Expression of androgen receptor mRNA affected by the functions of lung and trachea in animal model of Kunming mouse

Shuling Wang[1], Xiufang Chen[2]

[1]*College of Basic Medical Science*, *Zhengzhou University*, *Zhengzhou*, *Henan* 450001, *China*; [2]*Henan Xian Dai Medical Research Hospital*, *Zhengzhou*, *Henan*, *China*

Received January 24, 2009

Abstract

In this article, we demonstrate the experimental results of the expression of androgen acceptor mRNA correlate with the functions of lung and trachea in an animal model. This study depicts androgen acceptor as the connection between lung and kidney in Traditional Chinese Medicine. [Life Science Journal. 2009; 6(2): 48 – 50] (ISSN: 1097 – 8135).

1 Introduction

The function of lung is to breathe for the body. In traditional Chinese medicine, it belongs to gold in five lines. In contrary, kidney belongs to water in the concept of the five elements (of metal, wood, water, fire, and earth) used in ancient Chinese cosmology and later in herb medicine five lines. Based upon the philosophy of Traditional Chinese Medicine, elements in five lines are correlated to each other. Therefore, if the lung and kidney mutually being affected in pathology is true[1,2], the function of sex hormone can be through the special acceptor in target cell cytoblastema[3]. By using the real time polymerase chain reaction (RT-PCR) and the technique of TaqMan fluorescence examination for mouse trachea in lung organization to see if any androgen acceptor/Androgen Receptor (AR) mRNA expression can be found being compared to the AR mRNA founded in mouse testicle organization may be able to reveal the correlation between the lung and kidney. Also, by using immunity histochemical method carries of the AR being in lung is highly correlated to the function of kidney. The androgen level appearing the modification by kidney may be influenced through its acceptor in lung.

2 Materials and Methods

Ten animals of two months old healthy male Kunming mice with body weight 180 g to 200 g for the experiment of checking expression of AR mRNA in lung and trachea were provided by Henan Province Experimental Animal Center. The animal was anaesthetized by abdominal cavity injection with chloramine alkone (3 mg per Kg). Organs of trachea, lung and the testicle were rapidly taken and chopped to preserve in liquid nitrogen at – 70 ºC. Taking 50 mg sample for each organ from the freezer and putting it into 800 μl (10^{-6} liter) reagent A (guanidine thiocyanate-phenol solution) for vibrating 30 seconds, adding 200 μl reagent B (the chloroform: isoamyl alcohol in 24 : 1) for being in 14000 rpm centrifugal for 5 minutes. Supernatant should be carefully drawn 400 μl to mix with 400 μl isoamyl alcohols. Taking 50 μl mixed solution for 14000 rpm centrifugal 10 minutes, we draw off supernatant and added 500 μl 75% ethyl alcohol to sediment shaking uniformly and put it for 14000 rpm centrifugal 5 minutes. Then, we draw supernatant again to get the sediment in dry being ready for use. In the mean time, for each test tube, first making reverse transcript reactive solution by adding 1.5 μm random primer 5'-CTACTGCGCT-3' and 0.3 μm primer 5'-AGGCAGCTGCTCAGGGTGGC-3' mixed with buffer, and then 20 μl solution was taken to mix with 50 μl MLV keeping in 37 ºC incubator for an hour.

For reverse transcript reaction, each tube was added PCR reactive mixture 20 μl and 50 μl MLV-reverse transcriptase. For PCR reaction, making PCR reactive mixture including buffer solution, 2 mM $MgCl_2$, 200 μm

*Corresponding author. Email:

dNTP, 0.3 μM (primer 2) mixed with buffer as well as 0.3 μM 5'-TTACAGCAGAGGCAGGAGACT-3' (primer 1) and 1% off-ion formamide, taking 26 μl reactive mixture solution and 2 μl reverse transcript reactive solution and then added with 2 μl Taq DNA polymerase. Parameters used for the reaction were 94 ºC pre-denaturation for 5 minutes, 94 ºC for 45 seconds, 55 ºC for 45 seconds, 72 ºC for 45 seconds with 35 cycles and expended for 72 ºC for 5 minutes.

After preparing mixture solution by diluting one tenth of the product that was through PCR procedures, 15 μl mixture solutions were taken for 2% Agarose gel electrophoresis checking (0.05% Bromination second grade spindle at voltage of 5 V/cm). To isolate AR, 50 mg sample of mouse testicle was taken for experiment. The segment specificity of the sample can be shown at wavelength of 302 nm under ultraviolet exam. Meanwhile, male specimen has shown 260 bp in size at specificity of DNA segment.

In preparing of DNA specificity segment in tube for centrifuge and lysate being incubated at 37 ºC – 55 ºC for uniformly melting, we can mix 800 μl lysate with DNA, put the mixture in incubation at 55 ºC for 15 minutes, centrifuge at 14000 rpm for thirty minutes, take off supernatant fluid and add 75% alcohol, centrifuge again for another 30 seconds, take off supernatant fluid and add 600 μl TE buffer at 55 ºC gently for 15 minutes, put in 14000 rpm centrifugal for 3 minutes, dilute for 10 times dilution for fluorescent. The PCR amplification can be done by taking 2 μl mixtures for the process. In the mean time, Fluorescence PCR real time reaction for checking system can be performed by taking 2.5 μl buffer, 8 mM $MgCl_2$ and 100 μM dNTP, 2% as well as 3 pM fluorescent probes, 15 pM primer 2, double evaporate 2 μl template being added together with 50 μl distill water.

3 Results

In normal male Kunming mice, we investigated the expression of AR mRNA in trachea, lung tissue and testicles. The lowest expression of AR mRNA was found in lung. Real-time quantitative fluorescence PCR detection results show the *Ct* values at 9.880 ± 8.01, 12.831 ± 5.632 and 22.298 ± 2.35 in testicular and lung (Table 1). From the analysis of gel electrophoresis, size of 260 bp band of AR appearance of the specific segment is revealed.

Figure 1 to Figure 5 depicts the expressions of AR in testis of normal male rat, AR in testis of normal male rat, testis of male normal rat, expression of AR in trachea of normal male rat and the expression of AR in lung of normal male rat.

Table 1. the expression of AR in the trachea and lung of normal male rat

Tissues	*n*	Mean ash density	Mean optical density
Trachea	10	128.1 ± 4.11**	0.225 ± 0.04**
Lung	10	140.98 ± 9.58	0.15 ± 0.05

Compare the trachea with that of lung, **: $P < 0.01$.

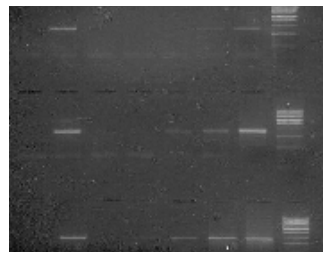

Figure 1. The expression of AR mRNA in the lung, testes and trachea of normal male mice.

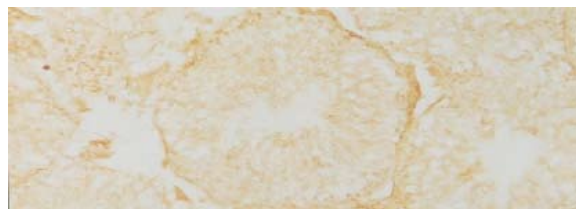

Figure 2. The expression of AR in testis of normal male rat (× 400). AR positive cell mainly appeared in the testis convoluted seminiferous tubule.

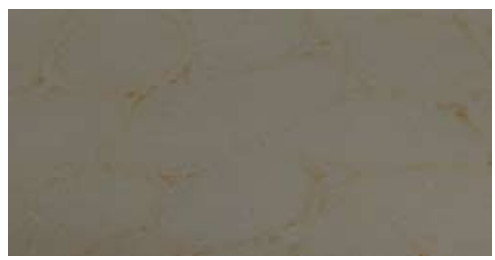

Figure 3. The testis of male normal rat (negative control) (× 400). This is PBS instead of first antibody (There were no AR positive cell in the testis of rat).

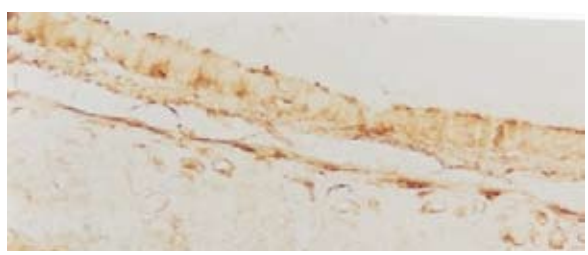

Figure 4. The expression of AR in trachea of normal male rat (× 400). AR positive cells appeared in the pseudo ciliated columnar epithelial cells and cartilage cell of male wistar trachea.

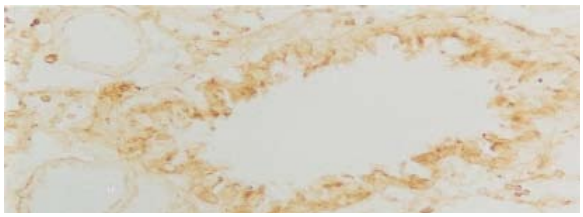

Figure 5. The expression of AR in lung of normal male rat (× 400). AR positive cells appeared in the ciliated columnar cell of each grade bronchus.

4 Discussion

These findings showed the AR mRNA expression can be observed in normal male mouse trachea and lung. Using of RT-PCR and the technology of molecular level, we found the expression of AR mRN in trachea. The AR mRNA expression quantity was stronger in testicle but lower in trachea and lung. The androgen may proliferate to enter the target as well as non-target organization, but only function in the target cells which the androgen receptor exists. Furthermore, the steroid hormone acceptor is located in the cell with AR. The androgen and its acceptor could be the bases of traditional Chinese medicine "the lung kidney mutual promotion of the five elements" and "the kidney host air intake".

The traditional Chinese medicine says that, the lung is the gold in five lines, the kidney is the water in five lines, between the lung kidney the passages through which vital energy circulates is connected. The kidney fine may moisten the lung in cloudy and the kidney may warm the lung. The androgen has the function through its acceptor to the lung and possibly being in the kidney as a warm aspect to the lung. Due to the decrease in the level of androgens, AR directly may affect its target organs for the senior. It may be one of the reasons of chronic bronchitis for senior being significantly higher than that of the incidence of young people[12]. According to the results of this study, many diseases such as asthma, lung cancer and other diseases for the seniors may have occurred with low level sex hormones in the trachea and lungs. Adjust the abnormal level of AR is probably the prevention and treatment of respiratory disease for seniors. Nonetheless, the function of body system is by various factors, and the adjustment mechanism is very complicated. Renal through its receptor on the lungs of androgens can adjust function of "kidney associated".

5 Conclusion

Our study found that the AR mRNA expressions in trachea and lung tissue in male normal mouse. The expression with AR mRNA is highest in testicular but lower in trachea and lowest lung tissue. AR expression in trachea and lung confirmed that trachea and lung are androgen target organ. Adjustment androgen and its acceptor level possibly can be the prevention and treatment for respiratory disease. Therefore, the androgen must have the influence like the traditional Chinese medicine says "the lung kidney mutual promotion of the five elements". The "kidney host air intake" can also possibly be one of material bases through its acceptor to the trachea and the lung to invigorate the kidney as one of the organ affecting respiratory disease.

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