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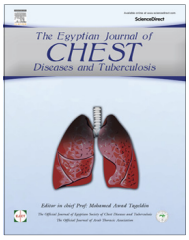

ORIGINAL ARTICLE

Is toll like receptor 4 a common pathway hypothesis for development of lung cancer and idiopathic pulmonary fibrosis?

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KEYWORDS

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Abstract Idiopathic pulmonary fibrosis (IPF) is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults and limited to the lungs. IPF is a disease with similarities and links to cancer biology whose main event is aberrant cell proliferation. Although toll like receptors (TLRs) are essential for protective immunity, inappropriate TLR responses contribute to inflammation. Chronic inflammation is one of the risk factors and features of cancer. It can affect any stage of tumorigenesis and migration of cancer cell.

Aim of work: To investigate the key role of TLR4 expression in the development and progression of lung cancer and IPF and its contribution as a common pathway in the development of both.

Methods: This study included 16 IPF patients, 20 lung cancer patients and 23 control subjects. All patients were subjected to full history taking, detailed clinical examination, radiological assessment, bronchoscopic biopsies and serum samples for measurement of TLR4 expression. TLR4 was measured in serum of all control subjects and in bronchoscopic biopsies for only five of them.

Results: TLR4 expression was higher in serum and tissue biopsies of IPF and lung cancer patients than that in the control group; however the highest level of LTR4 expression in serum was observed in the IPF group and the highest level in tissue biopsy was observed in the lung cancer group. TLR4 levels were not significantly different between the three studied groups. There was a significant association between TLR4 expression in tissue biopsy and distant metastasis among NSCLC cases ($p = 0.006$).

Conclusion: Our results support that TLR4 pathway may be a common contribution to both diseases. There was association between distant metastasis and TLR4 expression. Further studies are needed to evaluate the TLR4 prognostic value for tumor progression and its expression in precancerous lesions.

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Introduction

Idiopathic pulmonary fibrosis is a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults and limited to the lungs [1]. IPF is a disease with similarities and links to cancer biology. A number of pathogenetic hypotheses are shared by both fatal diseases, whose main event is aberrant cell proliferation [2]. Human TLRs are a family of trans-membrane receptors that consist of ten members and play a key role in innate immune defense, particularly in inflammatory response against various invading exogenous pathogens [3,4].

Although TLRs are essential for protective immunity against infection, inappropriate TLR responses contribute to acute and chronic inflammation, as well as to systemic autoimmune diseases [5,6]. Activation of TLR signaling in the steady state maintains tissue architecture. However, in the presence of deregulated inflammation and/or tissue injury, as occurs in fibrogenesis and tumorigenesis, the TLR-driven tissue response may promote tissue remodeling, neoangiogenesis and tumor growth by mechanisms that are still poorly defined [7].

TLRs are expressed in a variety of cells, including type II alveolar epithelial cells, airway epithelial, smooth muscle cells, and fibroblasts [8]. TLR4 is a key regulator of the pro-inflammatory transcription factor nuclear factor kappa B (NF-κB) and plays a dominant role in mediating sterile tissue damage. IκB kinase (IKK) activation leads to the dissociation of NF-κB from IκB and its subsequent activation [9].

The mechanism of TLR4 activation involves several auxiliary proteins as well as a co-receptor ''myeloid differentiation factor 2'' (MD-2) [10]. MD-2 is a soluble protein that represents the binding site for the acyl chains of lipid A. Lipid A is usually composed of 6 acyl chains, but only 5 of them bind to MD-2. The 6th acyl chain interacts with residues on TLR4. This MD-2/TLR4 heterodimerization is a prerequisite for the activation of the TLR4 signaling cascade [11,12]. The endotoxin/MD-2/TLR4 heterodimer can trigger the transcription of both proinflammatory cytokines as well as type I interferons [10].

It is therefore not too surprising that TLR4 activation affects not only the immune response against invading Gram-negative bacteria but is also involved in chronic inflammation, autoimmune diseases and malignancies. TLR4 signaling in cancer is considered a double-edged sword. If TLR4 is activated on immune cells, it can enhance anti-tumor immunity. On the other hand, chronic inflammation is a major risk factor in cancer development [13].

Chronic inflammation has emerged as one of the main risk factors and features of cancer. It can affect any stage of tumorigenesis, generating a microenvironment conducive to tumor development and progression, and promoting the survival, proliferation and migration of cancer cells. Thus, many cancers can arise from local irritation, inflammation and chronic infection. Changes in proteins or receptors involved in the inflammatory and immune responses may contribute to an increased risk of developing cancer. TLRs activate the NF-κB pathway, the main regulatory inflammation signaling pathway, and this activation is involved in the pathogenesis of cancer [14].

Emerging evidence suggests that chemoresistance is promoted by substances released from dead and damaged cells that activate the host repair program orchestrated by TLR4. TLR4 is often over-expressed in malignant and tumor infiltrating immune cells. TLR4 activation promotes local and systemic inflammation, leading to the induction of multiple circuits that create a regenerative environment favoring local recurrence and metastasis. Of particular importance is TLR4-mediated recruitment of endothelial progenitors derived from immature myeloid cells. These cells play a major role in rebuilding tumor-associated lymphatic and blood vessels, thereby promoting lymphatic and hematogenous metastasis [15].

The aim of the present study was to investigate the key role of TLR4 expression in the development and progression of lung cancer and IPF and to study TLR4 contribution as a common pathway in the development of both.

Patients and methods

The present study included 59 subjects who were sub-grouped into; sixteen patients with the diagnosis of IPF, twenty patients with bronchogenic carcinoma and twenty-three control subjects. All patients were recruited from chest departments, Cairo University and Fayoum University Hospitals in the period from January 2014 to June 2015. Informed consent was obtained from all patients who participated in the study. The study was approved by the research ethics committee, Faculty of Medicine, Cairo and Fayoum Universities.

IPF: They were diagnosed based on the guidelines of the international consensus statement produced as a collaborative effort from the ATS, ERS, JRS and ALAT [1]. All IPF patients were newly diagnosed and had not received any treatment and patients with any known cause of pulmonary fibrosis, such as a systemic connective tissue disorder, were excluded by both immunologic screening and rheumatological clinical evaluation. All IPF patients were subjected to full history taking including smoking and occupational history, detailed clinical examination, arterial blood gases analysis, spirometry, 6-min walk test, echocardiography with assessment of the pulmonary artery systolic pressure and high-resolution computed tomography of the chest. For evaluation of interstitial involvement with HRCT, fibrosis score or interstitial score as described by Gay et al. in (1998) [16] was used. In this method each lobe of the lung was separately scored for the presence, distribution and extent of honeycombing and interlobular septal thickening on a scale of 0–5 as follows: (0) no interstitial disease, (1) septal thickening without honeycombing, (2) honeycombing involving up to 25% of the lobe, (3) honeycombing involving 25–49% of the lobe, (4) honeycombing involving 50–75% of the lobe, (5) honeycombing involving $>75\%$ of the lobe. Honeycomb cysts were defined as localized areas of decreased attenuation with well defined walls. The lingula was scored as a separate lobe. After each lobe was scored individually, an average score for all lobes was obtained and used for the statistical analysis.

Bronchogenic carcinoma: All patients were subjected to full history taking including smoking history. Patients underwent radiological assessment to detect primary tumor site, pleural or mediastinal lymph nodes involvement and distant metastasis. The patients included in the study were diagnosed based on histopathological criteria from endobronchial biopsies and they had not received any treatment for lung cancer. The

patients were sub-classified according to histopathological diagnosis into small cell lung cancer (SCLC) and non small cell lung cancer (NSCLC). Patients with NSCLC were subdivided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma according to WHO classification in 1999 [17].

Control group: included 23 subjects; 5 were patients who underwent bronchoscopy for the investigation of hemoptysis, without any pulmonary comorbidities and with normal bronchoscopic findings while the remaining 18 subjects were healthy control.

Methods

- *Bronchoscopic biopsies for TLR4 detection:* TBLBs were obtained from the basal segments of the right lower lobe of IPF patients, while endobronchial biopsies from endobronchial tissue growth were performed for patients with bronchogenic carcinoma. Also bronchial mucosal biopsies were done for only the symptomatic control subjects (5 patients). For all patients biopsies were divided into 2 parts, one was immersed immediately in a bottle containing 10% formalin solution and sent for histopathological assessment while the other was put in 1 ml Phosphate Buffer Saline (PBS) for further lab processing and TLR4 determination.
- *Tissue biopsy sample processing for protein extraction:* Tissue biopsy punches were rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at −20 °C. After two freeze–thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 10 min at 5000*g*. The supernatant was stored at −80 °C until analysis. Total protein concentrations in the supernatant were determined using a protein in fluids assay kit (pyrogallol red/molybdate principle) (Dialab GmbH, Wiener Neudorf, Austria) and were used for normalization of tissue TLR4 levels where TLR4 in lung tissue was expressed as ng/mg protein.
- *Serum Sample for TLR4 level estimation:* Blood samples were withdrawn from all study groups. Samples were centrifuged, serum was separated and immediately stored at −80 °C until analysis
- *Determination of TLR4 levels in serum and tissue:* Was done using a commercially available enzyme-linked immunosorbent assay (ELISA) kit for quantitative determination of human toll like receptor 4 (TLR4) (Cusabio Biotech Co., Ltd., China), according to the manufacturer's instructions.
- *Statistical analysis of the results:* Data were statistically described in terms of mean ± SD, median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using the Mann Whitney *U* test for independent samples when comparing 2 groups and Kruskal–Wallis test with post hoc multiple 2-group comparisons when comparing more than 2 groups. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal variables/non-linear monotonic relation. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

Table 1 Characteristics of study groups.

	Study groups		
	Lung cancer (n = 20)	IPF (n = 16)	Control group (n = 23)
Male:Female (n)	16:4	10:6	15:8
Age			
(median) (years)	57	43	43
Range	35–77	20–70	20–70
Current smokers (No., %)	12 (60%)	3 (18.8%)	8 (34.8%)

Results

The study included 59 subjects who were sub-grouped into; sixteen patients with the diagnosis of IPF, twenty patients with bronchogenic carcinoma and twenty-three control subjects. The lung cancer patients (16 males and 4 females) had a median age of 57 years; IPF patients (10 males and 6 females) had a median age of 43 years and the control group (15 males and 8 females) had a median age of 43 years. Regarding smoking prevalence among the study groups, the numbers of current smokers were twelve patients in the lung cancer group, three patients in the IPF group and eight subjects in the control group (Table 1).

Table 2 presents the value of TLR4 in serum and tissue among the three studied groups. TLR4 expression was higher

Table 2 Statistical comparison between study groups according to the value of TLR4 in serum and tissue biopsies.

	Study groups			
	Lung cancer	IPF	Control group	p value
TLR4 in the serum (ng/ml)				
Number	20	16	23	
Median (25th–75th percentile)	3.99 (0.94–19.58)	12.46 (0.85–16.10)	1.07 (0.84–6.03)	0.682
TLR4 in tissue (ng/mg protein)				
Number	20	16	5	
Median (25th–75th percentile)	0.94 (0.33–2.56)	0.53 (0.29–4.05)	0.17 (0.14–0.79)	0.515

in serum and tissue biopsies of IPF and lung cancer patients than in the control group; however the highest level of LTR4 expression in the serum was observed in the IPF group (Fig. 1) and the highest level in tissue biopsy was observed in the lung cancer group (Fig. 2). TLR4 levels were not significantly different between the three studied groups.

During histopathological evaluation of the lung cancer group, SCLC was found in 15% (3 patients) while NSCLC (85%) was found in 85% (17 patients). NSCLC included several subtypes: adenocarcinoma (45%), large cell carcinoma (10%) and squamous cell carcinoma (30%). Among the lung cancer group; pleural involvement was present in 15%, mediastinal lymph node (L.N) involvement in 45% and distant metastasis in 25% as shown in Table 3.

In the lung cancer group (Table 4): it was observed that the TLR4 level was higher in the serum of patients who were current smokers and had pleural effusion, mediastinal L.N involvement and distant metastasis without statistical significance. On the other hand the TLR4 level in tissue biopsies was higher in patients with mediastinal L.N involvement and distant metastasis with statistical significance regarding distant metastasis ($p = 0.006$) (Figs. 3 and 4).

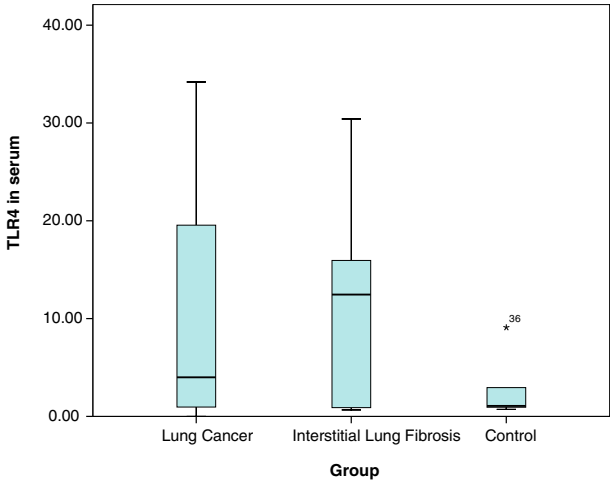

Figure 1 Comparison between the study groups according to TLR4 levels in serum samples.

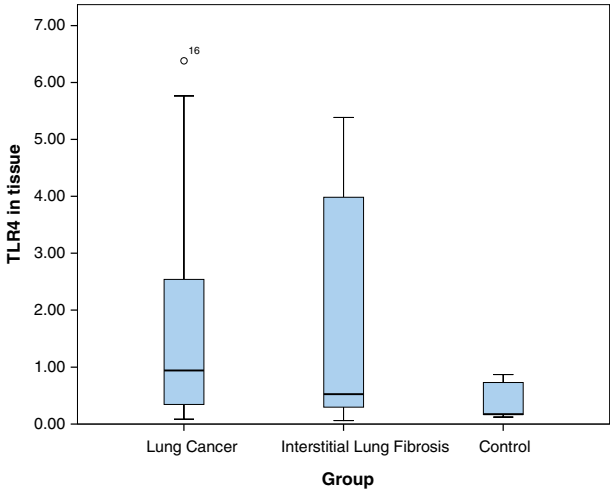

Figure 2 Comparison between the study groups according to TLR4 levels in tissue biopsies.

Table 3 Characteristics of the lung cancer group.

Lung cancer group ($n = 20$)	
Histopathological subtypes (No. %)	
SCLC	
NSCLC	3 (15.0%)
NSCLC subtypes:	17 (85.0%)
Adenocarcinoma	9 (45.0%)
Large cell carcinoma	2 (10.0%)
Squamous cell carcinoma	6 (30.0%)
Pleural involvement (No., %)	3 (15.0%)
Mediastinal L.N involvement (No., %)	9 (45.0%)
Distant metastasis (No., %)	5 (25.0%)

In the IPF group: TLR4 in the serum showed a negative correlation with PASP, $PaCO_2$ and HRCT score while it showed a positive correlation with PaO_2, SaO_2%, FVC%, FEV1%, FEF25–75% and 6MWD. Regarding TLR4 in tissue, there was negative correlation with PaO_2, SaO_2%, FVC%, FEV1%, FEF25–75% and 6MWD and a positive correlation with PASP, $PaCO_2$ and HRCT score. All correlations were insignificant as shown in Table 5.

Discussion

Recent observations support the hypothesis that IPF may not be just an inflammatory disorder but rather a complex process characterized by abnormal pneumocyte apoptosis and profound derangement of alveolar renewal, making it, at least in some aspects, more similar to malignant lung disease. TLR-driven inflammation seems to play a role in furthering malignant development in a wide context of ways [2]. TLR4 activation alters the balance of progrowth and antigrowth cytokines in the extracellular microenvironment, ultimately resulting in increased proliferation and growth [18].

TLRs are considered to be expressed in both immune cells and tumor cells. For this the present study aimed to investigate the key role of TLR4 expression in the development and progression of lung cancer and IPF and to study TLR4 contribution as a common pathway in the development of both.

In the present study, it was observed that TLR4 expression was higher in serum and tissue biopsies of IPF and lung cancer patients than in the control group; however the highest level of LTR4 expression in serum was observed in the IPF group and the highest level in tissue biopsies was observed in the lung cancer group. TLR4 levels were not significantly different between the three studied groups.

IPF is characterized by excessive scarring of the lung parenchyma. Despite considerable progress in defining the natural history of the disease, many features of IPF pathogenesis remain poorly understood. Several recent studies have highlighted links between pattern recognition receptors of innate immunity termed "Toll-like receptors" and the aberrant fibrogenesis that characterizes IPF [19].

There was a study done by Samara et al. in 2012 [20] on expression profiles of toll like receptors in non-small cell lung cancer and idiopathic pulmonary fibrosis. TLR expression was

Table 4 Relationship between serum and tissue biopsy levels of TLR4 and characteristics of the lung cancer group.

Character		Lung cancer (n = 20)	Serum TLR4* (ng/ml)	p value	Tissue TLR4* (ng/mg protein)	p value
Smoking history	Absent	8 (40%)	2.38 (0.81–12.98)	0.44	1.32 (0.35–3.51)	0.758
	Present	12 (60%)	9.78 (0.99–20.09)		0.85 (0.33–2.28)	
Pleural effusion	Absent	17 (85%)	1.48 (0.88–19.55)	0.186	1.04 (0.34–2.54)	0.634
	Present	3 (15%)	15.73 (3.29–34.18)		0.44 (0.17–3.84)	
Mediastinal L.N involvement	Absent	11 (55%)	1.48 (0.99–19.50)	0.569	0.84 (0.32–1.39)	0.732
	Present	9 (45%)	4.70 (1.08–20.29)		1.81 (0.28–3.21)	
Distant metastasis	Absent	15 (75%)	1.47 (0.99–17.85)	0.275	0.45 (0.31–1.39)	0.006**
	Present	5 (25%)	19.61 (1.24–20.61)		4.67 (1.81–6.07)	

* Data are presented as median (25th–75th percentiles).
** p value < 0.05 equal statistically significant.

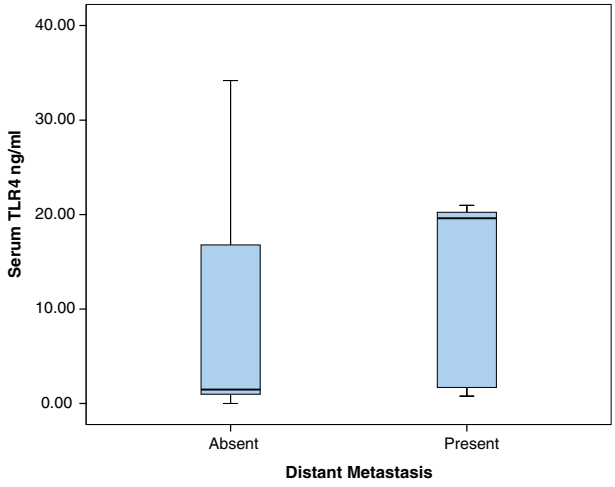

Figure 3 Relationship between serum TLR4 levels and distant metastasis.

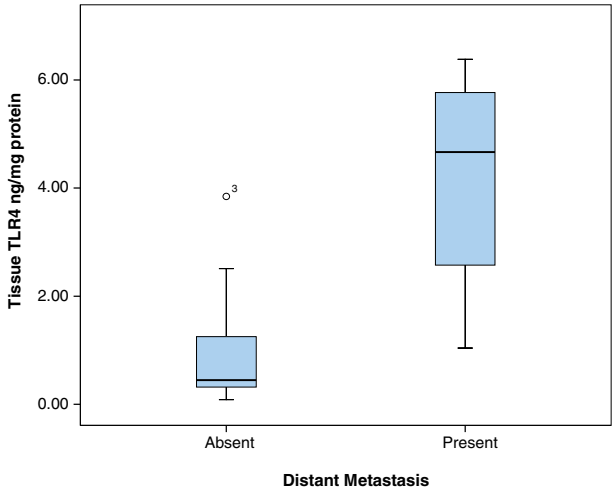

Figure 4 Relationship between tissue TLR4 levels and distant metastasis.

quantitatively measured by real-time reverse transcriptase polymerase chain reaction (RT-PCR) in bronchoalveolar lavage fluid of 16 IPF patients, 16 non-small cell lung cancer patients and 9 control subjects. They found that TLR4 expression was similar in both diseases and the control group as well.

Table 5 Correlation of TLR 4 in serum and tissue with the characteristics of the IPF group.

	TLR4 in the serum		TLR4 in the tissue	
	r	p	r	p
PASP mm Hg	−0.231	0.494	0.438	0.178
PaO_2 mm Hg	0.276	0.300	−0.301	0.257
$PaCO_2$ mm Hg	−0.276	0.301	0.186	0.489
SaO_2%	0.318	0.230	−0.232	0.387
HRCT score	−0.119	0.660	0.142	0.599
FVC%	0.346	0.190	−0.094	0.728
FEV1%	0.304	0.253	−0.028	0.917
FEF25–75%	0.158	0.559	−0.286	0.284
6MWD (m)	0.167	0.536	−0.409	0.116

In 2007, He et al. [21] described the expression of TLR4 in human lung cancer cells. Oblak and Jerala in 2011 [22] found that TLR4 is expressed on a variety of immune as well as tumor cells, but its activation can have opposing effects. While TLR4 activation can promote antitumor immunity, it can also result in increased tumor growth and immunosuppression.

TLR4 mediated signaling has been implicated in tumor cell invasion, survival and metastasis in several types of cancers [23]. Remarkably, the present study showed that the TLR4 level was higher in the serum of lung cancer patients (NSCLC cases) who had pleural effusion, mediastinal L.N involvement and distant metastasis without statistical significance. On the other hand the TLR4 level in the tissue biopsies was higher in patients with mediastinal L.N involvement and distant metastasis with statistical significance regarding distant metastasis.

Our findings agreed with Liu et al. in 2015 [24] who demonstrated a crucial role for TLR4 signaling to enhance the tumor progression and distant metastasis of NSCLC, which could further enhance the understanding of NSCLC pathogenesis and be helpful for developing novel therapeutics for NSCLC.

Also Boi and Elsawa in 2013 [25] mentioned that Nickel has been shown to be an agonist for TLR4, and Nickle could also contribute to the progression of human lung cancer by increasing the metastatic potential of the tumor cells by elevating levels of IL-8, TGF-β, MMP2, and MMP9. Moreover, the

down regulation of TLR4 could significantly inhibit the invasive potential of the cells when exposed to nickel.

Within the lung cancer group, it was found that 3 patients had pleural involvement (15%), 9 patients had mediastinal lymph node affection (45%) and 5 (25%) patients had distant metastasis (all of them were NSCLC cases). Tumor progression to pleura was in a fewer number of patients than the nodal spread or the distant metastasis.

Apoptosis is one of the main types of programed cell death, TLR4 modulates cell apoptosis by activating members of mitogen-activated protein kinase (MAPK) signaling pathways and leads to the activation of mitochondria-associated apoptotic cascades [26]. Modulating the proliferation of lung cancer cells and inhibiting apoptosis is poorly understood, TLR4 could coordinately improve the proliferation of tumor cells in vitro [27].

The pleural mesothelial cells (PMCs) have many defense mechanisms including the sialomucin complex on PMCs which acts as a defense layer. Also PMCs produce significant quantities of hyaluronan and endostatin which induce cell cycle arrest and apoptosis, inhibit endothelial cell migration, inhibit angiogenesis and reduce tumor growth [28].

In conclusion, the present study found that TLR4 is over expressed in serum and tissue of IPF and lung cancer patients than the control group, so the TLR4 pathway may be a common contribution to both diseases. There was association between distant metastasis and TLR4 expression so further studies are needed to evaluate its prognostic value for tumor progression. TLR4 expression in precancerous lesions and its use as a therapeutic target should be tackled in further studies. Also further studies are needed to highlight the link between TLR4 and aberrant fibrogenesis that characterize IPF.

References

[1] American Thoracic Society/European Respiratory society/Japanese Respiratory Society/Latin American Thoracic Association Statement, Idiopathic pulmonary fibrosis: evidence based guidelines for diagnosis and management, Am. J. Respir. Crit. Care Med. 183 (2011) 788–824.

[2] C. Vancheri, M. Failla, N. Crimi, G. Raghu, Idiopathic pulmonary fibrosis: a disease with similarities and links to cancer biology, Eur. Respir. J. 35 (2010) 496–504.

[3] S. Akira, K. Takeda, Toll-like receptor signalling, Nat. Rev. Immunol. 4 (7) (2004) 499–511.

[4] K. Takeda, T. Kaisho, S. Akira, Toll-like receptors, Annu. Rev. Immunol. 21 (2003) 335–376.

[5] S. Duncan, What is autoimmunity and why it is likely to be important in chronic lung disease?, Am J. Respir. Crit. Care Med. 181 (2010) 4–5.

[6] B. Salaun, P. Romero, S. Lebecque, Toll-like receptors' two-edged sword: when immunity meets apoptosis, Eur. J. Immunol. 37 (2007) 3311–3318.

[7] K. Shchors, E. Shchors, F. Rostker, E.R. Lawlor, L. Brown-Swigart, G.I. Evan, The Myc-dependent angiogenic switch in tumors is mediated by interleukin 1beta, Genes Dev. 20 (2006) 2527–2538.

[8] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on toll-like receptors, Nat. Immunol. 11 (2010) 373–384.

[9] K. Mollen, R. Anand, A. Tsung, J. Prince, R. Levy, T. Billiar, Emerging paradigm: toll-like receptor 4 sentinel for the detection of tissue damage, Shock 26 (5) (2006) 430–437.

[10] T. Kawai, S. Akira, TLR signaling, Cell Death Differ. 13 (5) (2006) 816–825.

[11] N. Resman, J. Vašl, A. Oblak, et al, Essential roles of hydrophobic residues in both MD-2 and toll-like receptor 4 in activation by endotoxin, J. Biol. Chem. 284 (22) (2009) 15052–15060.

[12] B. Park, D. Song, H. Kim, B. Choi, H. Lee, J. Lee, The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex, Nature 458 (7242) (2009) 1191–1195.

[13] A. Mantovani, P. Allavena, A. Sica, F. Balkwill, Cancer-related inflammation, Nature 454 (7203) (2008) 436–444.

[14] M. Proença, J. de Oliveira, A. Cadamuro, M. Succi, J. Netinho, E. Goloni-Bertolo, É. Pavarino, A. Silva, TLR2 and TLR4 polymorphisms influence mRNA and protein expression in colorectal cancer, World J. Gastroenterol. 21 (25) (2015) 7730–7741.

[15] S. Ran, The role of TLR4 in chemotherapy-driven metastasis, Cancer Res. 75 (12) (2015) 2405–2410.

[16] S. Gay, E. Kazerooni, G. Toews, J. Lynch, B. Gross, P. Cascade, D. Spizarny, A. Flint, M. Schork, R. Whyte, J. Popovich, R. Hyzy, F. Martinez, Idiopathic pulmonary fibrosis: predicting response to therapy and survival, Am. J. Respir. Crit. Care Med. 157 (1998) 1063–1072.

[17] W. Travis, T. Cobly, B. Corrin, Histological typing of tumors of lung and pleura, in: L. Sobin (Ed.), World Health Organisation, third ed., Springer-Verlag, Berlin, 1999, pp. 212–224.

[18] J. Faget et al, Early detection of tumor cells by innate immune cells leads to T(reg) recruitment through CCL22 production by tumor cells, Cancer Res. 71 (19) (2011) 6143–6152.

[19] D. O'Dwyer, M. Armstrong, M. Kooblall, S. Donnelly, Targeting defective Toll-like receptor-3 function and idiopathic pulmonary fibrosis, Expert Opin. Ther. Targets 19 (4) (2015) 507–514.

[20] K. Samara, K. Antoniou, K. Karagiannis, G. Margaritopoulos, I. Lasithiotaki, E. Koutala, N. Siafakas, Expression profiles of toll-like receptors in non-small cell lung cancer and idiopathic pulmonary fibrosis, Int. J. Oncol. 40 (5) (2012) 1397–1404.

[21] W. He, Q. Liu, L. Wang, W. Chen, N. Li, X. Cao, TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance, Mol. Immunol. 44 (11) (2007) 2850–2859.

[22] A. Oblak, R. Jerala, Toll-like receptor 4 activation in cancer progression and therapy, Clin. Dev. Immunol. 2011 (2011), 12 pages 609579.

[23] D. Li, Y. Jin, Y. Sun, J. Lei, C. Liu, Knockdown of toll-like receptor 4 inhibits human NSCLC cancer cell growth and inflammatory cytokine secretion in vitro and in vivo, Int. J. Oncol. 45 (2) (2014) 813–821.

[24] X. Liu, C. Pei, S. Yan, G. Liu, G. Liu, W. Chen, Y. Cui, Y. Liu, NADPH oxidase 1-dependent ROS is crucial for TLR4 signaling to promote tumor metastasis of non-small cell lung cancer, Tumor Biol. 36 (3) (2015) 1493–1502.

[25] S. Boi, S. Elsawa, Epigenetic regulation of toll-like receptor signaling: implications for cancer development, Med. Epigenet. 1 (2013) 19–30.

[26] J. Yang, X. Guo, J. Yang, J. Ding, S. Li, R. Yang, Z. Fan, RP105 protects against apoptosis in ischemia/reperfusion-induced myocardial damage in rats by suppressing TLR4-mediated signaling pathways, Cell Physiol. Biochem. 36 (2015) 2137–2148.

[27] X. Xu, H. Zhu, T. Wang, Y. Sun, P. Ni, Y. Liu, S. Tian, P. Amoah Barnie, H. Shen, W. Xu, H. Xu, Z. Su, Exogenous high-mobility group box 1 inhibits apoptosis and promotes the proliferation of lewis cells via RAGE/TLR4-dependent signal pathways, Scand. J. Immunol. 79 (6) (2014) 386–394.

[28] H. Batra, V. Antony, Pleural mesothelial cells in pleural and lung diseases, J. Thoracic Dis. 7 (6) (2015) 964–980.