促進細胞分化,促進神經細胞再生,抑制血管新生,促進血壓下降及情緒穩定,拮抗 ATR1 功能等。

吾人對第二型血管張力素受器第二型和 Aldosterone 之間的關係最有興趣。我們使用來自人類腎上腺皮質腫瘤的 H295R 細胞來做研究。經由 RT-PCR 我們已知 H295R 細胞並不表現 ATR2,所以我們希望能藉由使 H295R 細胞表現 ATR2,作為一個模式來研究 ATR2 在人體腎上腺所扮演的角色及訊息傳導方法。

吾人已成功的將 ATR2 成功的轉殖到 H295R 細胞,並且得到穩定的細胞株,並以第二型血管張力素處理 ATR2-H295R 細胞株,初步的結果是見到 ATR2-H295R 細胞株在經過第二型血管張力素處理之後,會造成皮質醛酮產量減少,不過仍需進一步實驗證實。

關鍵詞：第二型血管張力素第二類受器，皮質醛酮，人類腎上腺皮質腫瘤細胞-H295R cell,轉殖

ABSTRACT

Angiotensin II is an important hormone of renin-angiotensin-aldosterone system (RAA system). Many types of Angiotensin II receptors has been found, in which, type

I is best known due to its relationship to hypertension. In previous studies, we have known that there are several different physiological functions between ATR1 and ATR2. The general functions of ATR2 are: inhibition of and cell growth, stimulate cell apoptosis or differentiation, regenerate neural cell, inhibit angiogenesis, stabilize blood pressure, emotion and antagonizes ATR1 physiologically. We take H295R cell, a cell line derived from human adrenocortical carcinoma which was documented to have no ATR2 by RT-PCR. So we set up a model of ATR2 transfected H295 cell to explore the roles of ATR2 in adrenal cortex and its signal transduction.

So far, we have transfected ATR2 into H295R cell, and get stable cell line. Then, we treat ATR2-H295R cell with angiotensin II. The initial result is that aldosterone production decrease after ATR2 transfection, but we need further experiment to confirm this.

Keywords: Angiotension II receptor type 2, aldosterone, human adrenocortical carcinoma cell-H295R cell, transfection

INTRODUCTION

ATR2 has been cloned from several species, including human beings. As we know about ATR2, it couples to G proteins and has 7 trans-membranous domains. The molecular weight is about 41220 Dalton. ATR2 has 34% sequence homology with ATR1.

ATR2 is widely expressed during fetal development, but it regresses or disappears rapidly in early postnatal period except limbic system, several thalamic nucleus, adrenal medulla, zona glomerulosa, uterine myometrium etc. So, ATR2 was also suspected to involve in fetal development.

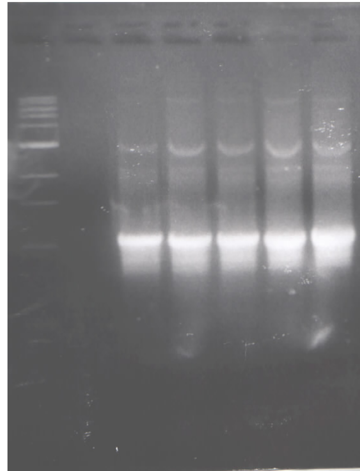
In adult adrenal gland, 40% of ATR2 is present in the zona glomerulosa; however, the significance of persistent expression of ATR2 is unknown. Besides, ATR2 mRNA was found in aldosterone-producing adenoma (APA), but H295R cell (derived from human adrencortical carcinoma) lacks ATR2 expression. The role of ATR2 in tumorigenesis was also doubtful.

We will clone ATR2 into an inducible vector (pIND(SP1)Hygro/pVgRXR), and transfec cloned vector into H295R cells to explore the effects on cell growth/apoptosis, aldosterone secretion. The mRNA and protein expression of ATR2 will be assessed by RT-PCR and Western blotting. Aldosterone concentration will also be analyzed. Finally, we will explore its signal transduction pathway.

RESULTS

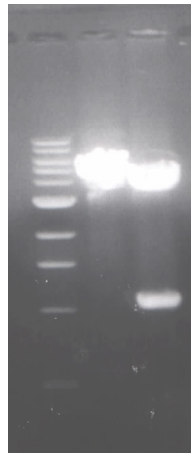
The encoding region of human ATR2 is amplified by RT-PCR with the primers of

AT2R2a with a BamHI cutting site (ATTGGATCCGATGAAGGGCAACTCCACCCTT); AT2R2b with a HindIII cutting site(ATTAAGCTTATTAAGACACAAAGGTCTCCAT). The PCR fragment was cloned into pRSET-B or pCMV-Tag 2A. (Fig 1a & Fig 1b)



M 1 2 3 4 5 6

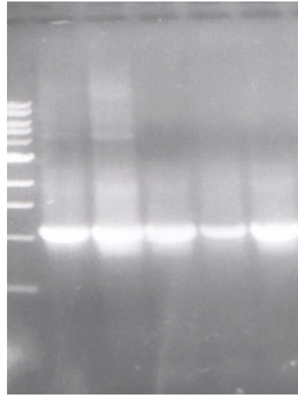
Fig 1a. ATR2 cDNA was cloned into pREST-B by RT-PCR (Lane M: 1Kb Marker Lane 1: Negative control, Lane 2~6: ATR2- pREST-B clone).



M 1 2

Fig 1b. ATR2 cDNA was cloned into pCMV-Tag 2A by enzyme double digestion (Lane M: 1Kb Marker, Lane 1: ATR2-pCVM-Tag 2A clone [uncut], Lane 2: ATR2-pCVM-Tag 2A clone [cut]).

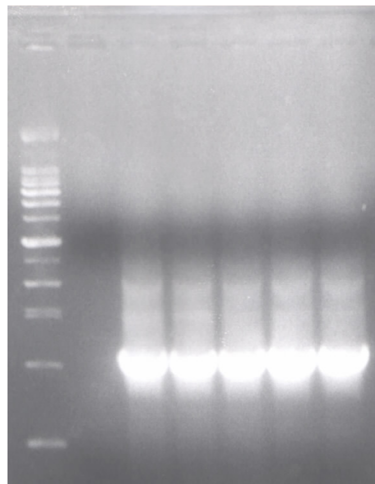
The AT2R2 accompanied with a His-tag in pRSET-B was amplified with the primers SETc3 with NheI cutting site (TTTGCTAGCTATGCGGGGTTCTCATCATC); SETb3 with ApaI cutting site (ATTGGGCCCGCTAGTTATTGCTCAGCGGTGG). The product was then cloned into the expression-inducible vector (pIND(SP1)Hygro/lacZ, *Invitrogen*^{TM})



M 1 2 3 4 5

Fig 2. ATR2 cDNA was cloned into pIND(SP1)Hygro/lacZ by RT-PCR (Lane M: 1Kb Marker, Lane 1 ~5: ATR2- pIND(SP1)Hygro/lacZ clone)

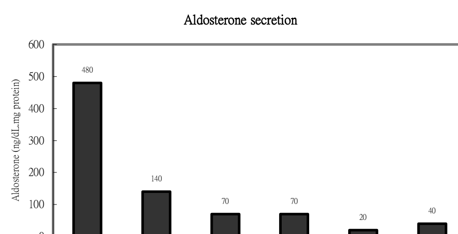
We prepared H295R cells which were plated at 50-60% confluent on 6-well plates with DMEM/F12 medium without serum. These cells were transfected with ATR2-pCMV-Tag 2A or, co-transfected with pVgRXR and ATR2-pIND(SP1)Hygro/lacZ by LipofectAMINE^{TM} 2000 Transfection Kit.



M 1 2 3 4 5 6

Fig 3. The ATR2 gene detected by RT-PCR analysis in H295R/ ATR2-pCMV-Tag 2A clones (Lane M: 1Kb Marker, Lane 1: H295R control cells, Lane 2~6: H295R/ ATR2-pCMV-Tag 2A transfected cells).

Then we treated ATR2-pCMV-Tag 2A transfected H295R cells and normal control H295R cells with angiotensin II respectively. Aldosterone concentration and total protein were analyzed.



ATR2	-	-	-	+	+	+
AgII	+	+	+	+	+	+

Fig 4. The Aldosterone secretion from H295R or H295R/ ATR2-pCMV-Tag 2A clones (treated with Ag II 10^{-6}M).

DISCUSSION

We transfected ATR2-pCMV-Tag 2A into H295R cell successfully and treated with angiotensin II. There is a trend that ATR2- transfected cell produced less aldosterone than normal control cell. We did not know exactly that the phenomenon was related to ATR2-induced cell apoptosis or other causes. If over-expression of ATR2 induces cell apoptosis, DNA fragmentation should be detected, however, we failed to demonstrate. Can ATR2 transfection induced cell apoptosis be questioned? We think this question only can be answered by using more specific way to detect DNA fragmentation. In apoptotic cell, cleaved caspase-3-7 will be detected. It is the evidence of cell apoptosis.

To evaluate the effect of ATR2 on cell function, we intend to block ATR1 by ATR1 blocker (valsartan) and treat control cells and ATR2-transfected cells with angiotensin II (10^{-7}~10^{-9}M) and specific ATR2 agonist, antagonist (PD123319) respectively. Valsartan is a highly specific ATR1 antogonist (blocking ratio is ATR1/ATR2= 20000/1), we use valsartan to block ATR1 and make added angiotensin II acts on ATR2 to evaluate the response by assessing ATR2 mRNA, total protein production, aldosterone concentration and aldosterone/total protein ratio. Further experiments will also be done by blockage ATR2 or ATR1 + ATR2.

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