



INTERFERON LAMBDA-3 AS A NEW CANDIDATE GENE INVOLVED IN THE PROGRESSION OF IDIOPATHIC PULMONARY FIBROSIS

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Idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing interstitial respiratory illness with an unknown origin, which leads to inevitably fatal outcomes in the majority of cases. The pro-inflammatory cytokine interferon lambda-3 (IFN- λ 3) has been recently identified as a risk factor for the development of lung fibrosis in scleroderma patients. In this study, we examined the involvement of IFN- λ 3 in IPF by utilizing the mouse model of BLM-induced pulmonary fibrosis. Our study identifies a remarkable overexpression in the mRNA of IFNL3, and a positive significant correlation between the IFNL3 mRNA levels and the various pro-inflammatory cytokines for instance NF- κ B, TNF- α , IL-1 β and TGF- β throughout the three-weeks study. These findings have proven the impact of IFNL3 on BLM-induced pulmonary fibrosis occurrence and progression. Moreover, the data suggest that the pro-inflammatory and pro-fibrotic capacities of IFNL3 may be provoked by additional pro-inflammatory cytokines NF- κ B, TNF- α , IL-1 β , and TGF- β . In conclusion, IFN- λ 3 looks like a promising target in the therapeutic development of new drug candidates for pulmonary fibrosis.

Keywords: idiopathic pulmonary fibrosis, interferon lambda-3.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic and incurable form of interstitial lung disease (ILD) with an undefined origin that leads to ongoing lung parenchymal scarring, which lowers life quality as well as survival rate. IPF is considered a disorder of advancing age, so the cost of IPF medical care could become catastrophic due to a much higher global longevity rate. The fibrotic mechanisms of IPF are still elusive, but popular theories indicate that alveolar epithelial cells are repeatedly subjected to micro-injuries. In genetically susceptible individuals, this leads to an irregular tissue repair response characterized by extensive collagen deposition^{1&2}.

Single intra-tracheal bleomycin instillation is the most extensively utilized as well as well-

studied animal model for IPF and many fibrotic ILDs. It is an inexpensive approach that produces a substantial fibrotic response with multiple histological features observed in IPF patients. The model operates by establishing an initial inflammatory phase that lasts 5–7 days before transitioning to a fibrotic phase that more closely resembles the hallmarks of IPF in humans. In mice, spontaneous fibrosis resolution occurs 3–4 weeks after intra-tracheal bleomycin administration³.

IPF's pathophysiology is thought to be associated with a breakdown in the communication between structural and inflammatory cells, which is influenced by a profusion of cytokines, chemokines, and growth factors that seem to be essential for maintaining tissue integrity and orchestrating injury responses⁴. Several cytokines have been

connected to various molecular mechanisms underpinning IPF, while some act as growth factors on top of that⁵. These cytokines and growth factors are intended to promote inflammation and fibrosis. For instance, TNF- α could potentially induce inflammation and mild fibrosis, while, TGF- β might possibly initiate minor inflammation but marked progressive chronic fibrosis, and IL-1 β is able to cause obvious inflammation, tissue destruction, and persistent fibrosis⁴. Lastly, NF- κ B could possibly be implicated in the production of inflammatory mediators and chemokines, drawing in more inflammatory immune cells and other related processes⁶.

IFN- λ 3, also known as interferon lambda-3, belongs to the type III interferon family, which is encoded by the IFNL3 or interleukin 28B gene (IL-28B)^{7&8}. IFNL receptors' patterns of expression are restricted to epithelial tissues, thus restricting the response to type III IFNs to these tissues. These tissues include the lungs, liver, and kidney⁹. IFN- λ 3 has now been thought to be a substantial predictor of liver inflammation and fibrosis despite the disease aetiology¹⁰.

IFN- λ 3 has recently been found to affect other epithelial organs, such as the lung and kidney. For example, in chronic obstructive pulmonary disease (COPD), circulating IFN- λ 3 has been linked to disease severity and consequences¹¹. Furthermore, according to previous research, IFN- λ 3 may raise the incidence of systemic sclerosis-associated pulmonary fibrosis¹². The pathologic fibrosis in numerous organs is anticipated to contribute to some standard stereotypes¹³. Hence, it is predicted that IFN- λ 3 could also affect pulmonary fibrosis risk in IPF and other fibrotic ILDs. Also, the exact mechanism of IFN- λ 3 in fibrogenesis is still illusive and needs further investigation.

During this investigation, we attempt to understand the relationship between IFN- λ 3 level and the development of IPF by employing an experimental design of bleomycin-induced pulmonary fibrosis and elucidating the proper mechanism of IFN- λ 3 in fibrogenesis.

MATERIALS AND METHODS

Animals

32 Male Balb/c mice weighing 25-32 g, all of them between the ages of 6-8 weeks. They

were supplied by the laboratory animal house (Assiut University, Assiut, Egypt). For a week, the animals were acclimated in a controlled environment between 18-25°C with 12:12 h light–dark cycle and unrestricted access to water and food. The Commission of Ethics of Scientific Research, Faculty of Pharmacy, Minia University, approved all experiments (project code number: ES-HV-02/2020).

BLM-Induced Pulmonary Fibrosis Mouse Model

Four groups of mice were divided at random (8 mice/group) according to the duration of bleomycin treatments as follows: a week group, two-week group, three-week group, and a sham-operated control group. To anesthetize, an intraperitoneal ketamine/xylazine combination of 100 mg/kg ketamine and 10 mg/kg xylazine has been used. The anesthetized animal was placed supine on a dissection dish that had been warmed with artificial lighting, and the skin on the neck was washed, shaved, and disinfected with iodine. A single sub-lethal dose of BLM (Bleocel 15, Celon, India) (3 mg/kg) was used to induce lung fibrosis. BLM was administered by intratracheal infusion in sterile isotonic saline (50 μ L per animal) to each animal at the beginning of the study (day 0)^{14&15}.

An intratracheal instillation of equal volume saline was given to the mice in the sham control group. The animals were sacrificed by cervical decapitation under ketamine/xylazine anesthesia after one, two, or three weeks, and a sham model operation, then lung tissues were collected and promptly washed with saline. After that, the lung was separated into two sections; one was preserved in a 10% balanced buffered formalin solution for histopathology examination, while the other was stored separately at -80 °C for molecular and biological assessment.

RNA isolation and quantitative real-time PCR

Total RNA was extracted from lung tissues using the RNeasy Mini Kit Reagent (Qiagen, Germany) as instructed by the manufacturer. A Nano-drop was used to measure RNA concentrations using spectrophotometry (Thermo Fisher Scientifics, Waltham, MA). Following the manufacturer's

instructions, double-stranded cDNA was produced using the High-Capacity cDNA Reverse Transcription kit (Qiagen, Germany).

According to the standard procedure, cDNA was processed to qPCR using The StepOnePlus™ Real-Time PCR apparatus (Applied Biosystems, USA) and SYBR Green qPCR Master Mix Maxima (Qiagen, Germany). Gene expression for IFNL3, NF-κB, TNF-α, TGF-β, and IL-1B was measured by qPCR. GAPDH was used as the house-keeping gene. Expression was measured using CT values, normalized to that of GAPDH ($\Delta CT = CT (GAPDH) - CT (target)$) and then expressed as $2^{\Delta CT}$. The primers used in the experiment are listed in **table (1)**.

Histopathological examinations of lung tissue

The lung tissue was rinsed with sterile water after being fixed in a 10% neutral buffered formalin solution and dehydrated with alcohol dilutions (methyl, ethyl, and absolute ethyl). The tissue was then cleared in xylene before being implanted in paraffin for 24 hrs in a heated air electric oven set to 56 °C. A microtome was used to slice paraffin bees wax blocks. The tissue was placed on a microscopic slide, deparaffinized, and stained with Masson's trichrome and hematoxylin & eosin (H&E) stain. The samples were then examined under a light microscope (Olympus, USA) for histopathological abnormalities by a histopathologist who was blinded to the group distribution during the analyses.

Statistical analysis

Version 9 of GraphPad Prism Software was employed to perform statistical analysis on the obtained data (GraphPad Software, San Diego, USA). The student t-test was used in the bivariate analyses. Each relevant result was visually displayed as mean \pm SEM (standard error of the mean). A p-value of 0.05 or less (typically 0.05) indicates statistical significance. The correlations between cytokines were evaluated by Spearman's correlation coefficient.

RESULTS AND DISCUSSION

Results

Histopathological assessment

The lung tissue in the sham-operated group had a normal histological structure with

normal bronchioles (B). The lung alveoli were normal (A) with normal alveolar cells and inter-alveolar septum (arrowhead) (H&E stain X100) as shown in figure 1A.

The lung tissue in the sham-operated group showed fine collagen fibers around the blood vessels and bronchioles (arrow). The interalveolar septum (arrowhead) around the alveoli appeared very thin and free from any collagen fibers (Masson's trichrome stain $\times 100$) as shown in figure 1B.

The lung tissue from the one-week bleomycin-treated group showed degenerative alterations in the lining epithelium of bronchioles (B) and a few alveoli (arrowhead), with the rest appearing normal (A). The lung tissue showed a thicker inter-alveolar septum (arrow) (H&E stain X100) as shown in figure 1C.

The lung tissue in the one-week bleomycin-treated group showed collagenic bundles around bronchioles (arrow) and in the inter-alveolar septum (arrowhead) (Masson's trichrome stain $\times 100$) as shown in figure 1D.

The lung tissue in the two-week bleomycin-treated group showed pulmonary tissue suffered from atrophy in some alveoli (A), while others appeared emphysematous (E). The inter-alveolar septum appeared thicker and contained cellular infiltration (arrow) (H&E stain X100) as shown in **figure 1E**.

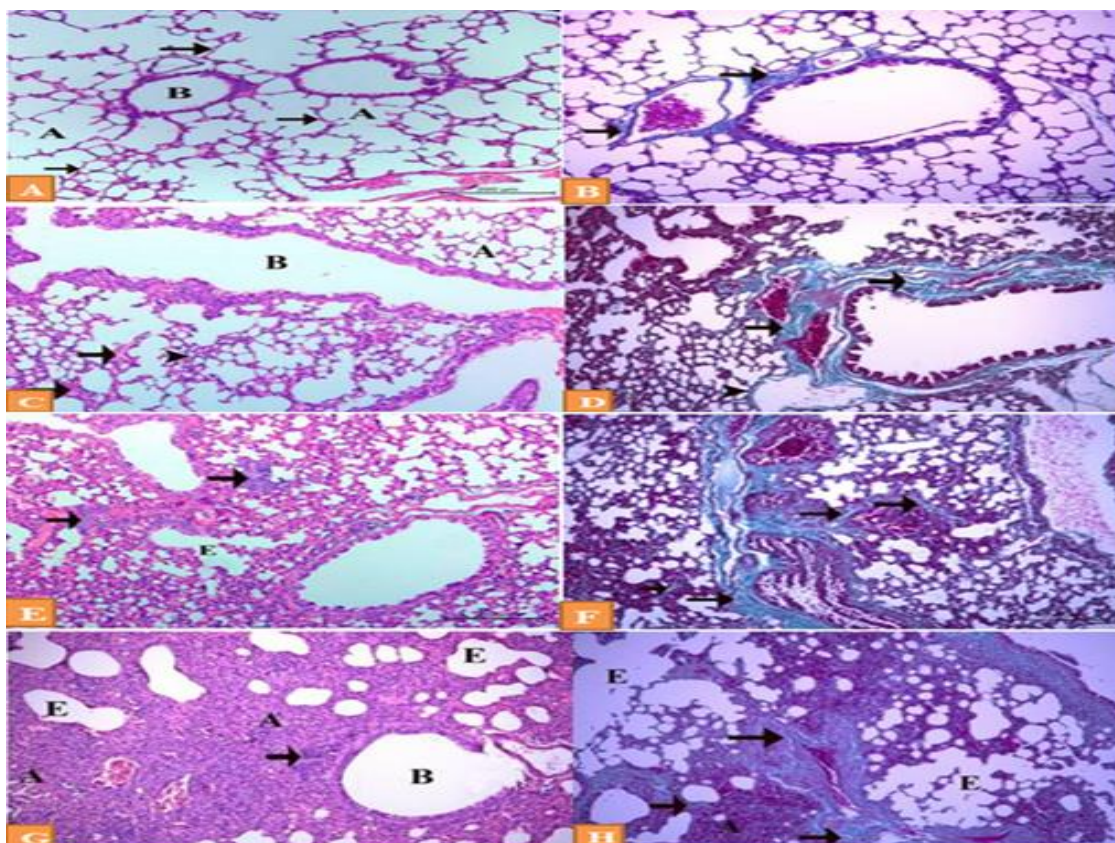
The lung tissue in the two-week bleomycin-treated group showed pulmonary tissue contained proliferative collagenic bundles around bronchioles and condensed around the alveoli in the inter-alveolar septum (arrow) (Masson's trichrome stain $\times 100$) as shown in figure 1F.

The lung tissue in the three-week bleomycin-treated group showed a significant number of atrophied alveoli (A) and a few emphysematous alveoli (E). Also, pulmonary tissue showed cellular infiltration (arrow) (H&E stain X100) as shown in figure 1G.

The lung tissue in the three-week bleomycin-treated group revealed that lung tissue healed by proliferative collagenic bundles around bronchioles and the alveoli (arrow). There are many atrophied alveoli (A) and a few emphysematous alveoli (E) (Masson's trichrome stain $\times 100$) as shown in figure 1H.

Table 1: primer sequences for mice IFNL3, NF-kB, TNF- α , TGF- β , IL-1B, and house-keeping gene GAPDH used for qRT-PCR analysis of their mRNA expression in BLM-induced pulmonary fibrosis in mice.

Primer	Sequence
IFNL3	Forward:5-TCAGCCCTGACCACCATC-3 Reverse:5-CTGTGGCCTGAAGCTGTGTA-3
NF-kBp65	Forward:5-GCCTCTTCTCATTTCCTGCTTG-3 Reverse:5-CTGATGAGAGGGAGGCCATT-3
TNF- α	Forward:5-GCCTCTTCTCATTTCCTGCTTG-3 Reverse:5-CTGATGAGAGGGAGGCCATT-3
TGF- β	Forward:5-TGGAGCAACATGTGGAAGCTC-3 Reverse:5-GTCAGCAGCCGGTTACCA-3
IL-1 β	Forward:5-AGTTGACGGACCCCAAAAG-3 Reverse:5-AGCTGGATGCTCTCATCAGG-3
GAPDH	Forward:5-GGGTTCCTATAAATACGGACTGC-3 Reverse:5-CCATTTTGTCTACGGGACGA-3

**Fig.1:** Histological analysis of lung tissue in adult male Balb/c mice in the sham-control group, one, two, and three weeks after BLM-induced lung injury. H&E X100 representative images are (A, C, E, and G) and Masson's trichrome stain X100 representative images are (B, D, F, and H).**Lung mRNA expression of IFNL3, TNF- α , TGF- β , NF-kB, and IL-1B**

First, the obtained data reflect a significant increase of IFNL3 mRNA level in the lung tissue after one-, two-, and three-weeks of BLM induction in comparison to the sham-operated group ($p = 0.003, 0.005, \text{ and } < 0.001$, respectively) as seen in figure 2A.

The mRNA level of NF-K β was significantly higher in the lung tissue of the two- and three-week groups compared to the sham-operated group ($P = 0.024 \text{ and } 0.006$, respectively). However, there was no significant difference in the mRNA level of NF-k β between the one-week group and the control group under sham surgery ($P = 0.07$) as shown in figure 2B.

Next, we explored the changes in in the TNF- α mRNA level in the lung tissue of BLM induced groups and the result showed that TNF- α mRNA increased rapidly in two- and three-week groups in comparison to the sham-operated group ($P < 0.001$ and $= 0.017$, respectively). Moreover, no significant difference in TNF- α mRNA levels was detected after a week of BLM induction compared to the sham-operated group ($P = 0.427$) as seen in figure 2C.

In addition, overexpression of mRNA level of TGF- β was indicated in the lung tissue of all experimental groups 1-3 weeks after BLM induction compared to the sham-operated

group ($P = 0.002$, < 0.001 , and $= 0.040$, respectively) as seen in figure 2D.

Finally, we found that the two- and three-week groups' lung tissue had a significantly higher level of IL-1B mRNA than the group that had a sham operation. ($P < 0.001$ and $= 0.01$, respectively). But no significant difference was noticed in the IL-1B mRNA level between the one-week group and the group under sham surgery ($P = 0.377$) as seen in figure 2E.

The student t-test was used to analyze the difference between the mean values. The data are shown as mean \pm SEM. As shown in table (2), P-values of less than 0.05 were regarded as statistically significant.

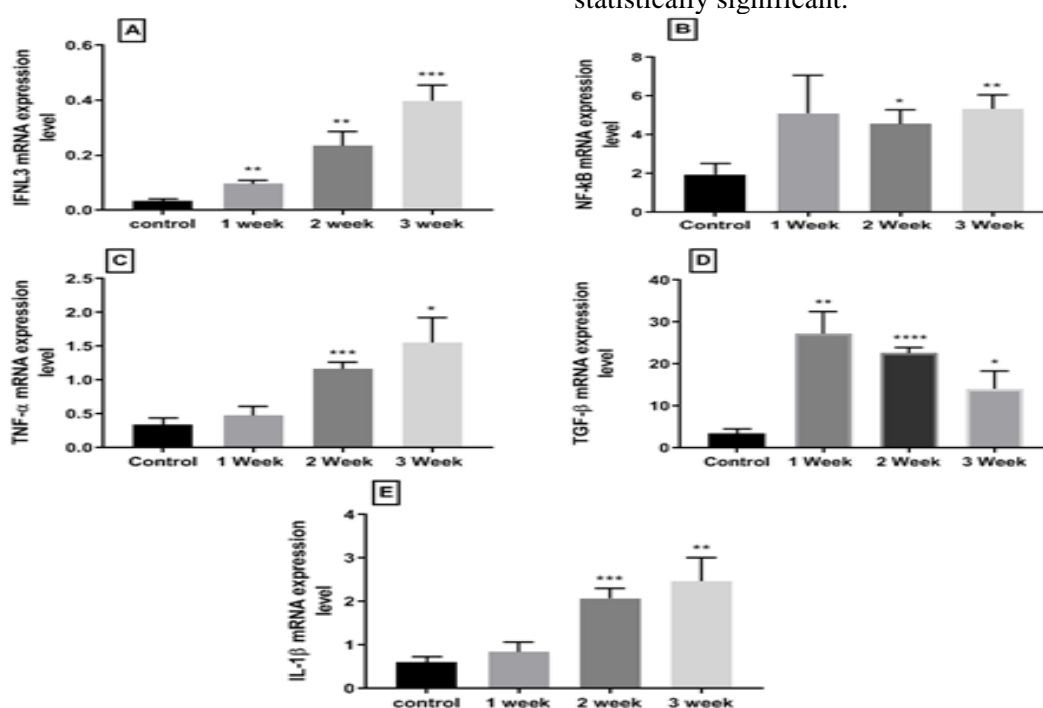


Fig. 2: Lung mRNA expression of IFNL3, TNF- α , TGF- β , IL-1B and NF-K β following BLM instillation in various groups was normalized to GAPDH mRNA expression; (A) Expression of IFNL3 mRNA, (B) Expression of NF-K β mRNA, (C) Expression of TNF- α mRNA, (D) Expression of TGF- β mRNA, (E) Expression of IL-1B mRNA. Data are presented as mean \pm SEM in comparison to the shamed control group.

Table 2: Quantification and correlation between mRNA expression of IFNL3 and other cytokines including TNF- α , TGF- β , IL-1B, and NF-k β genes in lung tissue of mice model.

Gene	Sham group	One-week group vs Sham group (t-test)		Two-weeks group vs Sham group (t-test)		Three-weeks group vs Sham Group (t-test)		Correlation with IFNL3 (Spearman's correlation coefficient)
IFNL3	0.034 \pm 0.007	0.096 \pm 0.013	$P = 0.003$	0.235 \pm 0.052	$P = 0.005$	0.398 \pm 0.057	$P < 0.001$	$r = 1.000^{**}$
TNF-α	0.341 \pm 0.101	0.481 \pm 0.132	$P = 0.427$	1.166 \pm 0.102	$P < 0.001$	1.557 \pm 0.368	$P = 0.017$	$r = 0.802^{**}$
TGF-β	3.472 \pm 1.088	27.253 \pm 5.264	$P = 0.002$	22.609 \pm 1.346	$P < 0.001$	18.918 \pm 3.686	$P = 0.040$	$r = 0.404$
IL-1B	0.608 \pm 0.126	0.848 \pm 0.223	$P = 0.377$	2.075 \pm 0.232	$P < 0.001$	2.473 \pm 0.540	$P = 0.010$	$r = 0.649^{**}$
NF-Kβ	1.957 \pm 0.567	6.164 \pm 2.124	$P = 0.07$	4.561 \pm 0.733	$P = 0.024$	5.342 \pm 0.721	$P = 0.006$	$r = 0.377$

Then, using the BLM-mice model, the association between the IFNL3 mRNA expression and the other pro-inflammatory cytokines was evaluated. The Spearman's rank correlation coefficient was done to understand the relationship between IFNL3 mRNA expression and TNF- α , IL-1 β , TGF- β , and NF- κ B. The mRNA expression level of the pro-inflammatory cytokines TNF- α and IL-1 β was found to be strongly positively correlated with IFNL3 mRNA expression ($r= 0.802^{**}$ and 0.649^{**} , respectively). While a medium correlation was found between IFNL3 mRNA expression and the pro-inflammatory cytokines TGF- β and NF- κ B mRNA expression ($r= 0.404$ and 0.377 , respectively).

Discussion

Interstitial lung disease is known as idiopathic pulmonary fibrosis (IPF) that strikes the lungs and frequently progresses to pulmonary fibrosis. The present concept of the pathogenic pathway of IPF has documented that fibrosis is following dysregulated repair responses to lung damage triggered by recurring epithelial cell micro-injuries that result in excessive extracellular matrix deposition¹⁶.

According to reports, IFN- λ 3 is a key IFN- λ subclass involved in regulating the progression of hepatic fibrosis and inflammation in both viral and non-viral diseases¹⁷⁻¹⁹. Nevertheless, IFN- λ 3 has an integral role in initiating fibrosis in other epithelial tissues. It has been linked to an increased risk of systemic sclerosis-related pulmonary fibrosis¹². Because of that, we assumed that IFN- λ 3 may participate in the activation of pulmonary fibrosis in IPF and other fibrotic ILDs. Other investigators showed that serum IFN- λ 3 levels were connected to clinical and immunological markers of liver inflammation and fibrosis, arguing that production of IFN- λ 3 may be adjusted by the status of liver injury²⁰.

Currently, because of its capacity to reproduce numerous aspects of IPF and many fibrotic ILDs, the mouse model of BLM-induced pulmonary fibrosis is the most widely utilized animal model. It is characterized by perfect reproducibility and an uncomplicated process of induction²¹.

In our work, we focus on the expression patterns of IFNL3 and other disparate inflammatory and fibrotic mediators that potentially influence the progression of fibrosis caused by BLM. Our findings demonstrate that over a course of time, inflammation becomes more serious and interstitial fibrosis is activated. The accumulation of collagen fibers in lung samples is a major sign of the growth of fibrosis. After three weeks of BLM instillation, the number of proliferative collagenic bundles around bronchioles and alveoli is ample, which confirms severe fibrotic lung destruction. Consistent with our results, prior studies have shown that the IFNL3 genetic variant as well as serum IFN- λ 3 levels possess pro-fibrotic effects in the BLM mouse model¹².

Our data demonstrated the augmented expression level of IFNL3 from the one-week group and reached its maximum level after three-weeks, might prove its supporting role in BLM-induced pulmonary fibrosis development and progression. Egli and his colleagues speculated that a rapid increasing in serum IFNL3 protein boosts the accumulation of pro-collagen 3, which is a primary biomarker of collagen formation²².

NF- κ B is a pro-inflammatory transcription factor that controls the production of various pro-inflammatory mediators such as chemokines and cytokines²³. NF- κ B performs a critical role in the production of transforming growth factor (TGF)- β 1²⁴. Furthermore, in lung fibroblasts, NF- κ B has been demonstrated to elicit the fibroblast-to-myofibroblast transition (FMT)²⁵, as well as elevated NF- κ B, has been associated with fibroblast aggregation in IPF patients' lung tissue²⁶, leading to enhanced extracellular matrix (ECM) deposition²⁷. We have documented an accelerated level of NF- κ B expression during the three-weeks of the study, which reached its highest level in the three-week group. Also, we detected a medium-strength positive correlation between IFN- λ 3 expression and NF- κ B expression. An earlier study found that IFN- λ 3 activated complex signalling pathways in epithelia during an antiviral response, primarily the NF- κ B signalling pathway and similar intracellular signalling transduction pathways activated by other inflammatory mediators²⁸.

TNF- α is a multifunctional pro-inflammatory cytokine that is required for

tissue remodeling and leukocyte growth^{29&30}. One of its principal aspects is stimulation of proliferation process, which results in epithelial overgrowth and lung fibrosis^{31&32}. Our data revealed a remarkable high level of TNF- α expression from the one-week group and continued to its maximum level after the third week of BLM. Furthermore, our data asserted a strong-positive correlation between IFNL3 expression and TNF- α expression.

IPF development is significantly influenced by the pro-fibrotic cytokine TGF- β 1. It regulates various pathophysiological mechanisms involved in IPF pathogenesis. TGF- β 1 may promote IPF by many pathways, such as activating Smads, inducing oxidative stress, and reducing the production of antioxidant substances³³. TGF- β 1 also boosts the formation of fibroblast extracellular matrix and governs the final differentiation of human lung fibroblasts³⁴. We observed that reinforced the expression of TGF- β 1 through the three-weeks of the study, demonstrating its integral part in fibrogenesis. Moreover, our results reported a medium-strength positive correlation between both IFN- λ 3 and TGF- β 1 expressions.

IL-1 β is a potent pro-inflammatory cytokine that causes fibroblast excitability via boosting of profibrotic cytokines such as TGF- β 1³⁵. The expression level of IL-1 β is strengthened and it achieved its peak level after three-weeks of BLM. In addition, our findings revealed a strong positive correlation between both of IFN- λ 3 and IL-1 β expressions.

Pharmacological research on profibrotic cytokines like TGF- β 1 and TNF- α in IPF has been conducted, and some brand-new medications have been designed that work toward the appropriate signaling pathways. These medicines include Nimbolide (TGF- β 1 inhibitor)³⁶, methylsulfonylmethane (TGF- β 1 and TNF- α inhibitor)³⁷, and etanercept (TNF- α inhibitor)³⁸. In addition to these agents that are under preclinical and clinical evaluation, some drugs such as pirfenidone (TGF- β 1 inhibitor)³⁹. The positive correlation between IFNL3 and the other pro-inflammatory cytokines suggests that IFN- λ 3 may be considered as a novel target for developing promising new drugs for pulmonary fibrosis.

In conclusion, our data revealed that as inflammation and fibrosis progress, they

stimulate the evolution of not only the level of IFNL3 mRNA expression but also other pro-inflammatory cytokines, including TNF- α , TGF- β 1, NF- κ β , and IL-1 β mRNA expression, verifying their critical role in BLM-induced lung fibrosis. Also, The mRNA expression levels of the additional pro-inflammatory cytokines TNF- α , TGF- β 1, NF- κ β , and IL-1 β have been found to positively correlate with the expression of IFNL3 mRNA level indicating that the pro-inflammatory, pro-fibrotic activity of IFN- λ 3 may be mediated by pro-inflammatory cytokines TNF- α , TGF- β 1, NF- κ β , and IL-1 β . However, for a deep understanding of these pivotal connection, further investigation is necessary.

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نشرة العلوم الصيدلانية جامعة أسيوط



جين إنترفيرون لامدا-3 مرشح جديد مشارك في تطور التليف الرئوي مجهول السبب

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التليف الرئوي مجهول السبب هو شكل مزمن وغير قابل للشفاء من امراض الرئة الخلالية وسبب حدوثه غير محدد ويؤدي إلى حدوث ندبات دائمة في نسيج الرئة ، مما يقلل من جودة و معدل البقاء على قيد الحياة. يعتبر هذا المرض من الأمراض المصاحبة للتقدم في العمر، وبسبب ارتفاع طول العمر العالمي فإن تكلفة الرعاية الطبية لتليف الرئة مجهول السبب يمكن أن تصبح كارثية. لا تزال الآليات المسببة لهذا المرض غير معروفة بعد، لكن النظريات الشائعة تشير إلى أن تعرض الخلايا الظاهرية للحوصلات الهوائية لإصابات صغيرة متكررة في الأفراد المعرضين وراثيا مما يؤدي إلى استجابة غير نظامية حيث تتحرف عملية ترميم الانسجة ويحدث ترسبات واسعة من الكولاجين في انسجة الرئة الخلالية.

التليف الرئوي المستحث بالبليوميسين هو النموذج الحيواني الأكثر استخداما لدراسة التليف الرئوي مجهول السبب والعديد من امراض الرئة الخلالية الليفية الأخرى حيث انه مدروس جيدا ويستخدم على نطاق واسع، وكذلك يتميز بانه غير مكلف وينتج استجابة ليفية مشابهة للعديد من الملامح التي لوحظت في مرضى تليف الرئة مجهول السبب، حيث يعمل النموذج من خلال إنشاء مرحلة النهائية أولية تستمر من خمسة إلى سبعة أيام قبل الانتقال إلى مرحلة ليفية تشبه إلى حد كبير السمات المميزة لتليف الرئة مجهول السبب في البشر.

ينتمي إنترفيرون لامدا-3 إلى عائلة الإنترفيرون من النوع الثالث. لقد اثبتت الدراسات السابقة علاقة الإنترفيرون لامدا-3 بالتهاب وتليف الكبد بغض النظر عن السبب، ومؤخرا وجد ان هذا التأثير يمتد الى الاعضاء الاخرى مثل الكلي والرئة، على سبيل المثال، تم ربط إنترفيرون لامدا-3 بشدة ومضاعفات مرض الانسداد الرئوي المزمن. ونظراً لأن التليف المرضي في العديد من الأعضاء من المتوقع أن يتشابه في بعض الصور النمطية القياسية، لذا من المتوقع أن يؤثر إنترفيرون لامدا-3 أيضاً على خطر الإصابة بتليف الرئة في التليف الرئوي مجهول السبب والعديد من امراض الرئة الخلالية الليفية الأخرى أيضاً، لا تزال الآلية الدقيقة لكيفية عمل إنترفيرون لامدا-3 في حدوث امراض الرئة الخلالية الليفية تحتاج إلى مزيد من الدراسة والبحث .

لقد قمنا في هذه الدراسة بفحص مشاركة إنترفيرون لامدا-3 في تليف الرئة مجهول السبب باستخدام نموذج التليف الرئوي المستحث بالبليوميسين في الفئران، حيث قمنا بقياس مستوى التعبير عن الحامض الريبوزي الرسول الخاص بإنترفيرون لامدا-3 بالإضافة إلى عدد من السيتوكينات الأخرى التي تعزز الالتهاب، مثل الانترلوكين-1 بيتا، والعامل النووي المعزز لسلسلة كابا (ب)، وعامل نخر الورم الفاء، وعامل النمو التحولي بيتا-1 في أنسجة الرئة وبعد مرور اسبوع واسبوعين وثلاثة اسابيع من التليف الرئوي المستحث بالبليوميسين قمنا بمقارنتها بأنسجة الرئة الطبيعية في الفئران. وقد وجدنا من خلال هذه الدراسة زيادة ملحوظة في الحامض الريبوزي الرسول الخاص ب إنترفيرون لامدا-3، والانترلوكين-1 بيتا، والعامل النووي المعزز لسلسلة كابا (ب)، وعامل نخر الورم الفاء، وعامل النمو التحولي بيتا-1 على مدار الثلاث اسابيع فترة الدراسة. ووجدنا أيضاً علاقة إيجابية بين مستويات الحامض الريبوزي الرسول الخاص ب إنترفيرون لامدا-3 وبعض السيتوكينات الأخرى التي تعزز الالتهاب، مثل الانترلوكين-1 بيتا، والعامل النووي المعزز لسلسلة كابا (ب)، وعامل نخر الورم الفاء، وعامل النمو التحولي بيتا-1. لقد اثبتت نتائج هذه الدراسة تأثير إنترفيرون لامدا-3 في التليف الرئوي المستحث بالبليوميسين وتطوره مما يساعد في فهم آلية حدوث المرض ويساهم في تطوير بعض الأدوية التي قد تستخدم لعلاج هذا المرض من خلال تأثيرها على هذه الآليات.