Immunobiology 224 (2019) 551–559

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Increased circulating Wnt5a protein in patients with rheumatoid arthritis-associated interstitial pneumonia (RA-ILD)

Miao Yu[a,b,1], Yuanyuan Guo[a,1], Peng Zhang[c], Jing Xue[c,d], Jiali Yang[a], Qian Cai[a], Xuehong You[a], Jia Ma[a], Dandan Yang[a], Yuanyuan Jia[a], Yujiong Wang[a], Feng Li[e], Shuhong Chi[e], Mengshu Cao[f,*], Juan Chen[c,*], Xiaoming Liu[a,b,*]

[a] Key Laboratory of Ministry of Education for Conservation and Utilization of Special Biological Resources in the Western, and College of Life Science, Ningxia University, Yinchuan, Ningxia 750021, China
[b] Department of Anatomy and Cell Biology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA
[c] Department of Pulmonary and Critical Care Medicine, General Hospital of Ningxia Medical University, Yinchuan, Ningxia 750004, China
[d] Institute of Human Stem Cell Research, General Hospital of Ningxia Medical University, Yinchuan, Ningxia 750004, China
[e] Department of Rheumatology, General Hospital of Ningxia Medical University, Yinchuan, Ningxia 750004, China
[f] Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu, China

ARTICLE INFO

Keywords:
Wnt5a
Rheumatoid arthritis
Interstitial lung disease
Usual interstitial pneumonia
Biomarker

ABSTRACT

An early diagnosis of interstitial lung disease (ILD) is important for guiding treatments of rheumatoid arthritis (RA)-associated ILD (RA-ILD) in clinical settings. The non-canonical Wnt signaling representative ligand Wnt5a was recently found to involve in idiopathic pulmonary fibrosis (IPF) and pathogenesis of RA. The goal of this study was to examine the clinical relevance of Wnt5a in RA-ILD. In this report, the clinical relevance of plasma Wnt5a protein was evaluated in 40 RA-ILD patients and 41 non-ILD RA cohorts. The results showed an elevated Wnt5a protein in plasmas of RA-ILD patients compared with non-ILD RA patients ($p < 0.01$), which was positively correlated with the plasma level of rheumatoid factor (RF). Of note, more abundant Wnt5a was also found in patients with usual interstitial pneumonia (UIP) than those with nonspecific interstitial pneumonia (NSIP) and other ILD patterns. More importantly, the disease severity was correlated with the circulating Wnt5a as ascertained by high-resolution computed tomography (HRCT)-UIP scores. The multiple-factor non-conditional logistic regression analysis further revealed that the age, RA duration, smoking and plasma Wnt5a were risk factors with clinical significance for RA-ILD. Interestingly, more Wnt5a-positive patients were identified in RA-ILD smokers relative to RA-ILD never-smokers, and longer smoking duration was strongly correlated with Wnt5a in RA-ILD patients. In consistence, ROC curve also suggested that the Wnt5a was a potential candidate biomarker for identifying patients with RA-UIP. These results demonstrate that the circulating Wnt5a may be a risk factor and potential biomarker for identifying UIP and accessing the severity and progression of ILD in RA patients.

Abbreviations: ACCP, anti-cyclic peptide containing citrulline; ACR, American College of Rheumatology; AEC, alveolar epithelial cell; AKA, anti-keratin antibody; AUC, area under the curve; CCPA, cyclic citrullinated peptide antibodies; CIA, collagen-induced arthritis; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; CTD, connective tissue diseases; DAD, diffuse alveolar damage; DAS28, disease activity score; DIP, desquamative interstitial pneumonia; ESR, erythrocyte sedimentation rate; Fzd, frizzled; GGO, ground-glass opacity; HC, honeycombing; HRCT, high-solution computed tomography; ILA, interstitial lung abnormalities; ILD, interstitial lung disease; IIP, idiopathic interstitial pneumonias; IL-6, interleukin 6; IPF, idiopathic pulmonary fibrosis; LDH, lactate dehydrogenase; NSIP, nonspecific interstitial pneumonia; LIP, lymphocytic interstitial pneumonia; OA, osteoarthritis; OP, organizing pneumonia; PCP, planar cell polarity; PN, postnatal; RA, rheumatoid arthritis; RF, rheumatoid factor; ROC, receiver operating characteristic; Ror2, orphan receptor 2; SD, standard deviation; SEM, standard error of mean; SMC, smooth muscle cell; UIP, usual interstitial pneumonia

* Corresponding authors at: College of Life Science, Ningxia University, 539 W. Helanshan Road, Yinchuan, Ningxia 750021, China.
E-mail addresses: miao-yu@nxu.edu.cn (M. Yu), gyy19954@163.com (Y. Guo), zhp357685122@163.com (P. Zhang), xuejing_0536@qq.com (J. Xue), yangjiali_123@163.com (J. Yang), caiqian1212@126.com (Q. Cai), yxh2527@163.com (X. You), majiajiaer@gmail.com (J. Ma), shengwuyangdan@163.com (D. Yang), liushenjyy@163.com (Y. Jia), wyj@nxu.edu.cn (Y. Wang), chi794613@163.com (S. Chi), mengshucao@126.com (M. Cao), chenjuan7419@163.com (J. Chen), xiaoming-liu@uiowa.edu (X. Liu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.imbio.2019.04.006
Received 10 January 2019; Received in revised form 31 March 2019; Accepted 13 April 2019
Available online 30 April 2019

1. Introduction

Connective tissue disease-related interstitial lung diseases (CTD-ILDs) are a heterogeneous group of disorders characterized by inflammation and/or fibrosis of lungs in patients with connective tissue diseases (CTD), including rheumatoid arthritis (RA) (Fernandez-Diaz et al., 2018). In this regard, 2–8% of RA patients eventually develop pulmonary fibrosis that is associated with a three-fold increased risk of mortality and has significantly economic implications (Bongartz et al., 2010; Hyldgaard et al., 2017; Raimundo et al., 2018).

Clinically, RA-ILD manifests many well-recognized phenotypes. One of the most notable distinctions of RA-ILD is a correlation with the usual interstitial pneumonia (UIP) pattern of phenotype, in which the UIP is the most common forms of ILD with a worse prognosis (Shaw et al., 2015). Indeed, in contrast to patients with other CTD-related ILDs (e.g., systemic sclerosis [SSc], idiopathic inflammatory myositis, and mixed connective tissue disease), in which a nonspecific interstitial pneumonia (NSIP) pattern is the most frequently phenotype. NSIP occurs in approximately one-third of patients with RA-ILD and is generally associated with a longer duration of articular manifestations, a lower risk of disease progression, a better response to treatment, and better overall outcomes compared with UIP (Kim et al., 2009). In addition, the organizing pneumonia (OP), lymphocytic interstitial pneumonia (LIP), diffuse alveolar damage (DAD), and desquamative interstitial pneumonia (DIP) are less frequent patterns of RA-related ILDs (Spagnolo et al., 2018). Among these patterns, UIP is the radiological form of ILD related to idiopathic pulmonary fibrosis (IPF) in clinical settings, and their terms are often interchanged each other. But UIP is not synonymous with IPF, and thus all known causes of pulmonary fibrosis need to be excluded for the diagnosis of IPF (Wuyts et al., 2014). This suggests that the disease activity and radiological pattern of ILD are the most important prognostic factors for patients with RA-ILD, and the identification of UIP in RA patients thus has an important clinical implication in guiding the treatment of RA patients with ILD.

In the case of RA-ILD patients, lung injury is induced by an autoimmune mechanism that may be controlled with timely immunosuppressive treatments (Vij and Strek, 2013). In a diagnostic standpoint, a multidisciplinary evaluation of clinical, radiological, and pathological disease features were recommended by internationally recognized guidelines for the diagnosis and management of ILD (Raghu et al., 2015; Travis et al., 2013). Of note, the high resolution computed tomography (HRCT) has emerged as an important tool in the evaluation of patients with RA-ILD. As a diagnostic modality, HRCT is widely available and is more acceptable with less risk over surgical lung biopsy (Caples et al., 2004). In addition, HRCT may provide direct images such as reticulation and honeycombing that have a good correlation with fibrosis in histopathological analysis. HRCT is also able to demonstrate ground-glass opacity change that correlate with inflammatory injury in lung tissue pathology analysis. Importantly, these HRCT images such as fibrotic images and ground-glass opacity can be quantitatively measured with indexes proposed by Kazerooni et al. (1997). With respect to RA-ILD, HRCT UIP pattern was recently found to strongly correlate with histological UIP in patients with RA-ILD, potentially obviating the need for lung biopsy in individuals with this HRCT finding (Raghu et al., 2015).

Apart from HRCT imaging, the surgical lung biopsy and bronchoscopic examination also have an important role in the diagnosis of UIP (Travis et al., 2013). Generally, a patchy interstitial fibrosis, collagen deposition, and architectural distortion characteristic of UIP pathologic pattern, evident by surgical pathology or at the macro scale by HRCT, is associated with poor prognosis and survival (Flaherty et al., 2002). Serial lung function testing has been routinely employed for monitoring the disease progression and outcome of treatments (King et al., 2001), but the clinical course of UIP is highly variable and unpredictable. Therefore, noninvasive blood biomarkers with diagnostic and prognostic utility may support the diagnosis of RA-UIP in clinic, particularly in settings with limited medical resources, and would help in the identification of vulnerable patients. With this respect, little is currently known about the biomarkers with diagnostic and prognostic value of UIP, although there was a study suggested that an increased fragmented was associated with UIP pattern patients with RA-ILD (Suhara et al., 2016).

A compelling body of studies recently demonstrated that the Wnt signaling is implicated in the pathogenesis of many diseases, including lung diseases and autoimmune diseases (Newman et al., 2016; Shi et al., 2016). The Wnt signaling pathways are well-established developmental signaling that play crucial roles in embryonic development, morphogenesis and organogenesis, as well as tissue regeneration and pathogenesis of many diseases (Nusse, 2005). Wnt signaling can be characterized as either the β-catenin-dependent canonical Wnt pathway or the β-catenin-independent non-canonical Wnt pathways include at least the planar cell polarity (PCP) pathway and Wnt/calcium pathway (Lim and Nusse, 2013). In this context, the Wnt5a is a representative ligand activating non-canonical Wnt signaling (Li et al., 2015). There are several studies supporting the role of Wnt5a in the pathogenesis of inflammatory diseases, such as RA (MacLauchlan et al., 2017; Pashirzad et al., 2017; Sen et al., 2002). For instance, increased transcripts of Wnt5a and Fzd5 were observed in RA synovial tissues, and blocking the Wnt-5a/Fzd5 signaling reduced the rheumatoid synoviocyte activation (Sen et al., 2001, 2000). Interestingly, a further study found that the pro-inflammatory cytokine IL-6 could induce Wnt5a which had implicated in synovial fibroblast hypertrophy in RA (Katoh and Katoh, 2007). Similarly, a growing body of evidence also revealed involvements of Wnt5a and non-canonical signaling in the pathogenesis of pulmonary fibrosis (Ahangari and Kaminski, 2018; Newman et al., 2016). For example, more abundant Wnt5a protein was observed in fibroblasts derived from lung tissues of IPF patients with UIP pattern, as determined by immunohistochemical staining (IHC) assay (Newman et al., 2016). These studies strongly support notions of involvements of non-canonical Wnt signaling and Wnt5a in the development of RA and pulmonary fibrosis.

In view of aforementioned findings of Wnt5a in RA and IPF, we therefore hypothesize that Wnt5a-mediated non-canonical Wnt signaling may have a clinical implication in the development of fibrosis in lungs of patients with RA-ILD, and an assessment of Wnt5a may offer clinical significances for identifying and monitoring ILD, particularly UIP in RA patients. To this end, the concentration of Wnt5a protein in plasmas of 40 clinically diagnosed RA-ILD patients from a single center was examined and analyzed by comparing with other clinical indexes. The results demonstrated a strong correlation of circulating Wnt5a protein with the disease severity of ILD in RA patients, particularly those with a UIP pattern.

2. Methods and materials

2.1. Ethics statement

Human blood samples were collected with a protocol approved by the Ethic Committee for the Conduct of Human Research at General Hospital of Ningxia Medical University (NXMU-GH-2016-073). Written consent was obtained from every individual for collecting blood samples and publishing the data according to the Ethic Committee for the Conduct of Human Research protocol. All participants were older than age of 21 years. The PI of this study maintains human research records, including signed and dated consent documents, for ten (10) years after the age of majority. The Ethic Committee the Conduct of Human Research at Ningxia Medical University approved the consent procedure for this study (NXMU-GH-2016-073).

2.2. Human subjects

Blood samples of 40 clinical diagnosed RA-ILD patients (21 females

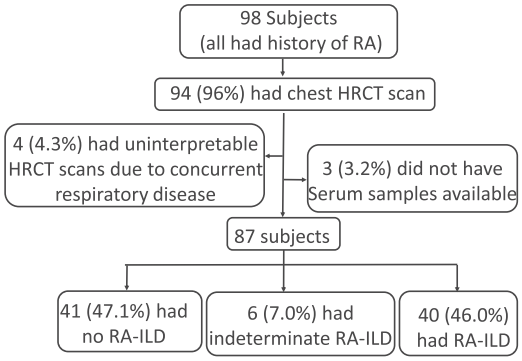

Fig. 1. Enrollment of patients in this study. A flow diagram of enrollment of participants categorized by HRCT patterns and clinical diagnostic data in this study. HRCT = high-resolution computed tomography; ILD = interstitial lung disease; RA = rheumatoid arthritis.

and 19 males) and 41 clinical diagnosed RA patients without a pulmonary complication were collected from the outpatient rheumatology and respiratory clinic of the General Hospital of Ningxia Medical University from December 2016 to January 2018 (Fig. 1). The RA was diagnosed according to the Guideline of American College of Rheumatology (ACR) 1987 criteria for the diagnosis of RA (Arnett et al., 1988). Seropositive RA was considered if the disease was associated with positive rheumatoid factor (RF) and/or cyclic citrullinated peptide antibodies (CCPA) in at least two independent tests (Fernandez-Diaz et al., 2018). Patients with ILD and IPF due to other causes, complications and/or chronic pulmonary diseases and infectious diseases such as chronic obstructive pulmonary disease (COPD), pulmonary infection, tuberculosis, and tumors in the lung were excluded from this study. Patients with severe heart, lung and renal dysfunction were also excluded. All bloods were collected in tubes with heparin, and plasmas were isolated and frozen in 100 μL aliquots at −80 °C until analyzed. There was no genetic relationship among these individuals. All the samples were collected under an informed consent.

2.3. Radiological re-categorization

The assessments of image of chest computed tomography (CT, DELINITION, Siemens Electrical Apparatus LTD, Germany)/high resolution computed tomography (HRTC) scan were independently analyzed by two senior radiologists in the General Hospital of Ningxia Medical University (with 6 and 11 years of experience, respectively). The radiologists had certificates for qualification of clinical radiological analysis issued by National Health Commission of the People's Republic of China (Beijing, China). The two radiologists evaluated independently and blindly to check the presence or absence of these CT/HRCT findings. The interpretation of differences in observed findings was determined by consensus. The radiological features of ILD were categorized according to the 2013 idiopathic interstitial pneumonia (IIP) classification (Fischer et al., 2015; Travis et al., 2013). The ILD and disease severity (no-ILD, mild-RA-ILD, moderate-RA-ILD) were assigned based on the distribution of interstitial lung abnormalities (ILA) consisting of septal lines, reticulation, traction bronchiectasis, cyst formation, and/or ground-glass opacity (GGO) image (Washko et al., 2010). The diagnostic criterion of HRCT images for no-ILD was normal or focal or unilateral GGO imaging, focal or unilateral reticulation, or patchy GGO abnormality involving < 5% of the lung (Fig. 2A). The image diagnostic criterion of mild-ILD was changes affecting > 5% of any lobar region with nondependent GGO or reticular abnormalities, diffuse centrilobular nodularity, non-emphysematous cysts, honeycombing, or traction bronchiectasis (Fig. 2B). The image diagnostic criterion of moderate-ILD was bilateral fibrosis in multiple lobes associated with honeycombing and traction bronchiectasis in a subpleural distribution (Fig. 2C) (Chen et al., 2015). No patient with severe ILD was included in this study. HRCT images were independently reclassified as UIP, NSIP, OP, and other patterns by the two radiologists according to the 2013 IIP classification (Fischer et al., 2015; Travis et al., 2013). All other patients those who did not fit the definition of any specific subtypes were classified in a group of other ILD pattern. Several representative images with different classified patterns in this study were provided in supplementary Fig. S1. The radiological RA-UIP criteria that were applied were those for the diagnosis of IPF (Raghu et al., 2015). RA-NSIP was defined as the predominance of GGO, possible visible subpleural sparing and possible fine reticulation with minor or no honeycombing. RA-OP was defined as single or multiple patchy consolidations.

2.4. HRCT imaging score

Both left and right lungs were divided into three imaging zones. The upper zones were at or superior to the aortic arch, the middle zones were between the aortic arch and pulmonary veins and the lower zones were at or below the pulmonary veins. The extents of GGO, reticulation and honeycombing were semi-quantitatively graded on a scale from 0 to 4 as follows: 0 = finding absent, 1 = minor peripheral scattered changes, 2 = uniform peripheral or minor central changes, 3 = substantial peripheral changes that penetrated deeply to the lung parenchyma, 4 = abundant peripheral and central changes. The total score of these three findings was obtained by summing the grades for all six zones, i.e. the maximum score 24 (Fig. 2D–F). Emphysema, traction bronchiectasis, architectural distortion and pleural plaques were scored using similar criteria, by summing given grades in all six zones, with exception of using score 0–3 (0–3; 0 = absent, 1 = single scattered changes, 2 = larger single changes or several minor changes, 3 = uniform or substantial changes) instead of the 0–4 scores in above GGO patterns, this therefore resulted in a score ranging from 0 to 18 (Fig. 2G–I) (Nurmi et al., 2018).

2.5. Detection of plasma Wnt5a by enzyme-linked immunosorbent assay (ELISA)

The concentration of plasma Wnt5a was measured using commercially available ELISA kit per manufacturer's instruction. The ELISA kit for Wnt5a was a product of mlbio Inc. (Shanghai, China). For detection of Wnt5a protein, the plasma was directly detected with stock suspension, and its concentration was presented as ng/mL through a standard curve. The demographic feature, serologic features, current treatment and clinical features, including medication history, the 28-joint Disease Activity Score (DAS28), current medication at baseline, plasma levels of rheumatoid factors (RF), anti-cyclic peptide containing citrulline (ACCP), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and anti-keratin antibody (AKA), lactate dehydrogenase (LDH), respiratory manifestations were also recorded.

2.6. Statistical analysis

Statistical analysis of data was performed using PRISM (version 5) (GraphPad Software, La Jolla, CA, USA) and/or SPSS for Windows (version 18.0) (SPSS Inc., Chicago, IL, USA). One-way ANOVA or Kruskal-Wallis test was employed for comparisons of more than two groups, and the *t*-test was conducted for comparison between two groups. ROC curve was used to determine the best cut of value and validity of certain variable. The multiple-factor non-conditional logistic regression analysis was performed with SPSS software. The association between qualitative variables was evaluated by Spearman correlation. Data was presented as the mean ± standard error of mean (SEM) or mean ± standard deviation (SD). A p value of less than 0.05 was considered statistically significant, which was denoted as *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.0001$.

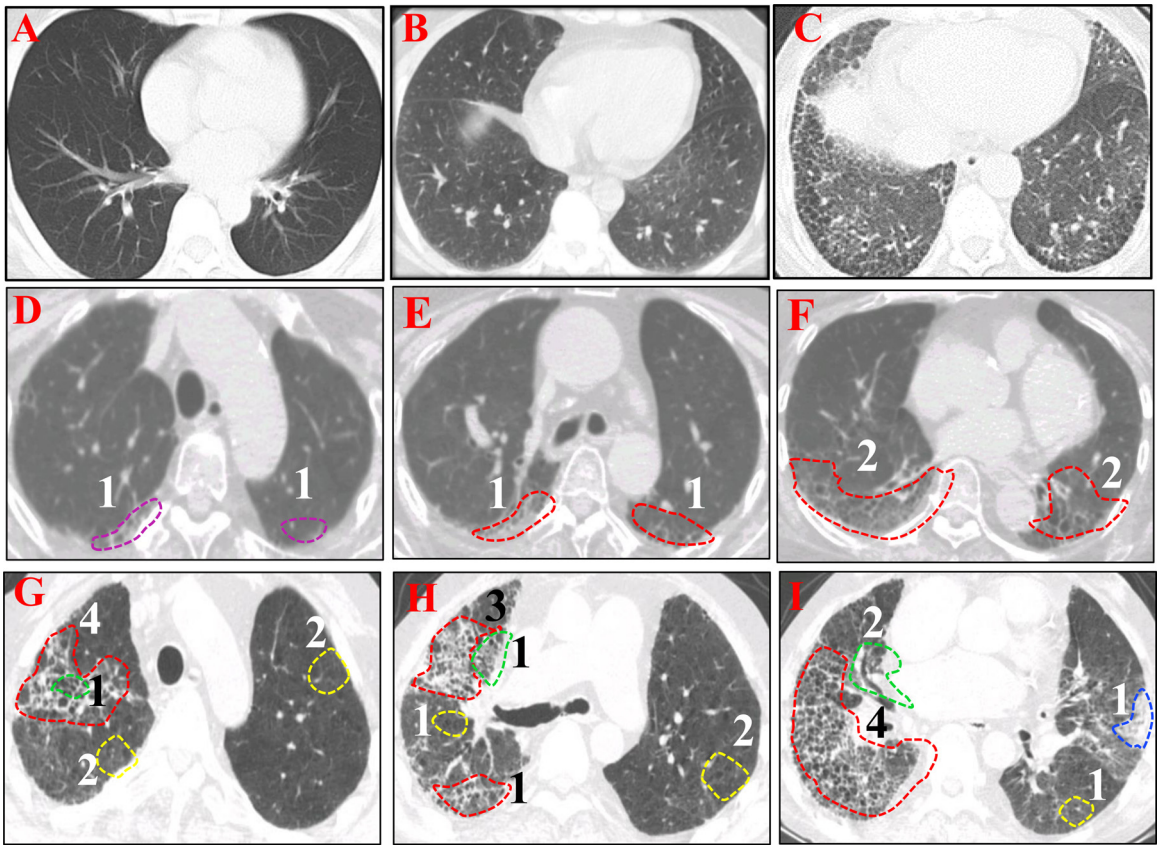

Fig. 2. Representative HRCT images with various severity of ILD and scoring criteria. (A–C) The severity of ILD was determined by senior radiologists based on the diagnostic criterion of HRCT images for no-ILD, mild-ILD and moderate-ILD. A. A representative HRCT image of no-ILD. B. A representative HRCT image of mild-ILD. C. A representative HRCT image of moderate-ILD. (D–F) An example of the mild honeycombing in the study represented axial HRCT images of a 59-year-old female with RA-UIP. D. Upper zone, at the level of the aortic arch. Pleural plaques was graded as score 1 on the right side and score 1 on the left, thus giving a total score of 2 for Pleural plaques in this zone. E. Middle zone, between the aortic arch and pulmonary veins. Honeycombing was graded as score 1 on the both sides, making the total honeycombing score to 2 at this point. F. Lower zone, below pulmonary veins. Honeycombing was graded as 2 on the both sides. In a total honeycombing score of 6, reflecting the extent of honeycombing. Magenta: pleural plaques; Red: honeycombing. (G–I). An example of the moderate honeycombing in the study represented axial HRCT images of a 58-year-old male with RA-UIP. G. Upper zone, at the level of the aortic arch. Emphysema was graded as score 2 on the both sides. Traction bronchiectasis and Honeycombing were graded as 1 and 4 on the right side, respectively. Thus giving a total score of 9 in this zone. H. Middle zone, between the aortic arch and pulmonary veins. Emphysema was graded as score 2 on the left side and 1 on the right side. Traction bronchiectasis and Honeycombing were graded as 1 and 4 on the right side, making the total score to 8 at this point. I. Lower zone, below pulmonary veins. Both of emphysema and reticulation were graded as score 1 on the left side. Honeycombing and traction bronchiectasis were graded as 4 and 2 on the right side, respectively. In a total honeycombing score of 12, reflecting the extent of honeycombing. Yellow: emphysema; Green: traction bronchiectasis; Red: honeycombing; Blue: reticulation (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3. Results

3.1. Elevated Wnt5a protein in the plasma of RA-ILD patients

To determine whether Wnt5a protein was correlated with ILD activity, plasma concentration of Wnt5a was evaluated in RA patients with and without ILD. More abundant plasma Wnt5a protein was found in RA-ILD patients (3.04 ± 0.50 ng/mL) than that of non-ILD RA cohorts (2.05 ± 0.22 ng/mL) ($p = 0.0354$) (Fig. 3A). In addition, even higher plasma Wnt5a level was determined in moderate-RA-ILD patients (4.26 ± 0.92 ng/mL) compared with non-ILD RA cohorts (2.05 ± 0.22 ng/mL) ($p = 0.0052$), and mild RA-ILD patients (2.14 ± 0.47 ng/mL) ($p = 0.0219$) (Fig. 3A). In addition, 27.5% (11/40) of RA-ILD patients and 4.8% (2/41) of RA cohorts were positive for Wnt5a, as determined using a cutoff of two standard deviations above the mean Wnt5a level in plasma of RA cohorts ($p < 0.000$).

3.2. Correlation between Wnt5a protein and RF in RA-ILD patients

The above data showed that plasma Wnt5a protein was more abundant in RA-ILD patients in comparison with that in RA patients without ILD, the correlation of Wnt5a protein and serologic features were analyzed. Interestingly, the Wnt5a protein was positively correlated with RA serological marker RF ($r = 0.4630$, $p = 0.0026$) (Fig. 3B). Further correlation analysis was performed for determination of the correlation coefficients of Wnt5a with other clinical serological biomarkers, including ESR, CRP, AKA, anti-CCP and LDH. The correlation coefficients between Wnt5a and ESR, Wnt5a and CRP, and Wnt5a and LDH were respective $r = 0.1463$ ($p = 0.3676$) (Fig. S2A), $r = 0.2387$ ($p = 0.1380$) (Fig. S2B) and $r = -0.1235$ ($p = 0.4487$) (Fig. S2C). No difference was detected between AKA positive and AKA negative (Fig. S3A), CCP positive and CCP negative in these patients (Fig. S3B).

3.3. Correlation of Wnt5a with clinical characteristics of RA-ILD patients

To determine whether Wnt5a protein was associated with demographic characteristics, serologic features, clinical features, or current treatments within the RA-ILD population, patients were grouped according to their Wnt5a protein status and variables collected at their baseline visit were compared (Table 1). 81.8% of Wnt5a positive was detected in current smokers and/or ex-smokers, this portion was significantly higher than those in the Wnt5a protein negative group

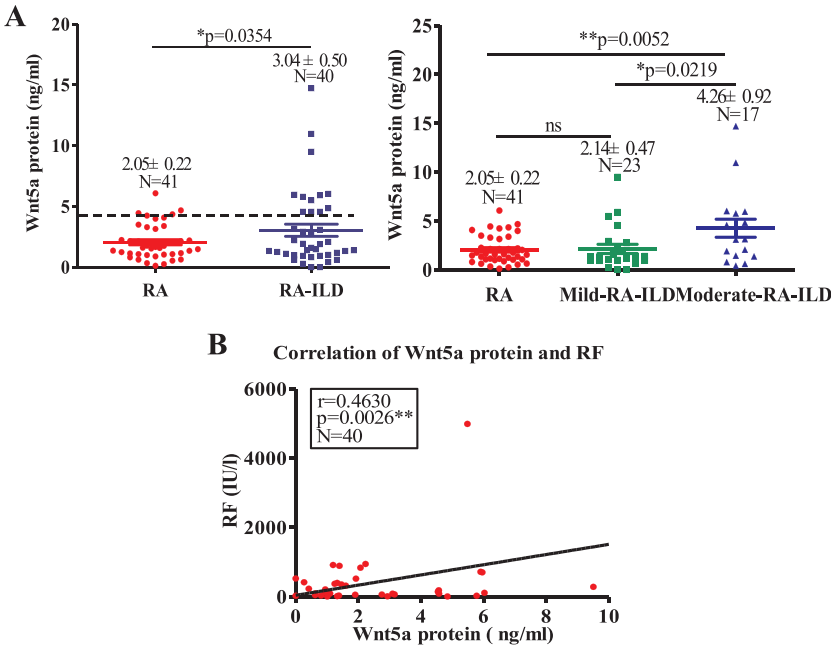

Fig. 3. Correlations of Wnt5a protein and other risk factor in RA-ILD patients. A. The plasma concentration of Wnt5a protein in RA patients with and without ILD. The concentration of Wnt5a protein was determined in sera of RA patients with and without ILD by an ELISA. Statistical differences were found between RA and RA-ILD patients (Left panel, $p = 0.0354$), RA and Moderate-RA-ILD patients (Right panel, $p = 0.0052$), moderate-RA-ILD and Mild-RA-ILD patients (Right panel, $p = 0.0219$). No statistical difference was detected between RA and Mild-RA-ILD patients (Right panel, $p = 0.163$). More abundant Wnt5a protein was detected in Moderate-RA-ILD patients relative to RA and mild-RA-ILD patients, and the highest concentration of Wnt5a protein was determined in moderate-RA-ILD patients. B. A positive correlation between Wnt5a protein and RF was detected by Spearman test. The cutoff for Wnt5a positivity in A was indicated (—) at 2-standard-deviations above the mean of the RA samples. **: $p < 0.01$; ***: $p < 0.001$. Data represent the mean ± SEM in each group. Spearman r and p values were displayed on graph ($r = 0.4630$, $p = 0.0026$, $N = 40$) in B.

Table 1
Characteristics of RA-ILD patients according to Wnt5a protein status.

Patient characteristics	Wnt5a negative (n = 29)	Wnt5a positive (n = 11)	p-value
Demographic features			
Ethnics (Chinese Han)	28 (96.6)	10 (90.9)	0.465
Age (mean ± SD) (yrs)	60.14 ± 1.67	61.09 ± 3.88	0.3961
Gender (male/female) (%female)	12 (41.4)	5 (45.5)	0.816
RA duration (mean ± SD) (range, yrs)	9.16 ± 1.88	12.64 ± 3.15	0.1625
Current/ever-smoker	13 (44.8)	9 (81.8)	0.036
Serologic features			
AKA (+) number (%)	10 (34.4)	3 (27.3)	0.664
RF (IU/ml)	597.88 ± 214.30	196.5 ± 71.2	0.1212
Anti-CCP (+) number (%)	27 (93.1)	9 (81.8)	0.288
ESR (mm/h)	53.55 ± 5.38	56.90 ± 8.51	0.3764
CRP (mg/dl)	36.64 ± 4.85	76.89 ± 16.78	0.0049
LDH (U/l)	237.42 ± 25.01	306.44 ± 118.30	0.2111
Current treatments			
NSAIDs	6 (20.1)	2 (18.2)	0.859
Glucocorticoids	3 (10.3)	1 (9.1)	0.906
MTX	7 (24.1)	1 (9.1)	0.288
DMARDs including MTX	14 (48.3)	6 (54.5)	0.723
CLINICAL FEATURES			
RA duration (mean ± SD) (yrs)	9.16 ± 1.78	12.64 ± 13.15	0.1625
DAS28 score	5.25 ± 0.62	5.50 ± 0.97	0.4195
Cough	27 (93.1)	10 (90.9)	0.814
Sputum	9 (31.0)	4 (36.4)	0.748
Dyspnea	26 (89.7)	8 (72.7)	0.181
Chest congestion	7 (24.1)	2 (18.2)	0.687
Dry rale	20 (69.0)	5 (45.5)	0.170
Bluish skin tinge	3 (10.3)	0 (0.0)	0.267
Clubbing	2 (6.9)	0 (0.0)	0.276

AKA: anti-keratin antibody; CCP: Cyclic citrullinated; CRP: C-reactive protein; LDH: Lactic dehydrogenase; DMARDs: disease-modifying anti-rheumatic drugs; DAS 28: 28-joint Disease Activity Score; ESR: Erythrocyte sedimentation rate; RA: Rheumatoid arthritis; RF: Rheumatoid factor.

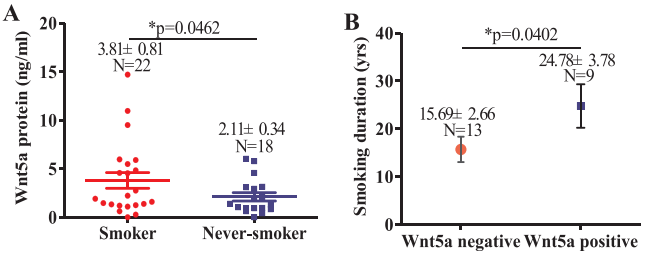

Fig. 4. Wnt5a protein was associated with smoking in RA-ILD patients. A. The concentration of plasma Wnt5a protein in RA-ILD smoker and RA-ILD Never-smoker. Statistical differences were found between RA-ILD smoker and RA-ILD Never-smoker ($p = 0.0462$). B. Smoking duration in Wnt5a positive and Wnt5a negative patients. Statistical differences were found between Wnt5a positive patients and Wnt5a negative patients ($p = 0.0402$). More abundant plasma Wnt5a protein was detected in RA-ILD smoker relative to RA-ILD Never-smoker. Longer smoking duration was observed in Wnt5a positive in comparison with Wnt5a negative patients.

(44.8%) ($p = 0.036$) (Table 1). Of note, the respective concentrations of plasma Wnt5a protein were 3.81 ± 0.81 and 2.11 ± 0.34 ng/mL in RA-ILD smokers and never-smokers ($p = 0.0462$) (Fig. 4A); Moreover, the plasma Wnt5a protein was also correlated with the smoking duration, patients with a longer smoking duration (24.78 ± 3.78 yrs) were more likes with plasma Wnt5a protein relative to those with a undetectable Wnt5a protein (15.69 ± 2.66 yrs) ($p = 0.0402$) (Fig. 4B). However, there was no significant difference in plasma Wnt5a protein was detected between RA-UIP smokers and never-smokers (Fig. S4A), and smoking duration was no correlation between RA-UIP patients with positive Wnt5a protein and negative Wnt5a protein (Fig. S4B). In addition, the titers of C-reactive protein (CRP) in RA-ILD patients with positive Wnt5a protein and negative Wnt5a protein were 76.89 ± 16.78 and 36.64 ± 7.85 mg/dl, respectively. Suggesting that CRP was significantly associated with positive Wnt5a protein ($p = 0.0049$). There was no significant correlation of frequencies of other serologic features, current treatments or clinical features to plasma Wnt5a protein was detected (Table 1).

3.4. Circulating Wnt5a protein correlates the progression of RA-ILD

In order to determine whether plasma Wnt5a protein was correlated

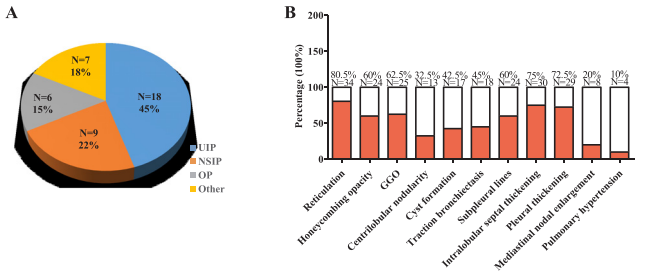

Fig. 5. CT-c or HRCT findings in RA patients with ILD. A. The HRCT image patterns were determined according to the diagnostic criterion for ILD. The proportions of UIP pattern (45%, n = 18), NSIP pattern (22%, n = 9), OP pattern (15%, n = 6) and other ILD pattern (17.5%, n = 7) were determined in RA-ILD patients (N = 40). B. The proportion of the ILD HRCT image patterns in patients with RA-ILD.

with the progression of disease severity, the clinical ILD patterns were first classified. 18 of 40 (45%) patients with imaging features of ILD were UIP, 9 of 40 (22.5%) cases were NSIP, 6 of 40 (15%) cases were OP, and the rest 7 (17.5%) cases were other ILD pattern, as determined by HRCT imaging (Fig. 5A). HRCT imageological analysis further revealed that reticulation (34/40, 85.0%) and pleural thickening (29/40; 72.5%), as well as intralobular septal thickening (30/40, 70.0%) were the most predominant patterns, followed by GGO (25/40, 62.5%) and honeycombing opacity (24/40, 60.0%), subpleural lines (24/40, 60.0%), traction bronchiectasis (18/40, 45%), cyst formation (17/40, 42.5%), centrilobular nodularity (13/40, 32.5%), mediastinal nodal enlargement (8/40, 20.0%), and pulmonary hypertension (4/40, 10.0%) (Fig. 5B). Results showed that the plasma Wnt5a concentrations were 5.12 ± 0.85 ng/mL, 1.17 ± 0.22 ng/mL, 2.24 ± 0.55 ng/mL and 0.78 ± 0.26 ng/mL for RA patients with UIP, NSIP, OP and other ILD pattern, respectively. Interestingly, more abundant Wnt5a protein was detected in the plasma of UIP patients relative to NSIP patients (p = 0.0018), which was also higher in comparison with those patients

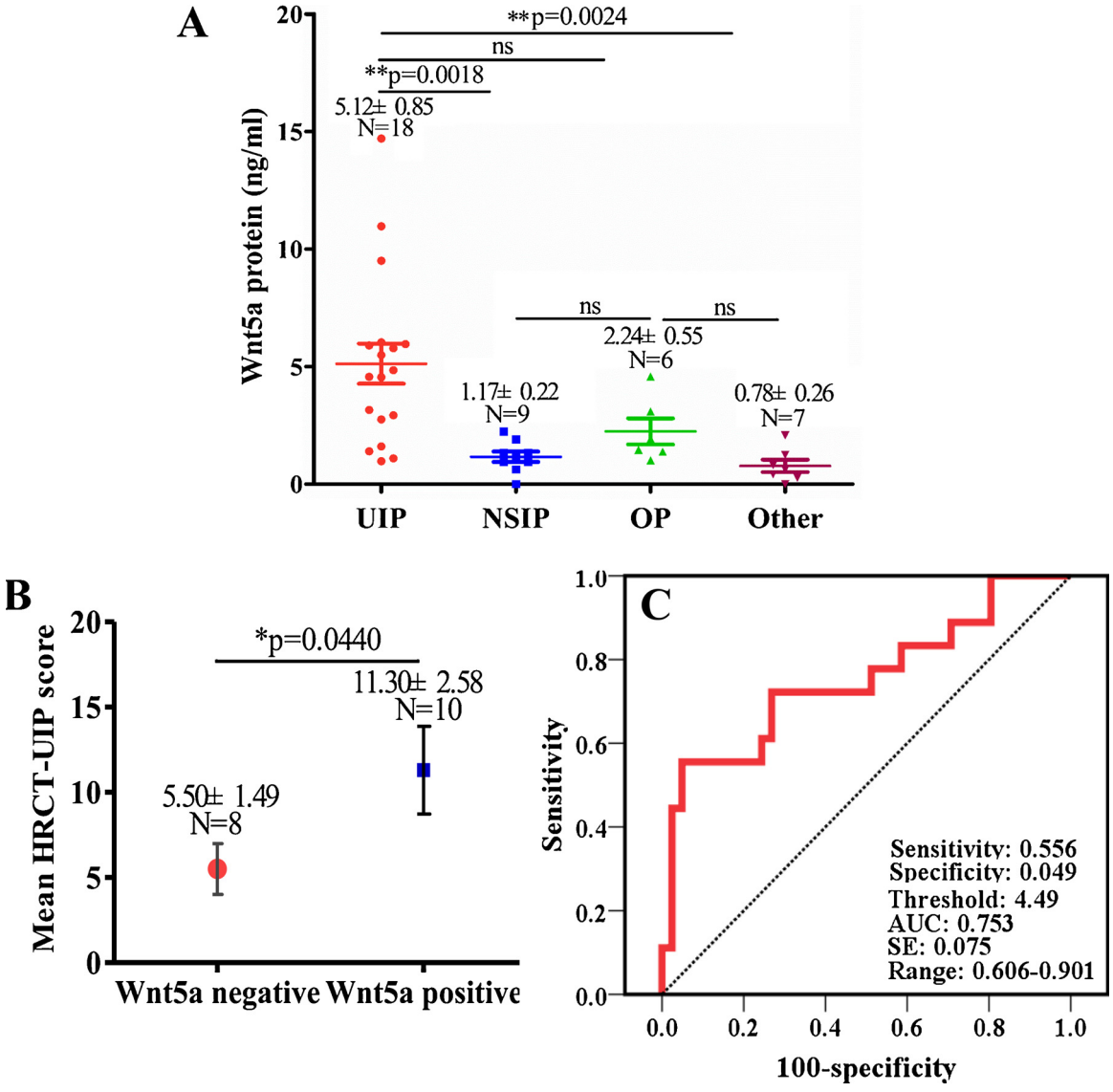

Fig. 6. Association of Wnt5a protein with the severity of RA-ILD. A. The plasma concentrations of Wnt5a protein in RA-ILD patients with different HRCT image patterns of ILD. The concentration of Wnt5a protein in UIP, NSIP, OP and patients with other ILD patterns. Statistical difference was detected between UIP and NSIP patients (p = 0.0018), UIP and patients with other ILD patterns. No Statistical differences were determined between UIP and OP (p = 1.141), NSIP and OP (p = 1.321), OP and other ILD patterns (p = 1.221). B. The mean HRCT-UIP score is shown according to Wnt5a protein status. Statistical differences were found between Wnt5a positive and Wnt5a negative patients (p = 0.0440). The higher HRCT-UIP score was observed in Wnt5a positive relative to Wnt5a negative patients. C. ROC curve for Wnt5a in rheumatoid arthritis-usual interstitial pneumonia.

with other pattern ($p = 0.0024$). However, no difference was detected in the plasma Wnt5a protein concentration between UIP and OP patients, NSIP and OP patients, OP and patients with other ILD patterns (Fig. 6A). Of interest, the UIP pattern was predominantly observed in the Wnt5a positive group (10 out of the 18 UIP patients, 55%) over the Wnt5a negative patients (8 of 18 UIP, 45%). Equally noteworthy, the abundance of Wnt5a protein was associated with the score of HRCT-UIP. The HRCT-UIP score in Wnt5a positive patients was higher compared to those with undetectable Wnt5a protein (11.30 ± 2.58 *vs* 5.50 ± 1.49; $p = 0.0440$, Fig. 6B).

3.5. Clinical significance of Wnt5a protein the identification of RA-ILD patients

More abundant plasma Wnt5a protein was found in RA-ILD patients than in non-ILD RA patients, which was even higher in RA-UIP patients than those with RA-NSIP and other ILD pattern. Of note, the HRCT-UIP score was higher in patients with detectable Wnt5a protein in plasma compared to those who with undetectable Wnt5a, suggesting that the Wnt5a could sever as an index for accessing the severity of UIP in RA patients. To evaluate the significance of the Wnt5a protein in clinical settings, we analyzed the sensitivities and specificities of these circulating factors in identified RA patients with UIP (Fig. 6C). The area under the curve (AUC) was 0.753 (SE: 0.075; range: 0.606–0.901; sensitivity: 0.556; specificity: 0.049) for Wnt5a protein. Interestingly, the multiple-factor non-conditional logistic regression analysis of impacts on RA-ILD suggested that plasma Wnt5a, age, RA duration and smoking were clinically significant factors for RA-ILD (Table 2). These results thus imply that the plasma Wnt5a protein may be an independent biomarker for the identification of UIP in RA patients, which can be an independent biomarker candidate for evaluating disease severity and progression of UIP in RA patients.

4. Discussion

In the present report, we examined Wnt5a protein in plasma of RA-ILD patients, and identified that plasma Wnt5a had a strong association with the severity and progression of UIP in RA patients with ILD relative to non-ILD. Even higher Wnt5a level in plasmas of RA patients with moderate ILD and UIP, in comparison with those with mild ILD and other patterns of ILD including NSIP and other undefined phenotypes, respectively. Intriguingly, the increased plasma Wnt5a in RA-ILD patients was positively correlated with rheumatoid factor (RF), and HRCT-UIP score. Additionally, a higher level of plasma Wnt5a protein was associated with smoking status in RA-ILD patients. Multiple-factor non-conditional logistic regression analysis suggested that the age, RA duration, smoking status and Wnt5a were clinically significant risk factors in RA-ILD. ROC curves further demonstrated that the plasma Wnt5a was a potential biomarker for the identification of severity and UIP in RA patients. This study thus implies that the circulating Wnt5a protein may be a valuable biomarker for identifying UIP and accessing the severity and progression of ILD in RA patients.

An early identification of pulmonary manifestations in RA patients has an importantly clinical significance for guiding treatments. As one of the most crucial signaling pathways in the morphogenesis and injury repair, an aberrant activation of non-canonical Wnt signaling has a strong implication in the pathogenesis of many diseases (Nusse, 2005). In this context, several key molecules in this signaling cascade have been identified to contribute to the pathogenesis of diseases (Abdelhamed et al., 2015; Ackers and Malgor, 2018; Uehara et al., 2018). Amongst these, the Wnt5a was one of the most extensively studied signaling molecules in both lung and autoimmune diseases (Pashirzad et al., 2017).

The Wnt5a, a representative ligand that activates non-canonical Wnt signaling in regulation of cell migration and polarity during embryonic morphogenesis, which is normally down-regulated in adult tissues. Owing to its pivotal role in embryonic morphogenesis, Wnt5a has thus been implicated in osteoclast dysregulation in RA. An increased Wnt5a concentration was detected in the synovium in patients with RA and osteoarthritis (OA) (Sen et al., 2000), and an inhibition of Wnt5a was able to reduce the proliferation of synovial fibroblasts derived from RA patients (Sen et al., 2001). In addition, a recent study showed the delivery of a soluble form of receptor-tyrosine orphan receptor 2 (ROR2), a candidate decoy receptor for Wnt5a, led to reduced radiographic severity of collagen-induced arthritis (CIA) murine model (Maeda et al., 2012). These data underscored the potential clinical value of Wnt5a in the pathogenesis of RA.

In the lung, the Wnt5a-induced non-canonical Wnt signaling is critical for pulmonary capillary patterning (Li et al., 2002), alveolar epithelial cell (AEC) differentiation (Ghosh et al., 2013), smooth muscle cell (SMC) function (Kumawat et al., 2013) and lung fibroblasts (Boucherat et al., 2007). With respect to lung fibroblasts, Wnt5a and Fzd1 were increased from postnatal day 2 (PN2) to PN7 fibroblasts in rat lungs, which were correlated with the stages before and during alveolar formation, respectively (Boucherat et al., 2007). Of great interest, Wnt5a exhibited potentials to promote cell proliferation, increase fibronectin expression and inhibit H_2O_2-induced apoptosis in lung fibroblasts. Therefore, the aberrant activation of Wnt5a-mediated non-canonical Wnt signaling may be instrumental in promoting lung diseases. In the present study, positive correlations between plasma Wnt5a and RF and HRCT-UIP score were also detected in RA-ILD patients in clinical settings. More interestingly, the concentration of Wnt5a protein was even higher in RA patients with UIP pattern of lung disease in comparison with who had NSIP or other ILD patterns. Consistently, the ROC curve also suggested that the plasma Wnt5a protein could be considered as a positive biomarker for the identification of UIP, and severity and/or progression of UIP in RA patients. Of note, less abundant plasma Wnt5a protein was found in patients with an OP pattern of ILD (Fig. 6A). Given the fact of that OP is an independent pattern different from UIP, as well as a small size of OP samples (6) analyzed in this study, this observation is required for further investigate with larger sample sizes.

Several lines of evidences have demonstrated that aging, male, smoking, increased RF and anti-CCP antibody are predictors for the development of RA-ILD (Dawson et al., 2002; Sathi et al., 2011). Indeed, smokers were reported at higher risk of developing lung diseases, which was associated with numerous molecular signaling pathways, including the non-canonical Wnt signaling (Vassallo, 2012). For instance, the cigarette smoke could induce Wnt5a-coupled PKC activity during lung carcinogenesis, which subsetantially induced Akt activity and anti-apoptosis in lung cancer (Whang et al., 2013). In line with these findings, more abundant Wnt5a protein was in plasmas of RA-ILD smokers than RA-ILD never-smokers, and the abundance of Wnt5a was strongly correlated with longer smoking duration in RA-ILD patients.

Table 2
Multiple-factor non-conditional logistic regression analysis of the impact of serological factors on RA-ILD.

	Male	Age	RA duration	Smoking	RF	LDH	ESR	AKA	Anti-CCP	CRP	Wnt5a
WALD	02.328	9.993	5.643	7.486	0.042	1.989	0.079	3.705	2.359	0.569	5.836
P	0.127	0.002	0.018	0.006	0.837	0.158	0.779	0.054	0.125	0.451	0.016
OR	4.629	1.131	1.182	15.926	1.000	1.004	0.995	7.183	5.620	1.016	2.285

Consistently, multiple-factor non-conditional logistic regression analysis also defined that the smoking status was a risk factor in development of ILD in RA patients.

Apart from the RF, several serological indexes, such as erythrocyte sedimentation rate (ESR) and cyclic citrullinated peptide antibodies (CCPA) also were reported to have diagnostic values for ILD in RA. For instances, an association between RA-ILD and increased ESR was detected in a group of 52 RA-ILD patients (Koduri et al., 2010), such an association was also identified CCPA and RA-ILD (Inui et al., 2008; Korkmaz et al., 2006). In agreement with their findings, positive correlations between the plasma Wnt5a protein and plasma RF and C-reactive protein (CRP) were detected in this study.

Histologically, distinct patterns of ILD have been originally defined, among which the UIP is the most frequent pattern in RA-ILD (Kelly et al., 2014). RA-ILD patients with UIP pattern appear to have a distinct clinical phenotype compared to those with other patterns. Patients with a UIP pattern were found to usually correlate to older, male, less responsiveness to conventional treatment, and a shortened survival time in RA-ILD patients (Kim et al., 2010; Lee et al., 2005; Nannini et al., 2008). Of note, the UIP was the prevalent ILD pattern in RA patients in this study, and the plasma Wnt5a had a stronger association with the UIP pattern than NSIP and other ILD patterns in RA-ILD patients. This was further supported by HRCT finding, i.e. the clinical HRCT-UIP score was higher in patients with detectable plasma Wnt5a protein compared to those with negative Wnt5a. Clinically, the most common abnormal chest radiological findings were reticulation, pleural thickening and intralobular septal thickening in this study, which was in line with the report from a recent study of 60 RA-ILD patients (Nurmi et al., 2018). They also found that the primary radiologic features of RA-ILD were reticular opacity, pleural thickening, GGO and intralobular septal thickening (Nurmi et al., 2018).

Collectively, this study analyzed the correlation of plasma Wnt5a protein and other serological biomarkers and clinical manifestations in 40 RA-ILD patients. More abundant Wnt5a protein was observed in RA-ILD patients compared with non-ILD RA patients, which was positively correlated with circulating RF. More importantly, the plasma Wnt5a concentration was correlated with the severity of UIP. In this regard, more abundant Wnt5a protein was detected in UIP patients than NSIP and other patients. Notably, such a higher level of Wnt5a protein was tightly associated with HRCT-UIP score in clinical settings. The ROC curve further revealed that the circulating Wnt5a was as a positive biomarker for the identification of UIP in RA patients. These data suggest that the Wnt5a protein may a serological biomarker for predicting the presence, severity and/or progression of RA-UIP, which warrants further investigation in clinical settings. However, there were several limitations in this study. First, the relatively small number of patients was included in this study, which limited our power to detect statistical differences in different lung imaging patterns. Second, clinical features lack of follow-up data such as a lack of pulmonary function and/or BALF testing. Third, most RA-ILD patients do not undergo surgical lung biopsy to confirm the pathological type. Fourth, we were unable to determine the ELISA kit's precise sensitivity. These limitations may partially explain the discrepancies between our study and other studies. Therefore, these findings require further confirmation in a larger and more selected population in the future.

Competing of interests

The authors declare that they have no competing interests. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (Nos. 31860318, 8176004 and 81860132), a grant from First-Class Discipline Construction Founded Project of Ningxia Medical University and the School of Clinical Medicine (NXYLXK2017A05), grants from the Natural Science Foundation of Ningxia (NZ17169 and NZ17161), and an internal supporting grant of Ningxia Medical University (XZ2017009). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imbio.2019.04.006.

References

Abdelhamed, Z.A., Natarajan, S., Wheway, G., Inglehearn, C.F., Toomes, C., Johnson, C.A., Jagger, D.J., 2015. The Meckel-Gruber syndrome protein TMEM67 controls basal body positioning and epithelial branching morphogenesis in mice via the non-canonical Wnt pathway. Dis. Model. Mech. 8, 527–541. https://doi.org/10.1242/dmm.019083.

Ackers, I., Malgor, R., 2018. Interrelationship of canonical and non-canonical Wnt signalling pathways in chronic metabolic diseases. Diab. Vasc. Dis. Res. 15, 3–13. https://doi.org/10.1177/1479164117738442.

Ahangari, F., Kaminski, N., 2018. WNT5a in extracellular vesicles–a new frontier for pulmonary fibrosis. Am. J. Respir. Crit. Care Med. https://doi.org/10.1164/rccm.201807-1321ED.

Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang, M.H., Luthra, H.S., et al., 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 31, 315–324.

Bongartz, T., Nannini, C., Medina-Velasquez, Y.F., Achenbach, S.J., Crowson, C.S., Ryu, J.H., Vassallo, R., Gabriel, S.E., Matteson, E.L., 2010. Incidence and mortality of interstitial lung disease in rheumatoid arthritis: a population-based study. Arthritis Rheum. 62, 1583–1591. https://doi.org/10.1002/art.27405.

Boucherat, O., Franco-Montoya, M.L., Thibault, C., Incitti, R., Chailley-Heu, B., Delacourt, C., Bourbon, J.R., 2007. Gene expression profiling in lung fibroblasts reveals new players in alveolarization. Physiol. Genomics 32, 128–141. https://doi.org/10.1152/physiolgenomics.00108.2007.

Caples, S.M., Utz, J.P., Allen, M.S., Ryu, J.H., 2004. Thoracic surgical procedures in patients with rheumatoid arthritis. J. Rheumatol. 31, 2136–2141.

Chen, J., Doyle, T.J., Liu, Y., Aggarwal, R., Wang, X., Shi, Y., Ge, S.X., Huang, H., Lin, Q., Liu, W., Cai, Y., Koontz, D., Fuhrman, C.R., Golzarri, M.F., Liu, Y., Hatabu, H., Nishino, M., Araki, T., Dellaripa, P.F., Oddis, C.V., Rosas, I.O., Ascherman, D.P., 2015. Biomarkers of rheumatoid arthritis-associated interstitial lung disease. Arthritis Rheumatol. 67, 28–38. https://doi.org/10.1002/art.38904.

Dawson, J.K., Fewins, H.E., Desmond, J., Lynch, M.P., Graham, D.R., 2002. Predictors of progression of HRCT diagnosed fibrosing alveolitis in patients with rheumatoid arthritis. Ann. Rheum. Dis. 61, 517–521.

Fernandez-Diaz, C., Loricera, J., Castaneda, S., Lopez-Mejias, R., Ojeda-Garcia, C., Olive, A., Rodriguez-Muguruza, S., Carreira, P.E., Perez-Sandoval, T., Retuerto, M., Cervantes-Perez, E.C., Flores-Robles, B.J., Hernandez-Cruz, B., Urruticoechea, A., Maiz-Alonso, O., Arboleya, L., Bonilla, G., Hernandez-Rodriguez, I., Palma, D., Delgado, C., Exposito-Molinero, R., Ruibal-Escribano, A., Alvarez-Rodriguez, B., Blanco-Madrigal, J., Bernal, J.A., Vela-Casasempere, P., Rodriguez-Gomez, M., Fito, C., Ortiz-Sanjuan, F., Narvaez, J., Moreno, M., Lopez-Corbeto, M., Mena-Vazquez, N., Aguilera-Cros, C., Romero-Yuste, S., Ordonez, S., Villa-Blanco, I., Gonzelez-Vela, M.C., Mora-Cuesta, V., Palmou-Fontana, N., Hernandez, J.L., Gonzalez-Gay, M.A., Blanco, R., 2018. Abatacept in patients with rheumatoid arthritis and interstitial lung disease: a national multicenter study of 63 patients. Semin. Arthritis Rheum. 48, 22–27. https://doi.org/10.1016/j.semarthrit.2017.12.012.

Fischer, A., Antoniou, K.M., Brown, K.K., Cadranel, J., Corte, T.J., du Bois, R.M., Lee, J.S., Leslie, K.O., Lynch, D.A., Matteson, E.L., Mosca, M., Noth, I., Richeldi, L., Strek, M.E., Swigris, J.J., Wells, A.U., West, S.G., Collard, H.R., Cottin, V., on behalf of the "ERS/ATS Task Force on Undifferentiated Forms of CTD-ILD, 2015. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. Eur. Respir. J. 46, 976–987.

Flaherty, K.R., Toews, G.B., Travis, W.D., Colby, T.V., Kazerooni, E.A., Gross, B.H., Jain, A., Strawderman 3rd, R.L., Paine, R., Flint, A., Lynch 3rd, J.P., Martinez, F.J., 2002. Clinical significance of histological classification of idiopathic interstitial pneumonia. Eur. Respir. J. 19, 275–283. https://doi.org/10.1183/13993003.00150-2015.

Ghosh, M.C., Gorantla, V., Makena, P.S., Luellen, C., Sinclair, S.E., Schwingshackl, A., Waters, C.M., 2013. Insulin-like growth factor-I stimulates differentiation of ATII cells to ATI-like cells through activation of Wnt5a. Am. J. Physiol. Lung Cell Mol. Physiol. 305, L222–228. https://doi.org/10.1152/ajplung.00014.2013.

Hyldgaard, C., Hilberg, O., Pedersen, A.B., Ulrichsen, S.P., Lokke, A., Bendstrup, E., Ellingsen, T., 2017. A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality. Ann. Rheum. Dis. 76, 1700–1706. https://doi.org/10.1136/annrheumdis-2017-211138.

Inui, N., Enomoto, N., Suda, T., Kageyama, Y., Watanabe, H., Chida, K., 2008. Anti-cyclic citrullinated peptide antibodies in lung diseases associated with rheumatoid arthritis.

Clin. Biochem. 41, 1074–1077. https://doi.org/10.1016/j.clinbiochem.2008.06.014.
Katoh, M., Katoh, M., 2007. STAT3-induced WNT5A signaling loop in embryonic stem cells, adult normal tissues, chronic persistent inflammation, rheumatoid arthritis and cancer (review). Int. J. Mol. Med. 19, 273–278.
Kazerooni, E.A., Martinez, F.J., Flint, A., Jamadar, D.A., Gross, B.H., Spizarny, D.L., Cascade, P.N., Whyte, R.I., Lynch 3rd, J.P., Toews, G., 1997. Thin-section CT obtained at 10-mm increments versus limited three-level thin-section CT for idiopathic pulmonary fibrosis: correlation with pathologic scoring. AJR Am. J. Roentgenol. 169, 977–983.
Kelly, C.A., Saravanan, V., Nisar, M., Arthanari, S., Woodhead, F.A., Price-Forbes, A.N., Dawson, J., Sathi, N., Ahmad, Y., Koduri, G., Young, A., British Rheumatoid Interstitial Lung, N, 2014. Rheumatoid arthritis-related interstitial lung disease: associations, prognostic factors and physiological and radiological characteristics–a large multicentre UK study. Rheumatology (Oxford) 53, 1676–1682. https://doi.org/10.1093/rheumatology/keu165.
Kim, E.J., Collard, H.R., King Jr., T.E., 2009. Rheumatoid arthritis-associated interstitial lung disease: the relevance of histopathologic and radiographic pattern. Chest 136, 1397–1405. https://doi.org/10.1378/chest.09-0444.
Kim, E.J., Elicker, B.M., Maldonado, F., Webb, W.R., Ryu, J.H., Van Uden, J.H., Lee, J.S., King Jr., T.E., Collard, H.R., 2010. Usual interstitial pneumonia in rheumatoid arthritis-associated interstitial lung disease. Eur. Respir. J. 35, 1322–1328. https://doi.org/10.1183/09031936.00092309.
King Jr., T.E., Tooze, J.A., Schwarz, M.I., Brown, K.R., Cherniack, R.M., 2001. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. Am. J. Respir. Crit. Care Med. 164, 1171–1181. https://doi.org/10.1164/ajrccm.164.7.2003140.
Koduri, G., Norton, S., Young, A., Cox, N., Davies, P., Devlin, J., Dixey, J., Gough, A., Prouse, P., Winfield, J., Williams, P., Eras, 2010. Interstitial lung disease has a poor prognosis in rheumatoid arthritis: results from an inception cohort. Rheumatology (Oxford) 49, 1483–1489. https://doi.org/10.1093/rheumatology/keq035.
Korkmaz, C., Us, T., Kasifoglu, T., Akgun, Y., 2006. Anti-cyclic citrullinated peptide (CCP) antibodies in patients with long-standing rheumatoid arthritis and their relationship with extra-articular manifestations. Clin. Biochem. 39, 961–965. https://doi.org/10.1016/j.clinbiochem.2006.06.004.
Kumawat, K., Menzen, M.H., Bos, I.S., Baarsma, H.A., Borger, P., Roth, M., Tamm, M., Halayko, A.J., Simoons, M., Prins, A., Postma, D.S., Schmidt, M., Gosens, R., 2013. Noncanonical WNT-5A signaling regulates TGF-beta-induced extracellular matrix production by airway smooth muscle cells. FASEB J. 27, 1631–1643. https://doi.org/10.1096/fj.12-217539.
Lee, H.K., Kim, D.S., Yoo, B., Seo, J.B., Rho, J.Y., Colby, T.V., Kitaichi, M., 2005. Histopathologic pattern and clinical features of rheumatoid arthritis-associated interstitial lung disease. Chest 127, 2019–2027. https://doi.org/10.1378/chest.127.6.2019.
Li, C., Xiao, J., Hormi, K., Borok, Z., Minoo, P., 2002. Wnt5a participates in distal lung morphogenesis. Dev. Biol. (Basel) 248, 68–81.
Li, C., Bellusci, S., Borok, Z., Minoo, P., 2015. Non-canonical WNT signalling in the lung. J. Biochem. 158, 355–365. https://doi.org/10.1093/jb/mvv081.
Lim, X., Nusse, R., 2013. Wnt signaling in skin development, homeostasis, and disease. Cold Spring Harb. Perspect. Biol. 5. https://doi.org/10.1101/cshperspect.a008029.
MacLauchlan, S., Zuriaga, M.A., Fuster, J.J., Cuda, C.M., Jonason, J., Behzadi, F., Duffen, J.P., Haines 3rd, G.K., Aprahamian, T., Perlman, H., Walsh, K., 2017. Genetic deficiency of Wnt5a diminishes disease severity in a murine model of rheumatoid arthritis. Arthritis Res. Ther. 19, 166. https://doi.org/10.1186/s13075-017-1375-0.
Maeda, K., Kobayashi, Y., Udagawa, N., Uehara, S., Ishihara, A., Mizoguchi, T., Kikuchi, Y., Takada, I., Kato, S., Kani, S., Nishita, M., Marumo, K., Martin, T.J., Minami, Y., Takahashi, N., 2012. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. Nat. Med. 18, 405–412. https://doi.org/10.1038/nm.2653.
Nannini, C., Ryu, J.H., Matteson, E.L., 2008. Lung disease in rheumatoid arthritis. Curr. Opin. Rheumatol. 20, 340–346. https://doi.org/10.1097/BOR.0b013e3282f798ed.
Newman, D.R., Sills, W.S., Hanrahan, K., Ziegler, A., Tidd, K.M., Cook, E., Sannes, P.L., 2016. Expression of WNT5A in idiopathic pulmonary fibrosis and its control by TGF-beta and WNT7B in human lung fibroblasts. J. Histochem. Cytochem. 64, 99–111. https://doi.org/10.1369/0022155415617988.
Nurmi, H.M., Kettunen, H.P., Suoranta, S.K., Purokivi, M.K., Karkkainen, M.S., Selander, T.A., Kaarteenaho, R.L., 2018. Several high-resolution computed tomography findings associate with survival and clinical features in rheumatoid arthritis-associated interstitial lung disease. Respir. Med. 134, 24–30. https://doi.org/10.1016/j.rmed.2017.11.013.
Nusse, R., 2005. Wnt signaling in disease and in development. Cell Res. 15, 28–32. https://doi.org/10.1038/sj.cr.7290260.
Pashirzad, M., Shafiee, M., Rahmani, F., Behnam-Rassouli, R., Hoseinkhani, F., Ryzhikov, M., Moradi Binabaj, M., Parizadeh, M.R., Avan, A., Hassanian, S.M., 2017. Role of Wnt5a in the pathogenesis of inflammatory diseases. J. Cell. Physiol. 232, 1611–1616. https://doi.org/10.1002/jcp.25687.
Raghu, G., Rochwerg, B., Zhang, Y., Garcia, C.A., Azuma, A., Behr, J., Brozek, J.L., Collard, H.R., Cunningham, W., Homma, S., Johkoh, T., Martinez, F.J., Myers, J., Protzko, S.L., Richeldi, L., Rind, D., Selman, M., Theodore, A., Wells, A.U., Hoogsteden, H., Schunemann, H.J., American Thoracic, S, European Respiratory, s, Japanese Respiratory, S, Latin American Thoracic, A, 2015. An official ATS/ERS/JRS/ALAT clinical practice guideline: treatment of idiopathic pulmonary fibrosis. An update of the 2011 clinical practice guideline. Am. J. Respir. Crit. Care Med. 192, e3–19. https://doi.org/10.1164/rccm.201506-1063ST.
Raimundo, K., Solomon, J.J., Olson, A.L., Kong, A.M., Cole, A.L., Fischer, A., Swigris, J.J., 2018. Rheumatoid arthritis-interstitial lung disease in the United States: prevalence, incidence, and healthcare costs and mortality. J. Rheumatol. https://doi.org/10.3899/jrheum.171315.
Sathi, N., Urwin, T., Desmond, S., Dawson, J.K., 2011. Patients with limited rheumatoid arthritis-related interstitial lung disease have a better prognosis than those with extensive disease. Rheumatology (Oxford) 50, 620. https://doi.org/10.1093/rheumatology/keq426.
Sen, M., Lauterbach, K., El-Gabalawy, H., Firestein, G.S., Corr, M., Carson, D.A., 2000. Expression and function of wingless and frizzled homologs in rheumatoid arthritis. Proc. Natl. Acad. Sci. U. S.A. 97, 2791–2796. https://doi.org/10.1073/pnas.050574297.
Sen, M., Chamorro, M., Reifert, J., Corr, M., Carson, D.A., 2001. Blockade of Wnt-5A/frizzled 5 signaling inhibits rheumatoid synoviocyte activation. Arthritis Rheum. 44, 772–781. https://doi.org/10.1002/1529-0131(200104)44:4<772::AID-ANR133>3.0.CO;2-L.
Sen, M., Reifert, J., Lauterbach, K., Wolf, V., Rubin, J.S., Corr, M., Carson, D.A., 2002. Regulation of fibronectin and metalloproteinase expression by Wnt signaling in rheumatoid arthritis synoviocytes. Arthritis Rheum. 46, 2867–2877. https://doi.org/10.1002/art.10593.
Shaw, M., Collins, B.F., Ho, L.A., Raghu, G., 2015. Rheumatoid arthritis-associated lung disease. Eur. Respir. Rev. 24, 1–16. https://doi.org/10.1183/09059180.00008014.
Shi, J., Chi, S., Xue, J., Yang, J., Li, F., Liu, X., 2016. Emerging role and therapeutic implication of Wnt signaling pathways in autoimmune diseases. J. Immunol. Res. 2016, 9392132. https://doi.org/10.1155/2016/9392132.
Spagnolo, P., Lee, J.S., Sverzellati, N., Rossi, G., Cottin, V., 2018. The lung in rheumatoid arthritis: focus on interstitial lung disease. Arthritis Rheumatol. 70, 1544–1554. https://doi.org/10.1002/art.40574.
Suhara, K., Miyazaki, Y., Okamoto, T., Ishizuka, M., Tsuchiya, K., Inase, N., 2016. Fragmented gelsolins are increased in rheumatoid arthritis-associated interstitial lung disease with usual interstitial pneumonia pattern. Allergol. Int. 65, 88–95. https://doi.org/10.1016/j.alit.2015.08.002.
Travis, W.D., Costabel, U., Hansell, D.M., King Jr., T.E., Lynch, D.A., Nicholson, A.G., Ryerson, C.J., Ryu, J.H., Selman, M., Wells, A.U., Behr, J., Bouros, D., Brown, K.K., Colby, T.V., Collard, H.R., Cordeiro, C.R., Cottin, V., Crestani, B., Drent, M., Dudden, R.F., Egan, J., Flaherty, K., Hogaboam, C., Inoue, Y., Johkoh, T., Kim, D.S., Kitaichi, M., Loyd, J., Martinez, F.J., Myers, J., Protzko, S., Raghu, G., Richeldi, L., Sverzellati, N., Swigris, J., Valeyre, D., Pneumonias, A.E.C.o.I.I, 2013. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am. J. Respir. Crit. Care Med. 188, 733–748. https://doi.org/10.1164/rccm.201308-1483ST.
Uehara, S., Udagawa, N., Kobayashi, Y., 2018. Non-canonical Wnt signals regulate cytoskeletal remodeling in osteoclasts. Cell. Mol. Life Sci. 75, 3683–3692. https://doi.org/10.1007/s00018-018-2881-1.
Vassallo, R., 2012. Diffuse lung diseases in cigarette smokers. Semin. Respir. Crit. Care Med. 33, 533–542. https://doi.org/10.1055/s-0032-1325162.
Vij, R., Strek, M.E., 2013. Diagnosis and treatment of connective tissue disease-associated interstitial lung disease. Chest 143, 814–824. https://doi.org/10.1378/chest.12-0741.
Washko, G.R., Lynch, D.A., Matsuoka, S., Ross, J.C., Umeoka, S., Diaz, A., Sciurba, F.C., Hunninghake, G.M., San Jose Estepar, R., Silverman, E.K., Rosas, I.O., Hatabu, H., 2010. Identification of early interstitial lung disease in smokers from the COPDGene Study. Acad. Radiol. 17, 48–53. https://doi.org/10.1016/j.acra.2009.07.016.
Whang, Y.M., Jo, U., Sung, J.S., Ju, H.J., Kim, H.K., Park, K.H., Lee, J.W., Koh, I.S., Kim, Y.H., 2013. Wnt5a is associated with cigarette smoke-related lung carcinogenesis via protein kinase C. PLoS One 8, e53012. https://doi.org/10.1371/journal.pone.0053012.
Wuyts, W.A., Cavazza, A., Rossi, G., Bonella, F., Sverzellati, N., Spagnolo, P., 2014. Differential diagnosis of usual interstitial pneumonia: when is it truly idiopathic? Eur. Respir. Rev. 23, 308–319. https://doi.org/10.1183/09059180.00004914.