

# Expression of Coxsackie and Adenovirus Receptor and its Significance in Human Lung Cancer

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**OBJECTIVE** To study the relationship between the coxsackie and adenovirus receptor (CAR) and the development of human lung cancer. To optimize adenovirus vector-based gene therapy.

**METHODS** The expression of CAR in 112 cases of lung cancer was examined using immunohistochemistry. At the same time, the relationship between CAR expression and clinicopathologic characteristics was analyzed.

**RESULTS** There is a little expression of CAR in normal lung tissue. Compared with paraneoplastic epithelial tissue of the lung, the expression of CAR is generally up-regulated in tumor tissues showing a significant difference ( $P < 0.01$ ). The positive rate of CAR expression in squamous cell carcinoma was 43.1%, and in adenocarcinoma 70.2%, with the difference between the two rates being statistically significant ( $P < 0.01$ ). Compared to the paraneoplastic tissues, the difference in CAR positive expression was 35.4% for squamous cell carcinoma and 38.3% for adenocarcinoma. But the difference in different stages of squamous cell carcinoma had no statistical significance ( $P > 0.05$ ). However, the expression of CAR was at a high level in the bronchioalveolar carcinomas as 80.4% were CAR positive. This research showed that there was a specially high expression of CAR in adenocarcinomas.

**CONCLUSION** CAR is expressed in human lungs at a low level and up-regulated in the tumor tissues, suggesting that there is a relationship between adenocarcinoma and CAR. This research provides a basis for planning a regimen of gene therapy using an adenovirus vector.

**KEYWORDS:** coxsackie and adenovirus receptor/CAR, lung cancer, gene therapy.

## INTRODUCTION

Lung cancer is one of the most common human malignancies, which is increasing in its incidence and morbidity. Angiogenesis of tumors plays an important role in all phases of tumorigenesis, development, infiltration and metastasis of solid tumors, and significantly influences the cancer biological behavior and prognosis of the patients<sup>[1]</sup>.

The coxsackie-adenovirus receptor (CAR) is a transmembrane receptor of an immunoglobulin superfamily that shows tissue and species specificity<sup>[2]</sup> and has a relationship with age<sup>[3]</sup>. The coxsackie adenovirus receptor (CAR) has primarily been studied in its role as the initial cell surface attachment receptor for coxsackie and group C adenoviruses. Recent reports suggest that CAR is associated with many tumors such as breast carcinoma, osteogenic sarcoma, fibroma sarcomatosum, hepatoma and so on. Many researchers<sup>[4,5]</sup> have characterized CAR as a global tumor suppressor. Others<sup>[6-8]</sup> have shown that CAR can help the development of

non-small cell lung cancer in vitro. At the same time, it is proposed that the expression of CAR is related to tumor proliferation, and in forming the cell types of a lung cancer. Our research was conducted to test the relationship between CAR and lung cancer by retrospective analysis of excised human lung cancer specimens.

## MATERIALS AND METHODS

### Selection of the cases

A total of 112 lung cancer cases, who were treated from January 2006 to December 2006, were selected for analysis. There were 81 male and 31 female patients of ages from 40 to 78 years, with an average of 62. According to the 2004 WHO Lung Cancer Classification, there were 65 squamous cell carcinomas and 47 adenocarcinomas. The histological stages of the squamous cell carcinomas were: I,11; II,42; and III,12.

### Contrast materials

All cases had excised paraneoplastic epithelial tissue which was 5 cm from the tumor tissue.

### Reagents

Human anti-CAR monoclonal antibody (Catalog 05-644) was purchased from the Upstate Co. USA; Streptavidin-peroxidase and DAB was obtained from Maixin. Bio, Beijing.

### Immunohistochemistry

Samples were fixed in formalin, embedded in paraffin and sectioned for preparation of polylysine-coated microscopy slides for immunohistochemical analysis. The sections were deparaffinized in xylene, 3× for 10 min, then dehydrated in a descending series of ethanol (100%, 96%, 70%), followed by washes in TBS (0.05 mmol/L Tris-buffer physiological saline, pH 7.4~7.6), 3× for 5 min. Antigen retrieval was achieved by heating the samples without boiling in 10 mmol/L sodium citrate buffer, pH 6.0 (200 ml) in a microwave oven. The incubation with the human anti-CAR antibody was conducted at 4°C overnight. After the incubation, the second and the third antibodies were added and the samples were held in a humid atmosphere for 30 min.

The final staining was conducted in a diaminobenzidine tetrahydrochloride (DAB) solution (49 ml TBS-buffer, 34 mg imidazole, 17 µl 30% hydrogen peroxide and 1 ml 30% DAB) for 5 min. The slides were washed with distilled water, with 70% ethanol for 1 min, and then with distilled water. The nuclei

were stained with Mayer's hemalum stain for 30 s. The slides were then transferred through an ascending ethanol series, and xylene before mounting.

### Assessment of the staining

All slides were examined, counted and assessed by two chief pathologists. Five high power fields (×400) were selected for counting the tumor positive cells at random. We scored both frequency and intensity of the positive-stained cells on an increasing categorical scale. The frequency score was: (0) for no positive-stained cells and (1+), (2+) and (3+) for positive cell frequencies at <20, 20~50, and >50%, respectively. The intensity score was: no staining/background of the negative control (0), weak staining above background (1+), moderate staining (2+), and intense staining (3+).

### Statistical analysis

The SPSS10.0 software package was used for statistical analysis using ANOVA ( $P<0.05$ ).

## RESULTS

In all of the 112 cases CAR staining was found on the cell membrane of the epithelial or tumor cells without intracellular staining.

### The expression of CAR in squamous cell carcinoma

Among the 65 squamous cell carcinomas, there were 28 CAR (+~++++) positive cases, with a positive rate of 43.1% (Table 1, Fig.1,  $\chi^2=21.4839$ ,  $P<0.01$ ). The results showed that there was a statistically significant difference in CAR expression between the tumor tissue and paraneoplastic epithelial tissue (a difference of 35.4%).

**Table 1. The expression of CAR in squamous cell carcinoma.**

Squamous cell carcinoma (number)	CAR expression		Positive rate (%) <sup>*</sup>
	—	+~+++	
Primary cancer(65)	37	28	43.1
Paraneoplastic(65)	60	5	7.7

<sup>\*</sup>Rates of expression were significantly different ( $P<0.01$ ).

### The expression of CAR in adenocarcinomas

There were 33 CAR (+~++++) positive cases among the 47 adenocarcinomas. The positive rate was 70.2% (Table 2, Fig.2,  $\chi^2=13.7935$ ,  $P<0.01$ ). These results showed that there was a statistically significant difference between the tumor tissue and the paraneoplastic

epithelial tissue (a difference of 38.3%).

**Table 2. The expression of CAR in adenocarcinoma.**

Adenocarcinoma (number)	CAR expression		Positive rate (%)*
	—	+~+++	
Primary cancer (47)	14	33	70.2
Paraneoplastic (47)	32	15	31.9

\*Rates of expression were significantly different ( $P<0.01$ ).

**The expression of CAR in subtypes of lung cancer**

Table 3 shows that there was a statistically significant difference in the rate of CAR expression between squamous cell carcinoma and adenocarcinoma,  $\chi^2=8.0987$ ,  $P<0.01$ . Expression in adenocarcinoma was 27.1% higher than squamous cell carcinoma.

**The expression of CAR in different stages of squamous cell carcinoma**

In Table 4, it can be seen that there was no significant difference in the rates of CAR expression among the different stages of squamous cell carcinoma.  $\chi^2=3.3682$ ,  $P>0.05$ .

**Table 3. The expression of CAR in the subtypes of lung cancer.**

Subtype of lung cancer	CAR expression		Positive rate (%)*
	—	+~+++	
Squamous cell carcinoma	37	28	43.1
Adenocarcinoma	14	33	70.2

\*Rates of expression were significantly different ( $P<0.01$ ).

**The expression of CAR in bronchioalveolar carcinoma**

There were 32 cases of bronchioalveolar carcinoma in the mixed adenocarcinomas, CAR expression was positive in 27 cases (84.4%) and 5 cases were negative.

**Table 4. The expression of CAR in different stages of squamous cell carcinoma.**

Stages	CAR expression		Positive rate (%)*
	—	+~+++	
I (11)	9	2	22.2
II (42)	22	20	47.6
III (12)	6	6	50.0

\*Rates of expression were not significantly different ( $P>0.05$ ).

**DISCUSSION**

With development of the technology, many gene therapy programs have moved into clinical trails. Gene therapy using adenovirus vector is developing rapidly because of the advancement of the technology and easy application. Ad-p53 has been employed in the treatment of many kinds of tumors where the adenovirus enters the cells through CAR.

CAR is a transmembrane receptor of the immunoglobulin superfamily which shows both tissue and species specificity<sup>[2]</sup>. It is expressed at a high level in the pancreas, prostate and small intestine, at a low level in the liver and lungs, and almost absent in the kidney, thymus and spleen. Hotta et al.<sup>[8]</sup> showed that there is a relationship between the level of CAR expression and age, i.e. there is a high level at birth and then it falls to almost no expression in the adult<sup>[8]</sup>.

CAR has primarily been studied concerning its role as the initial cell surface attachment receptor for coxsackie and group C adenoviruses. Recent reports suggest<sup>[9]</sup> that CAR is associated with many tumors such as breast carcinoma, osteogenic sarcoma, fibroma sarcomatosum, hepatoma and so on. Okegawa et al.<sup>[10]</sup> found that CAR expression was lacking or down-regulated in bladder carcinoma. Therefore, many researchers<sup>[4,5]</sup> have characterized CAR as a global tumor suppressor. However, CAR mRNA was expressed at high levels in osteosarcoma<sup>[11]</sup>, Ewing's sarcoma, neurofibroma and schwannoma<sup>[12]</sup>. Others

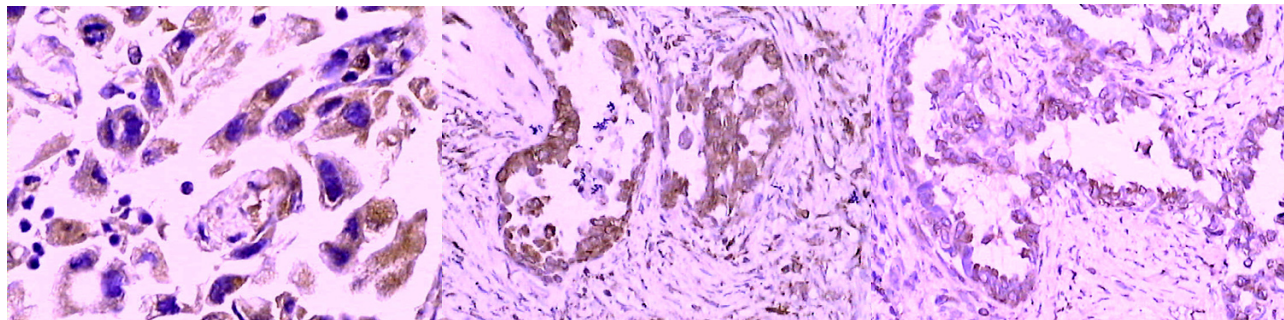


Fig.1. Squamous cell carcinoma.

Fig.2. Adenocarcinoma.

Fig.3. Bronchioalveolar carcinoma.

<sup>[13-17]</sup> have shown that CAR can help the development of non-small cell lung cancer in vitro. At the same time, it is proposed that the expression of CAR is necessary for tumor proliferation, and in forming the cell type of a lung cancer.

In our study, we analyzed CAR expression in primary cancer tissues, and paraneoplastic epithelial tissue of 112 lung cancer cases. There was only a little expression of CAR in normal lung tissue. Compared with the paraneoplastic epithelial tissue of the lungs, the expression of CAR is generally up-regulated in the tumor tissues. The difference in the rate of expression in squamous cell carcinoma was 35.4%, and in adenocarcinoma 38.3%, a significant difference ( $P < 0.01$ ) suggesting that there is a relationship between lung cancer and CAR. The positive rate of expression in squamous cell carcinoma was 43.1%, and in adenocarcinoma 70.2%, again a statistically significant difference ( $P < 0.01$ ). However, the difference in different stages of squamous cell carcinomas had no statistical significance ( $P > 0.05$ ). On the other hand, 80.4% of bronchioalveolar carcinomas were CAR positive.

Bronchioalveolar carcinoma grows along the bronchovesicular wall, resections are often not successful, and the tumor is resistant to chemotherapy. Our research provides a basis for planning a regimen of gene therapy using an adenovirus vector to treat bronchioalveolar carcinoma by dripping the virus vector through an endotracheal tube and it could supply the conventional lung cancer therapy.

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