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Research Article

# Type II Alveolar Epithelial Cells in Remodeled Areas of Idiopathic Pulmonary Fibrosis/ Usual Interstitial Pneumonia

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- Telomerase
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**Abstract**

In our previous study we showed that in normal areas of usual interstitial pneumonia (UIP), type II alveolar epithelial cells telomerase positive (AEC2T+) expression was significantly inferior when compared to normal lung tissue (NLT). Nevertheless, the density of AEC2 was the same in both UIP and NLT. An inversely significant correlation was observed between AEC2 density and AEC2 apoptosis in this area. These results led us to suggest that the pathogenesis of UIP after numerous mitoses is that AEC2 T- exhausts its telomeres and enters into apoptosis, in patients with small subpopulation of AEC2 T+. If this is correct, AEC2 T+ prevails in the remodeled areas of UIP. Thus, we hypothesized that in remodeled areas, AEC2T+ is predominant.

**Material and Methods:** We studied 24 open lung biopsies (12 male and 12 female) from our previous study with IPF/UIP disease [4]. Immunohistochemistry (IHC) analysis was performed to identify AEC2 by surfactant A protein and anti-telomerase antibodies. AEC2 density and telomerase expression in the remodeled areas of UIP were assessed at different time points in ten fields using the point-count technique. Forced vital capacity (FVC) was measured by Collins's computer spirometer in 15 patients

**Results:** The mean of AEC2 and with Telomerase did not present significant difference. There was a significant positive correlation between AEC2T+ density and FVC- %.

**Conclusion:** Our interpretation on the pathogenesis of IPF/UIP was confirmed by the predominance of AEC2T+. A positive correlation was found between AEC2T+ and FCV%.

## INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a disease characterized by progressive dyspnea and hypoxemia, restrictive pattern and decreased lung diffusion in pulmonary function tests. It occurs in patients at age sixty to seventy and despite treatment given, fatal evolution occurs after 2.5 to 3.5 years (1-3).

The histopathological pattern of IPF is Usual Interstitial Pneumonia (UIP) characterized by normal lung fibrosis, honeycomb changes, fibroblastic foci and alveolar collapse [1-3].

In a previous study of our research group we learned that although the density of AEC2 in normal areas of IPF/UIP was equal to normal lung tissue, type II epithelial alveolar cells telomerase positive (AEC2T+) expression was significantly reduced. Additionally, AEC2 density and AEC2 apoptosis were inversely correlated in a significant manner in the same areas [4].

Our interpretation of the pathogenesis of IPF/ UIP was that the AEC2T- after numerous mitosis exhausts its telomeres and enters into apoptosis. The main objective of this study is to verify the AEC2T+ predominance in the remodeled areas of UIP.

## MATERIAL AND METHODS

This research was approved by the Institutional Ethical and Scientific Committees of the University of São Paulo Medical School (FMUSP). The study included 24 open lung biopsies of patients (12 males and 12 females) with a mean age of 66 years (49 to 77 years), according to the criteria outlined in the American Thoracic Society/European Respiratory Society International

Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias [2]. Usual interstitial pneumonia (UIP), the histological pattern of IPF, was characterized by temporal heterogeneity with alternating areas of normal lung parenchyma, alveolar collapse, fibromyxoid tissue (fibroblastic foci, FF) and honey combing.

### Spirometry

The forced vital capacity (FVC) was realized on Collins's computer spirometer in 15 patients. The predicted value was of Knudson RJ [5].

### Immunohistochemistry Analysis

Immunohistochemistry analysis was performed to characterize AEC2 density [antibody surfactant apoprotein-A (SP-A), code scl3977; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; 1:6000 dilution] and telomerase expression (Calbiochem & Oncogene., Darmstadt, Germany; 1:250 dilution). In brief, the sections were deparaffinized and rehydrated with Tris-buffered saline (TBS: 0.0005 M tri. 0.15 M NaCl ), pH 7.6 for 10 minutes. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 5 minutes. Next, they were washed in TBS and incubated with primary antibodies at the appropriate dilutions for one hour. Biotinylated anti-mouse IgG was used as a secondary antibody (DAKO) followed by peroxidase-conjugated streptavidin (DAKO). The peroxidase reaction was developed using 3, 3 diaminobenzidine tetrachloride (0.25 mg dissolved in 1 ml of 0.02% hydrogen peroxide) for 3 minutes.

### Morphometry

AEC2 SP-A+ and AEC2T + were assessed in ten fields by point-count technique in remodeled areas of UIP, using a 100-point grid with a known area (62500 $um^{2)}$ at an 400x magnification attached to the ocular of the microscope [6]. At 400X magnification, the AEC2 SP-A+ and AEC2T+ in each field of remodeled areas was quantified according to the number of points hitting on positive cells. The density of these cells was determined as the number of positive cells divided by the remodeled area of each field. The final results were expressed as a percentage. These quantifications were obtained at different moments.

Interobserver comparisons were performed in 20% of the slides by two observers (ERP ad VLC). The variation coefficient for the interobserver error regarding cell count was < 5%. (Figures 1,2).

### Statistical Analysis

Statistical significance was evaluated using the independent t test for comparisons between AEC2 SP-A + and AEC2T+ expression.

Pearson test was used to study the relation between predicted percent of FVC and AEC2T+ density in 15 patients. The statistics program used was SPSS 18.0 (SPSS Inc., Chicago. IL, USA). A p value less than 0.05 was interpreted as statistically significant.

## RESULTS

No difference was observed between AEC2 SP-A+ (6.07 ± 2.03%) and AEC2 T+ (6.63 ± 2.04%) density by independent t test (p=0.35). The correlation between AEC2T+ (6.62 ± 2.20%) density and FVC predicted percent (65.70 ±10.31%) with the regression test was positive and significant (R=0. 637 p=0.01), Figure 3.

## DISCUSSION

In our former research study, we found that AEC2T+ density

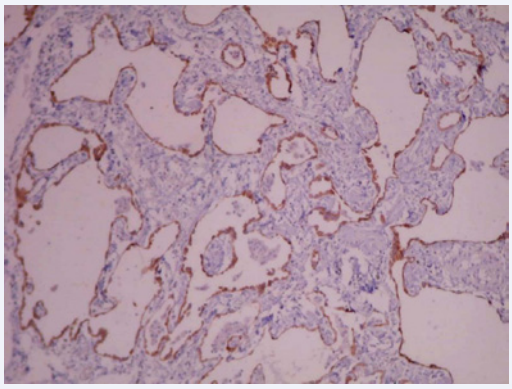

**Figure 1** Remodeled area with AEC2SP-A .100X magnification.

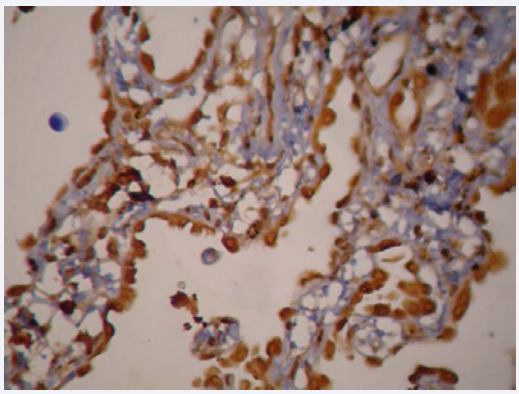

**Figure 2** Remodeled area with AEC2T+ .200X magnification.

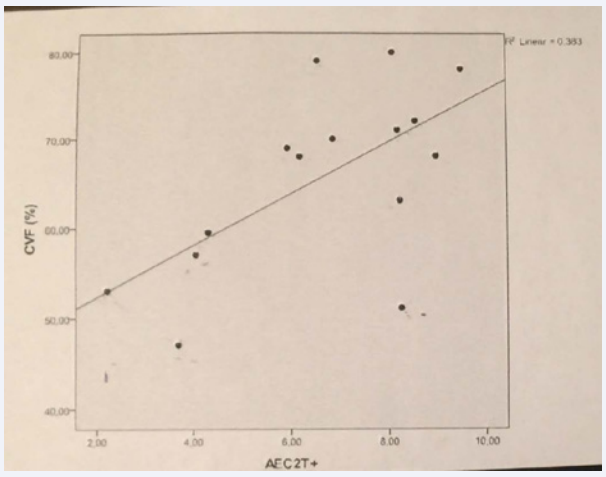


**Figure 3** AEC2T+X FVC%.

**Table 1:** Patient data and results.

| Nº | Name | Sex | Age | AEC2(SPA) | AEC2T+ | FVC (%) |
|---|---|---|---|---|---|---|
| 1 | JCC | M | 75 | 6.74 | 7.61 | |
| 2 | APC | F | 66 | 8.15 | 7.68 | |
| 3 | MCI | F | 74 | 4.68 | 7.39 | |
| 4 | SCC | M | 52 | 4.92 | 6.16 | 68 |
| 5 | TMR | F | 72 | 7.51 | 8.97 | 67 |
| 6 | ECA | F | 72 | 8.61 | 8.26 | 51 |
| 7 | AA | M | 55 | 4.93 | 7.36 | |
| 8 | MQS | M | 65 | 7.50 | 6.24 | |
| 9 | JN | M | 49 | 2.35 | 2.21 | 53 |
| 10 | SE | M | 71 | 5.17 | 6.84 | 70 |
| 11 | JPV | M | 61 | 8.80 | 8.23 | 63 |
| 12 | EFR | M | 69 | 5.52 | 4.04 | 57 |
| 13 | ELC | F | 59 | 4.10 | 8.07 | 80 |
| 14 | LFR | M | 69 | 7.17 | 6.51 | 75 |
| 15 | ELS | F | 77 | 4.15 | 4.29 | 59.5 |
| 16 | ARB | F | 67 | 2.25 | 3.69 | 47 |
| 17 | TMO | F | 76 | 8.15 | 9.53 | |
| 18 | JBPS | M | 68 | 8.49 | 5.90 | 69 |
| 19 | NOG | F | 74 | 6.76 | 8.18 | 71 |
| 20 | MJFO | F | 72 | 6.04 | 6.46 | |
| 21 | ACP | M | 69 | 6.94 | 8.54 | 72 |
| 22 | IMS | F | 71 | 2.86 | 2.83 | |
| 23 | TVS | M | 73 | 5.18 | 5.15 | |
| 24 | MEMG | F | 74 | 8.76 | 9.50 | 78 |

was significantly reduced in normal areas of UIP when compared to NLT [4]. In addition, AEC2 apoptosis was significantly inverse to AEC2 density. Our interpretation of these results in the pathogenesis of IPF/UIP was that after numerous mitoses, AEC2T- exhausted its telomeres and entered into apoptosis. Therefore, in remodeled areas, AEC2T+ is predominant. The t test was not significant, which leads us to believe that AEC2 SP-A and AEC2T+ are the same population, hence AECT+ are the predominant cells. This finding explains the high incidence of AEC2 in remodeled areas in spite of the AEC2T+ small sub-population in these patients.

The regression analysis of the AEC2T+ density and the predicted percent of forced vital capacity (FVC) were significant and positive in 15 patients (Figure 3) due to a larger pulmonary expansion caused by open alveoli AEC2T+. AEC2 also produces prostaglandin E2 [7], inhibiting fibroblastic proliferation, which in turn slows pulmonary fibrosis and reduces decrease in FVC.

Predominance of AEC2T+ in remodeled areas of UIP strengthens the hypothesis of apoptosis AEC2T- in the pathogenesis of IPF/UIP.

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