Yin Yang-1 (YY-1) expression in idiopathic pulmonary fibrosis

Giorgos A Margaritopoulos¹,², Katerina M Antoniou¹,², Giannoula Soufla³, Evi Vassalou², Demetrios A Spandidos³, and Nikos M Siafakas¹

¹Interstitial Lung Disease Unit, Department of Thoracic Medicine, University Hospital of Heraklion, Crete, Greece, ²Laboratory of Molecular and Cellular Pulmonary Medicine, Medical School, University of Crete, Heraklion, Crete, Greece, and ³Department of Virology, Medical School, University of Crete, Heraklion, Crete, Greece

Abstract
Context: Yin Yang-1 (YY-1) is implicated in the pathogenesis of lung cancer which can be complicated with idiopathic pulmonary fibrosis (IPF).

Objective: The aim of the study was to investigate whether YY-1 is involved in the pathogenesis of IPF and whether represents a common pathogenetic pathway which could explain the coexistence of these disorders.

Materials and methods: Lung tissue from 52 patients (37 with IPF and 15 controls) and bronchoalveolar lavage fluid (BALF) from 34 patients (25 with IPF and 9 controls) were studied and YY-1 mRNA expression was evaluated by real-time PCR.

Results: YY-1 was expressed in 8% (3/37) of IPF patients and in 6% (1/15) of healthy controls in tissue samples. In addition, 12% (3/25) of IPF patients and 33% (3/9) of healthy controls have expressed YY-1 gene in BALF samples. However, no statistical significant difference in mRNA expression between patients and controls has been detected in both tissue and BAL fluid samples.

Discussion and conclusion: Our results do not support the hypothesis of YY-1 involvement in IPF. However, similar expression of YY-1 gene in two biological samples cannot exclude a possible role of this polymorphic gene in the pathway of IPF. Further studies in a larger scale of patients are needed.

Keywords: Yin Yang-1, transcription factor, tumorigenesis, idiopathic pulmonary fibrosis, lung cancer

Introduction
Yin Yang-1 (YY-1) is a ubiquitous zinc-finger transcription factor that has the ability to initiate as well as to regulate the transcription through activation or repression (1). It has an important role in various biological processes such as cell-cycle control, embryogenesis, apoptosis, B-cell development and tumorigenesis (1).

A growing body of evidence has underlined the role of YY-1 in various steps during the process of tumorigenesis. YY-1 interacts with cell-cycle regulation via its association with the tumor suppressor gene p53 (2,3) and activates, when overexpressed, the protooncogenes c-myc which are implicated in the pathogenesis of various cancers (4,5). It also negatively regulates Fas transcription and expression and confers resistance to Fas-induced apoptosis (6) and moreover is implicated in tumor progression and metastasis (7,8). In addition, it might be responsible for the resistance of various cancers to chemotherapeutic agents (9).

Several studies in various types of lung cancer have shown that YY-1 is upregulated (10–12) suggesting a possible role of this factor in the pathogenesis of lung cancer. Idiopathic pulmonary fibrosis (IPF), another elusive lung disease with fatal outcome, can be complicated with lung cancer (13–15) raising questions regarding the similarity of IPF and lung cancer biology. Surprisingly, some pathogenetic hallmarks such as genetic alterations, uncontrolled and aberrant cellular proliferation and tissue invasion are common in both diseases leading to the hypothesis that IPF might be considered as a neoproliferative disorder of the lung (16).
However, the YY-1 gene expression has not been explored in IPF. The purpose of our study was to shed light on the role of YY-1 transcription factor in the pathogenesis of this lethal disorder.

**Patients and methods**

**Patients**

We studied the lung tissue from 52 patients (37 with IPF and 15 controls, prospectively collected in our institute from 2000 to 2010) and the bronchoalveolar lavage fluid (BALF) from 34 patients (25 with IPF and 9 controls, prospectively collected from 2006 to 2010).

Tissue specimens and BALF were obtained from consenting individuals in accordance with institutional review board approval. Patients with IPF had clinical and radiographic findings consistent with the diagnosis of IPF, or pathologic confirmation of the diagnosis of usual interstitial pneumonia. None of the patients in either the IPF or control group had been previously treated or were currently being treated with corticosteroids or other immunosuppressive agents. The control lung tissue specimens were from subjects undergoing thoracic surgery for reasons other than interstitial lung disease, mainly for lung cancer. The lung tissue was obtained from a site as distant as possible from the primary tumor as previously described (18) and was histologically free of neoplasm.

**RNA extraction and reverse transcription**

Total RNA was extracted from each lung specimen and from BAL fluid using a power homogenizer and the TRIzol® reagent (Invitrogen, Carlsband, CA) according to the manufacturer’s instructions. cDNA was synthesized using the Strascript reverse transcriptase kit (Stratagene, La Jolla, CA) as previously described (18).

**Real-time RT-PCR**

YY-1 mRNA expression was measured using a real-time RT-PCR assay with SYBR-Green I. Beta-actin (β-actin) was used as the internal control, in order to normalize YY-1 expression levels, as previously described (18). Primer sequences and their annealing temperatures are described in Table 1. For all the tested samples, the expression of both the target and the housekeeping gene was calculated, projecting, with the help of the standard curve, the Ct value of all unknown samples to a relative mRNA quantity. The mRNA gene expression of every sample was normalized by dividing the mRNA value of the target gene with the appropriate β-actin mRNA value according to the following formula:

\[
\text{Normalized Sample} = \frac{(1 + F_{\text{gene}})^{-\Delta C_t}}{(1 + F_{\text{Actin}})^{-\Delta C_t} \text{Actin}}
\]

**Statistical analysis**

YY-1 mRNA levels were first evaluated by the one-sample Kolmogorov-Smirnov goodness of fit test, in order to determine whether they follow a normal distribution pattern. The t-test was used to compare the mRNA expression of YY-1 after stratification for normal, lung and IPF samples. The Chi-square (χ²) test was used to evaluate the significant statistical differences in YY-1 mRNA expression status. All statistical analyses were performed with SPSS 11.5 (SPSS, Chicago, IL). Statistical significance was set at the 95% level (P<0.05).

**Results**

In tissue samples, YY-1 was expressed in 8% (3/37) of IPF patients and in 6% (1/15) of healthy controls (Table 2).

In BAL fluid samples, YY-1 was expressed in 12% (3/25) of IPF patients and in 33% (3/9) of healthy controls (Table 2).

We have not detected any statistical significant difference in mRNA expression between the study’s group in both tissue and BAL fluid samples.

**Discussion**

The aim of our study was to investigate the role of YY-1 transcription factor in the pathogenesis of IPF and, since it is well known that this factor is implicated in the process of tumorigenesis, to seek indirect evidence that its activity could explain the coexistence of IPF and lung cancer.

To the best of our knowledge, this is the first attempt to evaluate the expression of YY-1 in lung tissue and BAL fluid of patients with IPF. We have observed that YY-1 was expressed in a small number of patients and controls. Additionally, no statistical significant difference in mRNA expression has been found between patient’s group and controls in both types of samples. However, a similar rather low expression of the YY-1 gene has been detected in both biological samples, lung tissue and BAL fluid of patients with IPF. These results do not support our hypothesis but can not also exclude the involvement of YY-1 in the pathogenesis of IPF.

Table 1. Primer sequences used for quantitative real-time RT-PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer pair Sequence (5′→3′)</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>YY-1</td>
<td>For: GGAATATCCTGGCATTGACC</td>
<td>58°C</td>
</tr>
<tr>
<td></td>
<td>Rev: TCTTTGTGCAGCCTTTGAG</td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>For: CGGCATCGTCACCAACTG</td>
<td>58°C</td>
</tr>
<tr>
<td></td>
<td>Rev: GGCACACGCAGCTCATTG</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. YY-1 expression in IPF and controls in lung tissue and BAL.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample Type</th>
<th>Value</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>YY-1</td>
<td>(tissue)</td>
<td>3/37 (8%)</td>
<td>1/15 (6%)</td>
<td>NS</td>
</tr>
<tr>
<td>YY-1</td>
<td>(BAL)</td>
<td>3/25 (12%)</td>
<td>3/9 (33%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

χ² test: P < 0.05 is considered statistically significant. NS, not significant.
The role of YY-1 in cancer biology is contradictory because this factor can act as a transcriptional activator or repressor depending on the context in which it binds (1). YY-1 overexpression is associated with an increased malignant phenotype and poor outcome in breast cancer, cervical neoplasia, osteosarcoma, myeloid leukemia and Hodgkin’s and non-Hodgkin’s lymphoma (19). On the contrary, higher YY-1 protein levels were associated with a favorable prognosis in prostate and ovarian cancers (19).

Regarding lung cancer, gene expression analysis of YY-1 has revealed an increase of mRNA levels in squamous cell carcinoma and in adenocarcinoma in comparison to normal tissue (10–12). Unpublished data from our laboratory have shown that 4/17 (23.5%) of the patients with lung cancer have expressed YY-1 in BAL fluid with no significant difference in mRNA expression between patients’ group and controls.

Recently, our study group investigated the role of epidermal growth factor receptor (EGFR) and its downstream signalling pathways including the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) and Ras/Raf/MEK/ERK (mitogenactivated protein kinase (MAPK) pathway in the pathogenesis of IPF (18,20). EGFR as well as the Akt and MAPK pathways have a key role during tumorigenesis (21–24). We found no significant different in mRNA expression of EGFR between IPF and healthy subjects, whereas a downregulation of K-ras and an upregulation of B-ras has been observed in IPF patients in comparison to controls suggesting that despite the weak expression, these oncogenes could be implicated in the pathogenesis of IPF.

A growing body of evidence has pointed out several pathways common in these two disorders. Mutations of p53 gene, a tumor suppressor gene altered in most cases of cancer, have been demonstrated also in IPF (25). Mutations in the components of telomerase, an enzyme aimed to maintain telomere length, as well as telomere shortening, events so far almost exclusive of tumor development, have been described in both familial and sporadic IPF (26–28). The oxidant/antioxidant balance is implicated in the pathogenesis of IPF. Oxidative stress which activates the synthesis of several profibrotic cytokines leading to tissue injury and fibrosis, may cause alterations in DNA methylation patterns and specific histone modifications triggering the process of tumorigenesis (29). Intracellular signaling pathways crucial in the pathogenesis of lung cancer and mesothelioma (30) such as Wnt/bcatenin are fully involved in IPF (31).

**Conclusion**

Our findings do not support but can not also exclude the hypothesis that YY-1 gene is involved in the pathogenesis of IPF, representing a common pathogenetic pathway between IPF and lung cancer. The link between cancer and IPF is becoming intriguing and may give birth to new approaches for IPF. For years, it was believed that fibrogenesis was linked to a single pathogenetic pathway, and this belief led to the failure of trials targeting an individual pathway (32,33). The hypothesis of multiple pathogenetic pathways has been adopted in cancer biology, and targeting simultaneously these pathways is the main thinking in cancer therapy (34–36). Should this hypothesis be adopted in order to shed light in the pathogenesis and design therapeutic trials for fibrotic lung disorders and in particular for IPF (37)? Further investigations are needed to verify this hypothesis.

**Declaration of interest**

The authors report no declarations of interest.

**References**


