

# Aberrant Wnt/ $\beta$ -Catenin Pathway Activation in Idiopathic Pulmonary Fibrosis

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**To investigate the molecular events that may underpin dysfunctional repair processes that characterize idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP), we analyzed the expression patterns of  $\beta$ -catenin on 20 IPF/UIP lung samples, together with two downstream target genes of Wnt signaling, cyclin-D1, and matrilysin. In 18 of 20 cases of IPF/UIP investigated on serial sections, nuclear  $\beta$ -catenin immunoreactivity and abnormal levels of cyclin-D1 and matrilysin were demonstrated in proliferative bronchiolar lesions (basal-cell hyperplasia, squamous metaplasia, bronchiolization, honeycombing). The nature of these lesions was precisely defined using specific markers ( $\Delta$ N-p63, surfactant-protein-A, cytokeratin-5). Interestingly, nuclear  $\beta$ -catenin accumulation was also demonstrated in fibroblast foci in most (16 of 20) IPF/UIP samples, often associated with bronchiolar lesions. Similar features were not observed in normal lung and other fibrosing pulmonary diseases (diffuse alveolar damage, organizing pneumonia, nonspecific interstitial pneumonia, desquamative interstitial pneumonia). Sequence analysis performed on DNA extracted from three samples of IPF/UIP did not reveal abnormalities affecting the  $\beta$ -catenin gene. On the basis of these findings new models for IPF/UIP pathogenesis can be hypothesized, centered on the aberrant activation of Wnt/ $\beta$ -catenin signaling, with eventual triggering of divergent epithelial regeneration at bronchiolo-alveolar junctions and epithelial-mesenchymal-transitions, leading to severe and irreversible remodeling of the pulmonary tissue. (*Am J Pathol* 2003, 162:1495–1502)**

Idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP) is the most common and severe form among idiopathic interstitial pneumonias.<sup>1–4</sup> Many questions regarding this disease still remain unsolved in terms of etiology and natural history, and recent contrasting opinions have raised a stirring discussion regarding its pathogenesis.<sup>5–13</sup> The inflammatory theory of IPF/UIP has been challenged, and new models have been proposed based on the hypothesis that a dysregulated communication between mesenchymal and epithelial pulmonary components after tissue injury is key to the irreversible process of fibrosis and tissue remodeling.<sup>7,9,10</sup> This change in views appears particularly intriguing because it might provide the rationale for new treatment approaches, aimed at contrasting fibroblast proliferation and/or inducing fibroblast apoptosis.<sup>7,14,15</sup> Nevertheless, the centrality of fibroblasts/myofibroblasts in IPF/UIP still remains controversial and unproven, and little is known about the molecular mechanisms involved in the pathogenesis of this disease. Critical arguments regard the epithelial target of early injury, as well as the molecular features characterizing abnormal mesenchymal/epithelial cross-talking. In this regard, we have recently observed that bronchiolar epithelial cells and bronchiolo-alveolar junctions are a relevant target of cell injury in IPF/UIP, and that abnormal bronchiolar proliferations (including hyperplasia, metaplasia, bronchiolization, and honeycombing) may, in fact, represent substantial features of this disease.<sup>16</sup>

Much evidence suggests that the study of the molecular pathways regulating lung development and morphogenesis may provide important information regarding the pathogenesis of pulmonary diseases.<sup>8,16</sup> Early phases of lung development are dependent on complex molecular networks that include a series of stimulatory and inhibitory pathways including *Fgf*, *Egf*, *TGF $\beta$ /Activin*, *Wnt*, *Hedgehog*, *Hox*, *SOX*, *sprouty*, and others.<sup>17–22</sup> It is reasonable to hypothesize that these molecular pathways can in part be recapitulated during postnatal life, because the complex lung architecture can reform precisely after extensive damage, as observed in various pulmonary diseases.

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In this study we focused on Wnt signaling and its effector  $\beta$ -catenin for a number of reasons. First, this relevant signaling pathway is involved in lung development and organogenesis in mammals.<sup>19–22</sup> Second, the metalloproteinase matrilysin/MMP-7, which is a target of  $\beta$ -catenin transactivation, has recently been revealed as a key regulator of pulmonary fibrosis.<sup>23–25</sup> Third, the Wnt pathway has been implicated in the pathogenesis of some human fibrosing diseases.<sup>26,27</sup> Finally, direct evidence has been recently provided for a role of  $\beta$ -catenin signaling in the induction of epithelial-mesenchymal transition (EMT), an important process occurring during critical phases of embryonic development, tumor progression, and fibrotic tissue repair after injury.<sup>28–32</sup>

It is widely accepted that either activation of the Wnt pathway or abnormalities affecting the  $\beta$ -catenin-transactivating functions can be demonstrated *in situ* by specific expression patterns of  $\beta$ -catenin accumulation. In particular, cytoplasmic/nuclear accumulation of  $\beta$ -catenin, as shown by immunohistochemistry, represents a reliable means to demonstrate posttranslational stabilization of  $\beta$ -catenin.<sup>33,34</sup> In this study we have investigated the expression patterns of  $\beta$ -catenin in a series of IPF/UIP samples, together with two gene products, cyclin-D1 and matrilysin/MMP-7, whose expression is under  $\beta$ -catenin control.<sup>23,24,35</sup>

## Materials and Methods

### Study Population

The study group consisted of 20 previously untreated patients with clinical, radiographical (chest radiograph and high resolution computed tomography (HRCT)), physiological, and bronchoalveolar lavage findings consistent with the diagnosis of IPF. Histological examination of surgical lung biopsies revealed all of the major features of UIP, according to the recently defined criteria.<sup>1–4</sup> Five samples of normal lung (fragments of unaffected tissue from patients submitted to large excisions for lung carcinoma), two samples of fetal lung (12 and 15 weeks of gestation), 10 samples from patients with organizing pneumonia (OP/BOOP), 4 samples of nonspecific interstitial pneumonia (NSIP), 2 samples of acute interstitial pneumonia with diffuse alveolar damage (AIP/DAD), and 2 samples of desquamative interstitial pneumonia (DIP), defined according to the recent consensus criteria,<sup>4</sup> were analyzed as controls. All samples were fixed in buffered formalin and paraffin-embedded following standard protocols.

### Immunohistochemical Staining and Antibodies

All cases were immunostained with a pan- $\beta$ -catenin monoclonal antibody (clone 15B8; Sigma Chemical Co., St. Louis, MO). Heat-induced antigen retrieval was performed using a microwave oven and 0.01 mol/L of citrate buffer, pH 6.0, for 30 minutes. All samples were processed using a sensitive avidin-streptavidin-peroxidase technique (Biogenex, San Ramon, CA) in a automated cell staining system (GenoMx i6000, BioGenex).

To better define the nature and differentiation level of the epithelial and mesenchymal lesions, we used antibodies recognizing low-molecular weight cytokeratin 8/18/19 (clone 5D3, Biogenex); cytokeratin-5 (CK5, clone XM26; Novocastra, Newcastle, UK) expressed in bronchiolar basal cells; urine protein 1, a rabbit antibody (DAKO, Glostrup, Denmark) recognizing CC10 antigen in Clara cells; SP-A monoclonal antibody (clone PE-10, DAKO) recognizing surfactant protein-A; 1A4 monoclonal antibody (DAKO) recognizing  $\alpha$ -smooth muscle actin (no antigen retrieval); TN2 monoclonal antibody (DAKO) recognizing tenascin. In addition, a panel of antibodies was selected to thoroughly investigate the molecular network characterizing lesions expressing nuclear  $\beta$ -catenin: mAb clone 4A4 (Santa Cruz Biotechnology, Santa Cruz, CA), reacting broadly with all known variants of human p63; p40 antibody (Oncogene Research Products, Boston, MA), a polyclonal rabbit antiserum specifically recognizing the truncated  $\Delta$ N-p63 isoforms lacking the transactivating domain;<sup>36</sup> p53-specific monoclonal antibody (clone DO-1, DAKO); monoclonal antibody recognizing p21<sup>WAF1</sup> (clone SX118, DAKO). Details regarding these molecules in normal lung and IPF/UIP samples have been previously described.<sup>16,37</sup> Finally, to better evaluate the function of Wnt/ $\beta$ -catenin pathway activation in IPF/UIP samples we immunohistochemically investigated on serial sections the expression of cyclin-D1 (clone DCS-6; Progen Biot., Heidelberg, Germany) and matrilysin/MMP-7 (clone 141-7B2; Chemicon, Temecula, CA).

### Evaluation of Immunostaining

Normal pulmonary tissue structures were used, when present, as internal controls for immunostaining with  $\beta$ -catenin, and only preparations in which normal bronchial/bronchiolar segments were present showing clear-cut membrane-bound  $\beta$ -catenin expression (linear pattern), were considered as suitable for interpretation. Nuclear staining was defined as negative when void nuclei were evident, together with clear-cut membrane immunostaining, and as positive when nuclei were immunoreactive. The nuclear pattern was better evaluated on lightly hematoxylin-counterstained preparations, in which positive nuclei changed their color from blue to brown.

Epithelial lesions were defined as bronchiolar or bronchiolo-alveolar-junctional when the cell phenotype included CK5 and  $\Delta$ N-p63 expression.<sup>16</sup> Only nuclear staining was interpreted as positive for p63,  $\Delta$ N-p63, p53, p21<sup>WAF1</sup>.

### Molecular Analysis: $\beta$ -Catenin Gene Sequencing

Three representative samples of IPF/UIP were used for molecular analysis. DNA was extracted from 10- $\mu$ m paraffin sections with the DNeasy Tissue kit (Qiagen, Chatsworth, CA). For sequence analysis, Exon 3 of the  $\beta$ -catenin gene was amplified by polymerase chain reaction using primers synthesized with an Applied Biosystem synthesizer (Foster City, CA). Primer sequences (ATTTGATGGAGTTGGACATGGC and CCAGCTACTTGTTCT-

TGAG TGAAGG) were as previously described.<sup>38</sup> Polymerase chain reaction was performed in a 10- $\mu$ l standard reaction mixture containing 50 ng of DNA, 5 pmol of each primer, 2  $\mu$ mol/L dNTPs, 1.5 mmol/L MgCl<sub>2</sub>, 0.5 U of TaqDNA polymerase (Promega, Madison, WI). For sequence analysis a 50- $\mu$ l polymerase chain reaction was gel-purified with the QIAEX gel extraction kit (Qiagen). Sequence reactions were performed using the Applied Biosystem Dye Terminator Cycle-Sequencing kit (Perkin Elmer, Foster City, CA) and analyzed on a Applied Biosystem model 373 automated DNA sequencer (Perkin Elmer). Four pilomatrixoma samples harboring  $\beta$ -catenin mutations were included as positive controls.<sup>39</sup>

## Results

### *$\beta$ -Catenin Intracellular Expression Pattern in Normal Adult and Fetal Lung*

In the fetal lung  $\beta$ -catenin nuclear immunostaining was demonstrated in budding alveolar structures as previously described (Figure 1a).<sup>21</sup> In the normal adult lung,  $\beta$ -catenin expression was strictly confined to cell membranes in all endothelial and epithelial cells, as shown by a clear-cut linear pattern of immunostaining (Figure 1b). Nuclear accumulation was evident in a small proportion of cuboidal alveolar cells, recognized as type II pneumocytes by morphology and immunophenotype, characterized by expression of CK8/18/19 and SP-A, and lack of CK5 and  $\Delta$ N-p63 (not shown).

### *$\beta$ -Catenin Intracellular Expression Pattern in IPF/UIP Samples*

In IPF/UIP patients the number of cells expressing nuclear  $\beta$ -catenin was highly increased, especially in areas where abnormal remodeling of lung architecture was evident.

#### *Bronchiolar Lesions*

A striking number of epithelial cells expressing  $\beta$ -catenin nuclear accumulation were demonstrated in proliferative bronchiolar lesions in most (18 of 20) samples (Figure 1; c, d, and j). Nuclear  $\beta$ -catenin accumulation was heterogeneously distributed in these abnormal structures, mainly occurring in clusters of hyperplastic basal cells. The presence of nuclear  $\beta$ -catenin was particularly evident in bronchioles exhibiting honeycomb modifications (Figure 1, g and i) and/or bronchiolization (a process of migrating bronchiolar cells progressively colonizing alveolar spaces). Interestingly, at the same sites nuclear overexpression of p53 and p21<sup>waf1</sup> could be demonstrated as previously described.<sup>16</sup> The bronchiolar nature of all these lesions was confirmed on serial sections by the use of antibodies recognizing  $\Delta$ N-p63 and high-molecular weight cytokeratin CK5 (Figure 1, h and i), and by the absence of both surfactant-A and CC10 antigens as previously demonstrated.<sup>16</sup>

#### *Alveolar Structures*

Cells expressing nuclear  $\beta$ -catenin were found lining damaged alveolar structures, recognized as cuboidal type II pneumocytes by morphology and immunophenotype on serial sections (surfactant-A-positive and  $\Delta$ N-p63-negative). The number of positive cuboidal pneumocytes progressively increased from normal to severely affected alveoli (Figure 2, a and b).  $\beta$ -catenin nuclear expression was observed in all enlarged and/or atypical cuboidal cells.

#### *Fibroblast Foci*

Nuclear expression of  $\beta$ -catenin was observed in spindle cells forming fibroblast foci present in 16 of 20 samples in which these lesions could be clearly identified and immunohistochemically analyzed on serial sections (Figure 2, c and e). These foci, characterized as myofibroblastic by intense  $\alpha$ -smooth muscle actin and tenascin immunoreactivity on serial sections (Figure 2d), were frequently intramural and located under abnormal bronchiolar segments, as forming strictly related lesions (Figure 2e). This pattern was different from that observed in intra-alveolar inflammatory polyps (Masson's bodies) present in OP/BOOP (Figure 2f) and interstitial fibroblasts of AIP/DAD (Figure 2g) samples used as control, in which only a minority (less than 10%) of spindle cells expressed nuclear  $\beta$ -catenin.

### *Expression of Cyclin-D1 and Matrilysin*

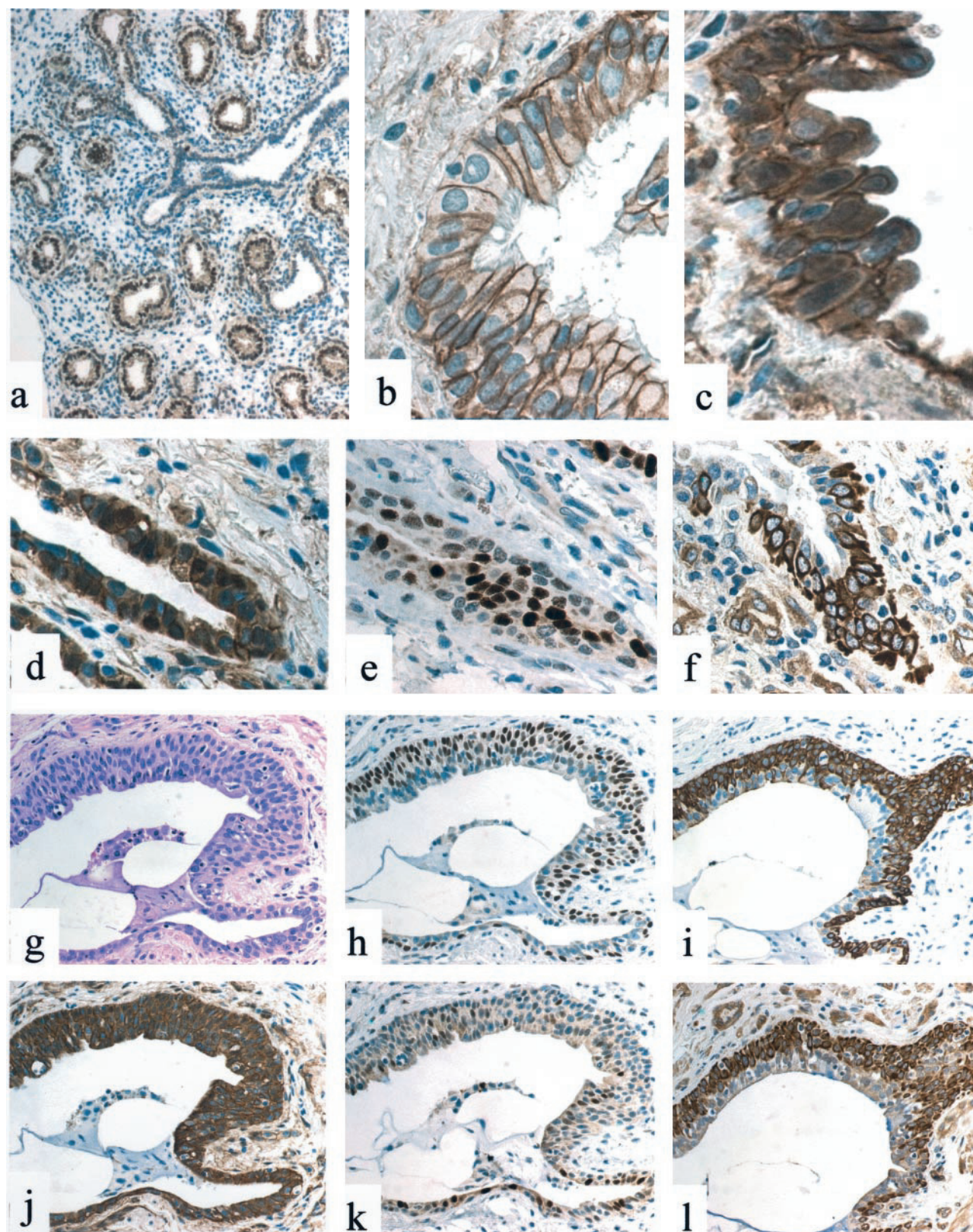
A high number of cells expressing both cyclin-D1 and matrilysin could be demonstrated in IPF/UIP samples by immunohistochemical analysis on serial sections (Figure 1; e, f, k, and l). Matrilysin immunoreactivity was evident in all types of proliferative bronchiolar lesions, with particular intensity in hyperplastic and atypical basal cells (Figure 1, f and l). This pattern was decidedly different from normal and pathological control samples in which expression of matrilysin appeared as inconsistent (Figure 2i).

Both cyclin D1 and matrilysin were clearly located at sites corresponding to nuclear overexpression of  $\beta$ -catenin, although the distribution of the three molecules was not identical on serial sections of proliferative epithelial lesions. In fact,  $\beta$ -catenin nuclear and/or cytoplasmic overexpression was evident in the vast majority of cells (Figure 1, d and j), whereas matrilysin and cyclin-D1 were only expressed in a proportion of cells (Figure 1; e, f, k, and l). The reason for this finding is not clear, but could be ascribed to undefined differentiation signals. In line with this view is the observation that both matrilysin and  $\Delta$ N-p63 stop to be expressed in more superficially located cells of bronchiolar lesions (Figure 1, h and i).

### *$\beta$ -Catenin Intracellular Expression Pattern in Other Interstitial Pneumonias*

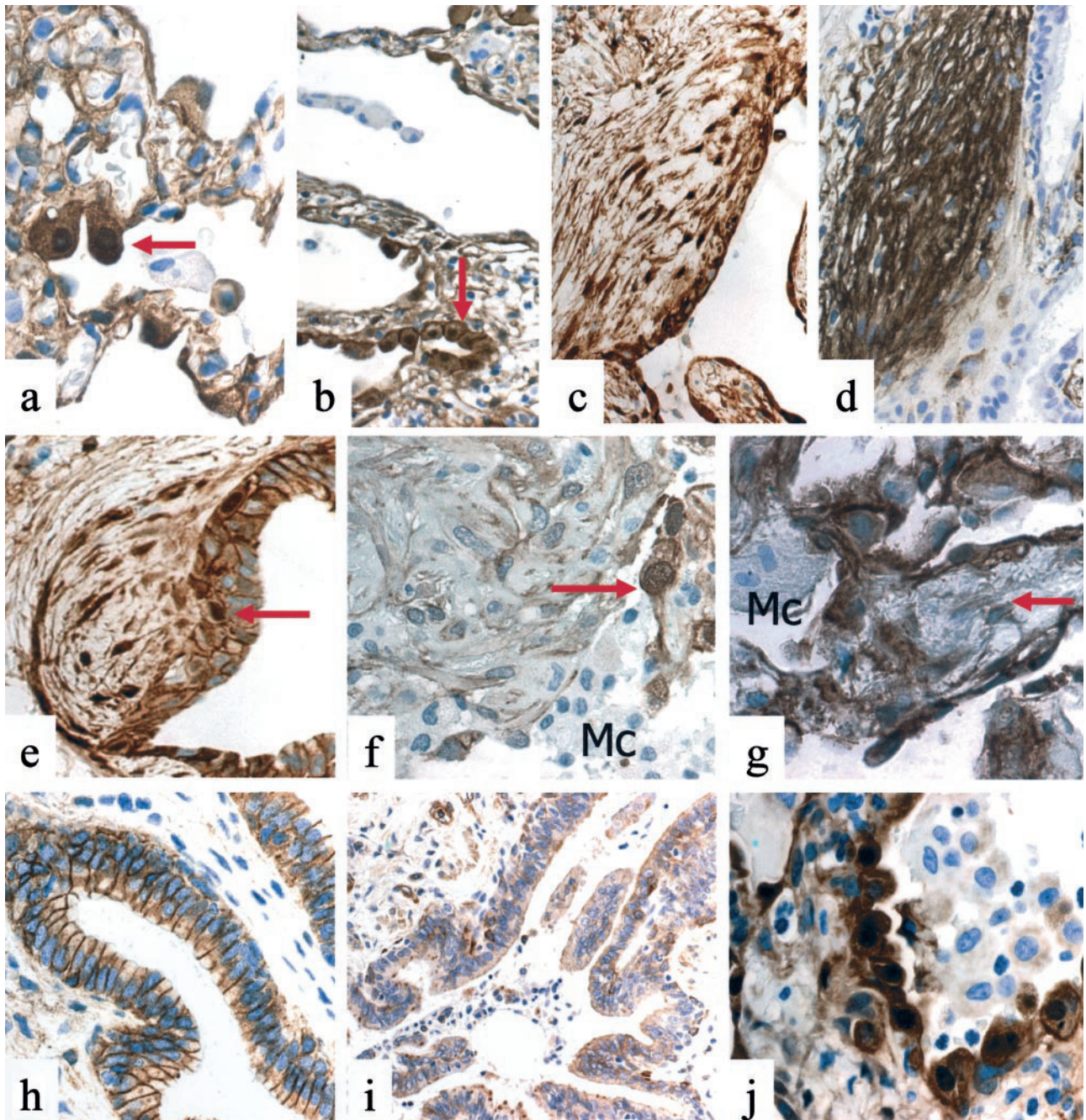
In all cases of OP/BOOP, AIP/DAD, NSIP, and DIP analyzed, bronchiolar changes were extremely rare and no





**Figure 1.** **a:** Fetal lung (12 weeks): nuclear expression of  $\beta$ -catenin is evident in alveolar buds, but not in airway cells. **b:** Normal lung: discrete membrane immunoreactivity of  $\beta$ -catenin in basal and ciliated cells in a bronchiole. **c:** IPF/UIP: aberrant nuclear accumulation of  $\beta$ -catenin in a proliferative bronchiolar lesion. **d:** IPF/UIP: nuclear expression of  $\beta$ -catenin in basal cells of an abnormal bronchiole. **e:** IPF/UIP (serial section to **d**): cyclin-D1-expressing cells. **f:** IPF/UIP (serial section to **d**): matrilysin/MMP-7 abnormal expression. **g:** IPF/UIP: H&E appearance of a small honeycombing bronchiolar lesion. **h:** IPF/UIP (serial section to **g**): basal cell hyperplasia as evidenced by  $\Delta$ N-p63 nuclear expression. **i:** IPF/UIP (serial section to **g**): basal cell hyperplasia as evidenced by CK5 expression. **j:** IPF/UIP (serial section to **g**): abnormal intracellular expression of  $\beta$ -catenin in both basal and luminal epithelial cells. **k:** IPF/UIP (serial section to **g**): increased expression of cyclin-D1. **l:** IPF/UIP (serial section to **g**): aberrant expression of matrilysin/MMP-7 in basal cells.





**Figure 2.** **a:** IPF/UIP: nuclear accumulation of  $\beta$ -catenin in cuboidal type II pneumocytes (arrow) in scarcely affected alveolar structures. **b:** IPF/UIP: nuclear accumulation of  $\beta$ -catenin in cuboidal type II pneumocytes (arrow) in severely affected alveolar structures. **c:** IPF/UIP: nuclear accumulation of  $\beta$ -catenin in spindled fibroblasts in subepithelial fibroblast foci. **d:** IPF/UIP (serial section to **c**): strong tenascin expression in spindled fibroblasts in subepithelial fibroblast foci. **e:** IPF/UIP: nuclear accumulation of  $\beta$ -catenin in spindled cells of subepithelial fibroblast foci. Note nuclear expression of  $\beta$ -catenin also in bronchiolar basal cells (arrow). **f:** OP/BOOP: nuclear  $\beta$ -catenin expression is not observed in spindled cells of an intraluminal Masson's body and in macrophages (Mc). Note intense nuclear immunostaining in hyperplastic type II pneumocytes (arrow). **g:** DAD: nuclear  $\beta$ -catenin expression is evident in alveolar pneumocytes. Interstitial fibroblasts (arrow) and macrophages (Mc) lack evident immunoreactivity. **h:** NSIP: membrane  $\beta$ -catenin expression in bronchiolar cells. **i:** NSIP: scarce expression of matrilysin/MMP-7 in bronchiolar cells. **j:** NSIP: nuclear  $\beta$ -catenin expression in many alveolar pneumocytes.

abnormal expression of  $\beta$ -catenin could be demonstrated by immunohistochemical analysis (Figure 2h). Accordingly, matrilysin overexpression was not observed in bronchiolar epithelium (Figure 2i). On the other hand, the proportion of alveolar pneumocytes expressing nuclear  $\beta$ -catenin was prominent in samples obtained from patients with pulmonary diseases in which extensive alveo-

lar damage and repair take place, such as AIP/DAD, OP/BOOP, and NSIP (Figure 2j).

### Sequence Analysis of $\beta$ -Catenin Gene

Evidence of mutations could not be found in the three samples of IPF/UIP after sequencing of the amplified region.

All four pilomatricoma samples analyzed as known positive controls in the same experiments carried missense mutations in the third exon of the  $\beta$ -catenin gene.

## Discussion

In this work, we provide evidence of a previously unrecognized involvement of the Wnt/ $\beta$ -catenin signaling pathway in IPF/UIP, as evidenced by extensive nuclear accumulation of  $\beta$ -catenin at different involved sites. We used the immunohistochemical approach to reveal subcellular localization of  $\beta$ -catenin, from the membrane location toward the nucleus. This morphological approach is able to detect intracellular redistribution and nuclear accumulation of  $\beta$ -catenin with high sensitivity, and has been widely used to demonstrate the activation status of the Wnt pathway in human development and pathology.<sup>21,33,34,40–42</sup> To further support the functional significance of  $\beta$ -catenin nuclear immunoreactivity we investigated *in situ* the expression of two target genes of the Wnt/ $\beta$ -catenin pathway, cyclin-D1 and matrilysin, demonstrating significant overexpression of both molecules at the same involved sites overexpressing nuclear  $\beta$ -catenin.

Finally, we searched for mutations affecting the  $\beta$ -catenin gene, showing that  $\beta$ -catenin gene abnormalities are probably not implicated in the observed activation of the Wnt pathway, although our technique might miss mutations occurring in a small percentage of cells. Further studies are warranted to investigate in IPF/UIP samples other genetic and/or expression abnormalities affecting the complex array of molecules involved in the Wnt/ $\beta$ -catenin cascade, which include APC, Axin, and GSK3 $\beta$ , as well as Wnt ligands and *frizzled* receptors.<sup>43</sup> In fact, on the basis of our data it is not possible to define whether Wnt/ $\beta$ -catenin activation is causative or secondary to the disease. Nevertheless, the absence of similar features in all other interstitial lung diseases investigated in this study strongly suggests the pathogenic relevance of Wnt/ $\beta$ -catenin aberrant activation in IPF/UIP.

In this study nuclear  $\beta$ -catenin accumulation could be demonstrated at three different involved sites in IPF/UIP samples: 1) bronchiolar proliferative lesions, 2) damaged alveolar structures, and 3) fibroblast foci. Bronchiolar proliferative lesions, including basal-cell hyperplasia, squamous metaplasia, honeycombing, and bronchiolization, are common in IPF/UIP, and represent well-recognized peculiar features of this disease.<sup>1–4,16</sup> According to our study, the aberrant activation of the Wnt pathway was particularly evident and extended in these lesions, involving in most cases the basal cell compartment. This observation is intriguing, because basal cells are considered the renewal component of bronchial and bronchiolar structures. In addition, these findings are unique, because bronchioles in normal lung samples and in various interstitial diseases other than IPF/UIP herein investigated with the same methods showed only membranous  $\beta$ -catenin immunoreactivity. On the other hand,  $\beta$ -catenin nuclear expression observed in cuboidal hyperplastic pneumocytes can be considered as part of a physiological response to alveolar damage, because type II pneu-

mocytes exhibit this pattern in a variety of conditions in which alveolar proliferation/regeneration takes place, including fetal development,<sup>21</sup> and a variety of pulmonary diseases such as AIP/DAD and OP/BOOP, as shown in this study.

Nuclear  $\beta$ -catenin localization was also observed in spindle cells forming intramural fibroblast foci (fibroproliferative plaques) occurring in IPF/UIP, at variance with intra-alveolar Masson's bodies in cases of organizing pneumonia and interstitial myofibroblasts in AIP/DAD. This finding is relevant because abnormal activation of the Wnt pathway could in fact provide autocrine survival signals necessary to induce the peculiar resistance to apoptosis characterizing intramural fibroblast foci of IPF/UIP.<sup>7,44–46</sup> In this regard, it is worth noting that nuclear accumulation of  $\beta$ -catenin because of gene mutations is a central feature in the pathogenesis of aggressive fibromatosis (desmoid tumor), a class of mesenchymal lesions that share with fibroblastic foci of IPF/UIP some morphological and phenotypic features, including aberrant activation of the Wnt pathway, as shown in this study.<sup>47,48</sup> Further support to our hypothesis has been recently provided by an experimental study in which  $\beta$ -catenin stabilization is able to dysregulate mesenchymal cell proliferation, motility, and invasiveness causing proliferative fibroblastic lesions in transgenic mice.<sup>49</sup>

As described above, the distribution of  $\beta$ -catenin in a pattern of abnormal nuclear accumulation involves adjacent compartments, focused on bronchiolo-alveolar junctions. This peculiar zonal distribution could explain, in our view, some typical histological features of UIP such as the temporal and spatial heterogeneity, and honeycomb architectural derangement.<sup>1</sup> In our view, these data are consistent with a pathogenic model in which the variegated appearance, from early lesions to extensive fibrosis and remodeling characterizing IPF/UIP lesions, is produced by the progressive interference on physiological tissue repair mechanisms because of abnormal  $\beta$ -catenin activation, starting from foci of ongoing injury and repair processes, as following a gradient of Wnt signal concentration. Deregulated expression of Wnt target genes could exert divergent effects on different airway components (namely bronchiolar and alveolar), eventually leading to alveolar loss on one hand, and bronchiolar proliferation on the other. Accordingly, in different systems and cell types, the activation of the Wnt pathway is able to either trigger or inhibit survival and death by modulating the availability of cyclin-D1 and *c-myc*, two proteins that play roles in both cellular proliferation and apoptosis.<sup>50,51</sup> In this model, alveolar cells could be particularly vulnerable to deranged Wnt signaling, because diverse differentiation and death inducing signals, including p53, p21<sup>waf1</sup>, and transactivating isoforms of p63 are simultaneously expressed in repairing alveoli after injury.<sup>16,52–55</sup> On the other hand, bronchiolar basal cells could be protected from apoptosis by the constitutive expression of truncated dominant-negative  $\Delta$ N-p63 isoforms exerting potent anti-apoptotic signals.<sup>16,56</sup>

The pathogenic role of Wnt pathway activation in IPF/UIP is further supported by the *in situ* demonstration of abnormal expression of the metalloproteinase matrilysin



(MMP-7), another important downstream target gene of  $\beta$ -catenin/LEF-1 signaling. An abnormal increase of matrilysin expression in IPF/UIP has also been demonstrated in a recent microarray gene expression analysis.<sup>25</sup> Further support is also provided by experimental studies, because matrilysin knockout mice are protected from bleomycin-induced pulmonary fibrosis.<sup>25</sup> The role of matrilysin in the development of bronchiolar proliferative lesions and lung remodeling can be ascribed to the peculiar multifunctional roles of this metalloproteinase, including the induction of epithelial cell migration, apoptosis, and metaplastic conversion.<sup>57,58</sup>

A final relevant topic to be discussed in this context is the possible involvement of EMTs in the pathogenesis of IPF/UIP. These intriguing phenomena, known to occur in embryogenesis and carcinoma progression, allow cells to dissociate from the epithelial tissue from which they originate and to migrate freely.<sup>29</sup> In addition, definitive experimental evidence has been provided that fibroblasts can directly derive from epithelial cells in tissue fibrosis by epithelial to mesenchymal transition.<sup>30,32</sup> Interestingly,  $\beta$ -catenin-signaling plays a relevant role in inducing a mesenchymal phenotype in epithelial cells, as shown in experimental EMT,<sup>28,31</sup> thus it is possible to argue that the aberrant nuclearization of  $\beta$ -catenin observed in bronchiolar lesions of IPF/UIP can be involved in a EMT-related process increasing basal-cell motility (eg, by altering the expression of metalloproteinases), thus promoting bronchiolization and tissue remodeling. Another interesting possibility in this model, is that part of the abnormal fibroblasts in IPF/UIP could directly derive from epithelial basal cell precursors at sites of ongoing injury/repair processes, forming the peculiar lesions in which fibroblast-foci and abnormal bronchiolar segments are strictly associated (as shown in Figure 2, c and e). Interestingly, EMT can be experimentally induced by cytokines (transforming growth factor- $\beta$ 1, fibroblast-growth-factor-2, insulin-like growth-factor, interleukin-8), whose expression can be tuned by complex regulatory loops with  $\beta$ -catenin signaling and which are potentially involved in the pathogenesis of pulmonary fibrosis.<sup>28,59–64</sup>

In conclusion, although the precise molecular mechanisms leading to abnormal activation of the Wnt pathway observed in IPF/UIP could not be defined in this study, our findings can contribute to decipher the molecular mechanisms involved in the pathogenesis of this disease, and might also help in the search for new pharmacological strategies to counteract irreversible lung remodeling. In fact, intense investigation is currently focused on molecules exerting modulatory and inhibitory actions on the Wnt pathway.<sup>65–67</sup> These molecules could represent potential candidates for new treatment strategies for cancers in which the Wnt pathway is deranged, as well as in IPF/UIP, a deadly disease in which conventional treatments have proved to be unsatisfactory.

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