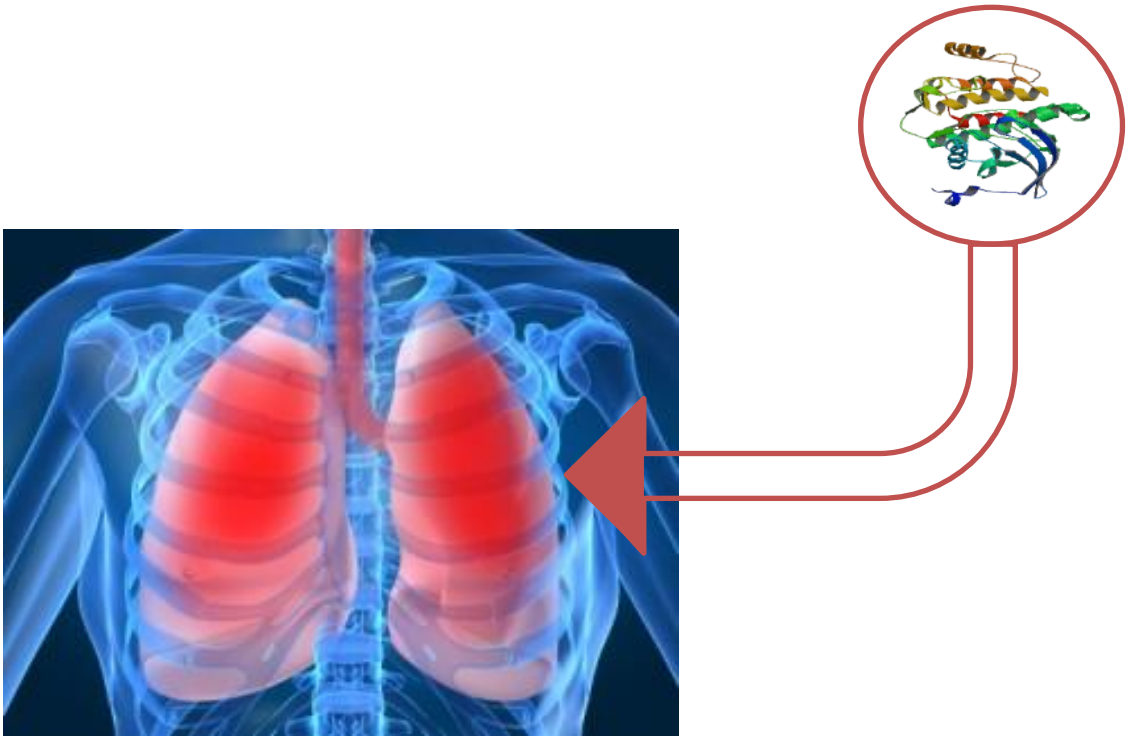
LCK, a potential target for COPD?



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Summary

Chronic obstructive pulmonary disease (COPD) is a serious inflammatory disease that results in the deterioration of the lungs' proper function. The progression coincides with the increase of mucous in the lumen and penetration of the inflammatory immune cells by the innate and adaptive immune system producing lymphoid follicles, which triggers the repair mode that thicken the airway walls. What is mostly understood of COPD has not given way to develop a proper treatment of the lungs or even a cure. The stimulation of certain genes and specific T-cell activity via response to an antigen, most likely to inhalation of cigarette smoke and other irritants, are important factors in this complex lung disease. The lymphocyte-specific kinase LCK is involved in the phosphorylation of components required for the initiation of the T-cell receptor (TCR)-signalling pathways. In a normal system, LCK is critical for the differentiation of naïve T-cells to obtain full effector function. However, those who are predisposed to this disease, LCK activity may have been disrupted. Therefore, this review has based its focus on gaining insight about the role of tyrosine kinase protein (LCK) and whether there is link to COPD pathogenesis and exacerbations Understanding the connection between the activation of LCK and the progression of COPD can give an opportunity in the future to scientifically evaluate the effectiveness of the newly-designed inhibitory drug. Inhibiting the activity of LCK may potentially lead to the reduction in the inflammation that may be causing the destruction in the lungs.

1. Introduction

An irreversible lung disease that is progressing to the point of being the fourth leading cause of death world-wide is COPD, affecting the amount of continuous airflow due to tissue damage otherwise known as emphysema and chronic bronchitis (Chen & Wang, 2011). These two can either be developed separately or together. The air sacs and airway walls tend to lose elasticity, become thick, and inflamed producing more mucous to the point of obstructing the air passage (Saetta et al., 2001). This usually leads to wheezing, persistent coughs, and even sputum. A major contributor to COPD is tobacco smoke, triggering an inflammatory response within the lungs and disrupting the function of the lungs. However, not all smokers have shown to develop COPD, and the reasons are not thoroughly understood (Ali et al., 1986; Barnes, 2008). Evidently, the cessation of smoking entirely, after diagnosed with COPD, does not reverse the disease nor prevent the continuation of the ultimate destruction of the lungs. The inflammation remains and continue to augment is likely due to infections (bacterial, viral, or both), which was noticed by the pathogens found in the lungs with COPD (Sullivan et al., 2005). As of yet, no appropriate therapy has been discovered. However, the existence of a novel drug, that is meant to target LCK by inhibiting its proper function, has not been researched to show its potential use. Therefore, the focus on understanding the function of LCK protein as an upstream mediator of T-cell activity and defining its relationship within the pathogenesis of COPD, may lead to possible targets in halting the unrelenting severity in this type of lung disease.

2. COPD Pathogenesis

Approximately 4 to 6% of individuals aged more than 45 years have developed COPD (Szulakowski et al., 2006). The destruction of the alveolar walls contributes to the lack of proper airflow in those affected by emphysema (Barnes, 2008). The damage inflicted on the lungs can occur from the free radicals, which initiate oxidative stress, or the cytokine release due to the inflammation from smoke inhalation (Ali et al., 1986). On a genetic standpoint, COPD can also be a result of a deficiency in the anti-protease enzyme gene α-1-anti-trypsin. This genetic disorder can also be triggered by exposure to cigarette smoke (Agusti et al., 2011).

2.1 Oxidant Stress

Smoking is found to be the culprit in the imbalance of oxidant/antioxidant levels due to oxidative stress, which activates transcription factors like NF-κB. It appears that individuals who smoke yet have relatively healthy lungs have the capacity to promote the DNA-binding activity of NF- κB just like those with COPD and continue to smoke. Although DNA in the lung fibroblasts and human bronchial epithelial cells become increasingly damaged due to the smoke, necrosis/apoptosis is somehow not visualised in the tissue (Ali et al., 1986). In the breath and serum of those with COPD, certain markers are noticeably high that give an indication of oxidative stress helping to orchestrate the pathogenesis of COPD like hydrogen peroxide, 8-isoprostane, and lipid peroxides.

2.2 Action of proteases and mucous hypersecretion

Type 1 pneumocytes are the products of these elastin-attacking enzymes which destroy the fibres of the alveolar walls and leaving the alveolar cells that make up the epithelial cell layer of the wall to undergo cell death. Neutrophil elastase, an enzyme that causes this type of degradation, also appears to promote extreme mucous production (Barnes, 2008; Pesci et al., 1998). The central airways are found to be the primary place of mucous secretion, with high level of neutrophils in the bronchial glands (Saetta et al., 2001).

2.3 Apoptosis parallel with aging

The effects from inhaling smoke indicate a connection between the aging organism and maintaining the structure of the organ. The damaged lung via alveolar destruction and poor gas exchange observed with emphysema correlate with that of a typically aged lung. An aging lung is known to also develop characteristics that would induce lung tissue destruction via elastin fibre fragmentation and a reduction in the air-capillary exchange. Prolonged energy applied to protect and repair an injured lung against oxidative stress via infiltrating free radicals (increase due to mitochondrial weakening or malfunction in an aged individual) (Tuder et al., 2006). Therefore, long-term smokers with emphysema tend to have a higher degree of proliferating cytotoxic T-cells and thus apoptosis of the structural cells of the lungs, which correlates to the fact that high levels of oxidants age the lung significantly (Cosio et al., 2002).

2.4 Genetics

A deficiency of glycoprotein α_1-antitrypsin would be a disadvantage and a possible risk for developing COPD, considering this protein is derived from liver and necessary to block the activity of proteolytic enzymes (Gupta et al., 2005).According to genetic studies, early-onset of COPD correlated with the phenotypes based on certain regions found on the chromosomes (Croxton et al, 2002).

3. Diagnosis & Treatment

Assessment of the severity and the method of treatment are aided by the 'level of airflow limitation'. COPD can be diagnosed by applying spirometry along with chest X-rays and blood tests in order for it to not be mistaken for other diseases like asthma or congestive heart failure. Spirometry measures forced expiratory volume of air in one second of breath and the forced vital capacity (FEV1/FVC ratio). A ratio less than 0.9 suggest a person has COPD and less than 0.3 is a person at a very severe stage. Usually the treatments involve bronchodilators that are inhaled like salmeterol, formoterol, salbutamol and terbutaline, β_2 agonists, to help with the breathing by improving airflow. Corticosteroids in the form of an inhaler are used in more moderate to severe cases of COPD. Nevertheless, this type of therapy appears to not respond well-enough as it does in asthma patients (Chen & Wang, 2011; Agusti et al., 2011). Suppression of the disease or arresting the progression does not appear to be effective with this type of treatment (Löwenberg et al., 2005).

4. Immunology

The mechanism of T-cell recruitment has yet to be figured out, but T cells have a crucial role when it comes to the understanding of the pathogenesis of chronic inflammatory lung disease. A variety of cytokines and chemokines are released and regulated by NF- κB in COPD, leading to macrophages in the airways (Barnes, 2008; Sullivan et al., 2005). When the neutrophils are subjected to travel into the lung tissues, they become activated in the capillaries of pulmonary microcirculation which may lead to capillary sequestering of neutrophils upon activation of endothelium and cause damage to lung, increasing inflammation (Drost & MacNee, 2002).

4.1. Histopathology

The analyses of the biopsies of bronchial airways, small airways, and lung parenchyma from COPD patients, there was an indication of a great amount of T-cells and neutrophils infiltrating into the airway lumen. It also showed a considerable amount of fibrosis surrounding the smaller airways perhaps establishing a reason behind the incapability of reversing the narrowing of the airways. Many other indicators like type 1 pneumocytes, increasing levels of elastolytic enzymes like neutrophil elastase and matrix metalloproteinases (MMP), and the diminishing amounts of anti-proteinases like α_1-antitrypsin in the lungs (Barnes, 2008).

4.2 Macrophages & Granulocytes

The sputum of patients with COPD does reveal large amounts of neutrophils, which associate with the severity of COPD (Celli & Barnes, 2007). The smokers with COPD, instead of the 'healthy smokers', appeared to have these neutrophils and macrophages, which derive from monocytes that circulate in the blood and migrate to lungs according to certain chemoattractants (e.g. CCL2 and CXCL1 act upon CCR2 and CXCR2, respectively) (Barnes, 2008). Previous research has shown that macrophages promote inflammation by secreting these chemokines which subsequently attract the neutrophils, T-cells, and proteases like that of MMP9, MMP12, and MMP-13. In addition, macrophages in combination with endothelial cells tend to release transforming growth factor-B, causing the proliferation of fibroblasts in the airways (i.e. fibrosis production) (Sullivan et al., 2005; Lee et al., 2009; Pesci et al., 1998).

4.3 Immunological responses of CD8+ and CD4+ T cells

Mature T-lymphocytes can be divided into two subtypes: helper inducer cells (CD4+) and cytotoxic suppressor cells (CD8+). The CD8+ T-cells recognise class I major histocompatibility complex (MHC) determinants as restriction elements on antigen-presenting cells while class II MHC are recognised by CD4+ T-cells (Veillette et al., 1988). T cells have been found to be linked to the inflammation development. CD4+ are found to accumulate in the airways and lungs (of COPD individuals) as Th1 cells, in order to release certain molecules to help B-cells in combatting microbes by producing macrophages and antibodies. As for the type 1 cytotoxic cells, which are a subset of CD8+ T- cells, they have been proven to secrete IFN-γ (inflammatory cytokine) and express CXCR3 (in order to bind cytotoxic chemokines), linking them to persisting inflammation of the lungs (Barnes, 2008). Since the amount of CD8+ cells in the patients'

lungs with COPD have been shown to be in high quantities, this subset of T-cells are implicated in the tenacious airway inflammation and the onset of COPD. The auto-reactivity of these CD8+ cells can push the cytotoxicity levels until the point of damaging the lung tissue. It is known that the role of CD8+ cells produce pro-inflammatory cytokines such as IL-2, IFN-γ, and TNFα and chemokines like CXCL10 and CCL5, which lead to more inflammatory cells. It has been demonstrated previously that even more inflammatory cytokines are released in the existence of COPD (Grundy et al., 2013). Toll-like receptors (TLR) on CD8+ T-cells were found in the lung tissues with COPD, according to the research by Nadigel *et al*., and it showed that cigarette smoke triggered specifically TLR4 andTLR9 on these T-cells. And so, the outcome was a high cytokine production from the CD8+ cells, capable of playing a part in the COPD pathogenesis (Nadigel et al., 2011).

COPD may be connected to an adaptive immune system, by the fact that CD247 decreases in activity by leaving the cells consistently exposed to antigens (e.g. vial, bacterial, etc.) and unable to return to normal levels during disease progression. Consequently, the predisposition to frequent infections becomes a factor when the TCR-signalling molecules are down-regulated in pulmonary CD8+ cells. Diseases like COPD that involve autoimmune processes seem to indicate a decrease in the expression of TCR-ζ (i.e. CD247) and LCK, which result in the dysfunction of T-cells, thereby producing infections making patients even more susceptible to exacerbations. The outcome in the CD8+ cells from the lungs of COPD appears to differ significantly with the pulmonary CD8+ cells from smoker's lungs, which was demonstrated by using qRT-PCR and immunofluorescence (Grundy et al., 2013). Smokers with emphysema have greater amounts of CD8+ T-cells, and a higher degree of apoptosis. The lungs of non-smokers tend to have variable amounts of CD4 and CD8 cells, in general. As for those smokers who have not developed COPD showed a normal pattern of T-cells but with a 'shifting trend' towards CD8+ cells (Majo et al., 2001; Saetta et al., 2001). Therefore, these results indicated that emphysema development are linked with increasing CD8+ T cells; also, the length of time (i.e. after 30 years) involved in smoking cigarettes is an essential factor for the symptoms to be obvious, meaning the T-cells need time to elevate in number before inflammation progresses towards COPD (Majo et al., 2001).

5. Role of LCK in T-cell activation

Lymphocyte-specific tyrosine protein kinase (LCK) is considered to be part of the Src family of protein tyrosine kinases. The 58 kDa non-receptor protein has been discovered to play a significant role in

intracellular signalling pathways exclusively in lymphocytes for the regulation and maturation of T-cells. LCK can bind to cell surface glycoproteins on the cytoplasmic side of co-receptors CD4 and/or CD8 (Zhu et al., 1999; Löwenberg et al., 2005).

LCK is known to be necessary for the function of mature T cell responses and are highly expressed in T-lymphocytes, predominantly in CD4+ and CD8+ cells. After the myristylation and palmitoylation on the amino terminus which anchors the protein to the internal section of the plasma membrane, LCK has been shown to cross-link with CD4+ and CD8+ cells. The coupling of CD4+ and TCR generates the activation of LCK by inducing tyrosine phosphorylation (Straus & Weiss, 1992).The lipid anchorage strategy is to bring LCK nearby the TCR complex, thereby commencing its function in phosphorylating the tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAMs) of the cytoplasmic tail of the TCR-ζ chain (Veillette et al., 1988; Zhu et al., 1999). This step is a requirement for the binding of ZAP-70, which release the 'TCR signalling cascade' by exposing sites for other kinases, adaptor proteins, etc. The activation of the T-cell antigen receptor (TCR) based on the recognition of an antigen induces T-cells to proliferate and differentiate that are needed to activate the phosphatidylinositol pathway, ras/mitogen-activated protein kinase pathway, and rho/rac pathway. The trigger of the first pathway eventually leads to the increase in the levels of inositol phosphates and diacylglycerol causing the discharge of calcium, as drawn in Figure 1 (Straus & Weiss, 1992; Zhu et al., 1999).

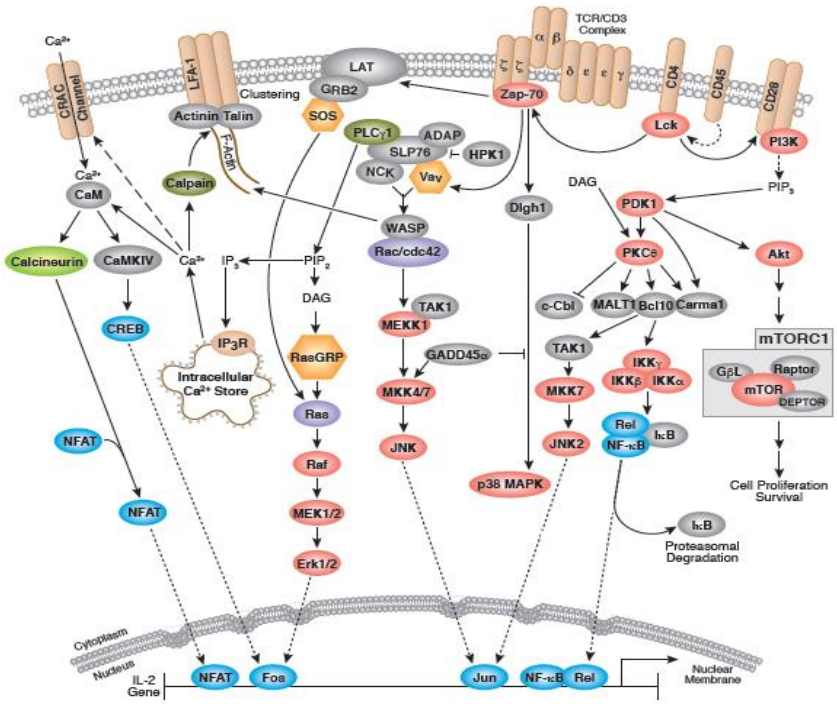

Figure 1. TCR-signalling pathway showing the activation of LCK leading to activation of several cascades which determine cell fate [extracted from Cell Signalling Technology Inc., 2012].

Another way the stimulation of LCK can occur is by initiating contact with the cytoplasmic section of the CD2 receptor resulting in the extreme phosphorylation of the tyrosine kinase (Isakov & Biesinger, 2000). This tyrosine kinase has two possible phosphorylation sites: residue 394 autophosphorylates in order to triggers the activation of the protein; and on residue 505, C-terminal src kinase phosphorylates to allow the inhibition of LCK. Once LCK activity is inhibited, the result is normally a decrease in the number of thymocytes and mature T-cells. Such was the case when LCK immunoprecipitated with TCR in one cell line showing that an activated LCK actually stimulated signalling via ζ-chain of the TCR complex. It was demonstrated that losing the expression of the entire LCK, in human Jurkat T-cells, did cause a disruption in the TCR signalling. In addition, completely dismantling the LCK gene had prevented thymocyte maturation (Grundy et al., 2013; Zhu et al., 1999).

5.1 Impact of LCK expression in disease and COPD

LCK has been identified as a key enzyme in influencing the antigen response through the TCR signalling pathway. According to research by Molina *et al*. in 1993, knock-out of the LCK gene in mice and subjecting them to lymphocytic choriomeningitis virus (LCMV) or vaccinia virus had proved that CD4+ and CD8+ antiviral responses were difficult to achieve, and signals mediated by LCK were necessary for proper T-cell function. However, the low presence of T-cell maturation, that caused a decrease in CD4+ and CD8+ cells, LCK did not impede the T-independent B-cell stimulation (Molina et al., 1993).

Studies performed by O'Hara *et al.,* in human lung adenocarcinoma, had indicated that active LCK tyrosine kinase in alveolar cells lead to respiratory diseases after exposing bronchial epithelial cells to low levels of chromium (VI). This caused a delay in the activation of the transcription factor STAT3 (signal transducer and activator of transcription) where LCK is required to stimulate a prolonged tyrosine phosphorylation of STAT3, and increasing levels of IL-6. Their results established that STAT3 cannot be phosphorylated without the presence of LCK, and the induction of

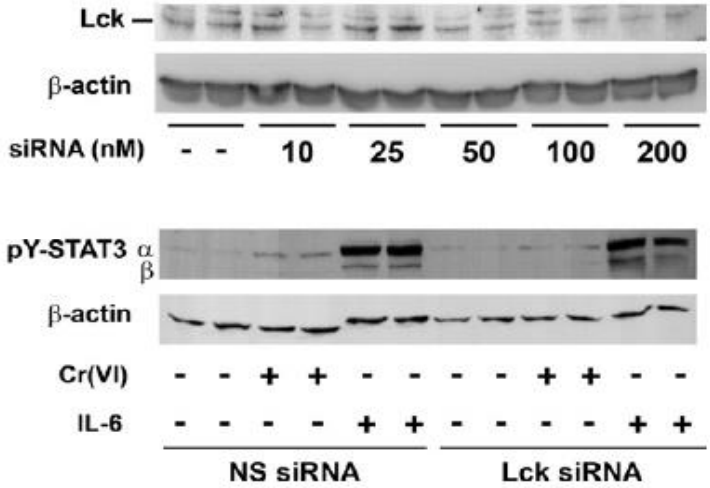

Figure 2. Western blot analysis depicting the necessity of LCK for complete chromium (VI)-stimulated STAT3 phosphorylation [reported in (O'Hara et al., 2007).

an inflammatory cytokine IL-6 also required LCK. However, the stimulation of IL-6 was accomplished after a prolonged activation of STAT3, not due to direct effects of chromium. Inhibitory experiments with siRNA (small interfering RNA), thereby halting LCK expression entirely, had evidently shown isoforms of STAT3 could not be phosphorylated and the IL-6 production was prevented (as displayed in Figure 2). The alpha isoform of STAT3 was necessary for transmission of IL-6, yet the beta isoform was implicated in anti-inflammatory pathways after its deficiency was evaluated to promote inflammation. This signalling pathway has shown to be associated with progression of pulmonary diseases even though it was suggested that it may be attempting to protect the lungs, since this pathway is considered part of the innate system (O'Hara et al., 2007).

In contrast, a more recent research conducted with pulmonary COPD samples by Grundy *et al*. had established clearly an accumulation of CD8+ T-cells along with lower level protein expression of LCK and CD247 (Figure 3). These results were found to be statistically significant ($p<0.05$) between the COPD and smoker control samples analysed via quantitative real time-PCR. The down-regulation of these specific genes in COPD patients in comparison to the controls may be the reason for the many challenges in fighting against viral or bacterial infection. COPD has been associated with exposure to antigens in the most persistent manner (Grundy et al., 2013). Their research only reached to the point of analysing the genes expressed in the CD8 cells in COPD patients, healthy smokers, and non-smokers. The magnitude of the changes in protein expression in terms of their function was not studied.

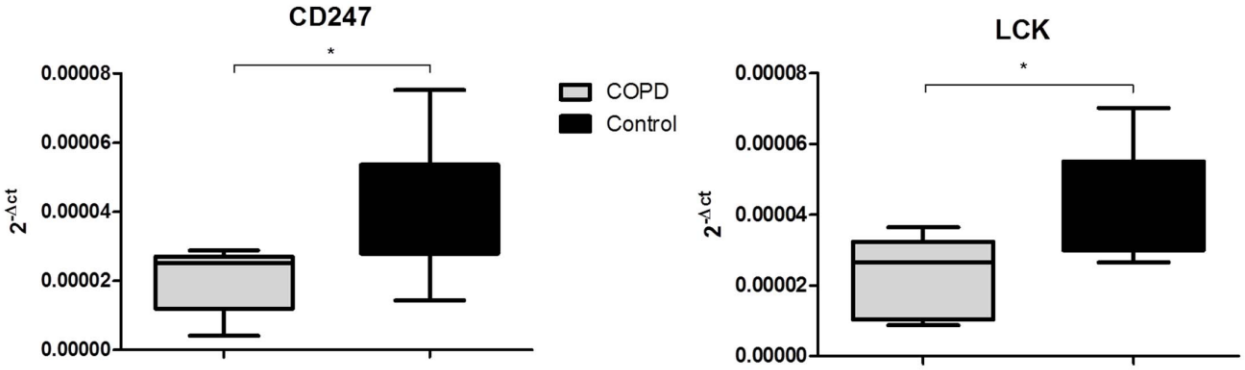

Figure 3. Transcript levels of TCR signalling genes LCK and CD247 in COPD and Smoker pulmonary CD8 cells. (COPD, n = 6) and smokers with normal lung function (control, n = 6) obtained from (Grundy et al., 2013).

5.2 The connection between IL-6 and COPD

Patients with chronic lung inflammation like COPD have expressed IL-6 in their breath condensate (Bucchioni et al., 2003). The role of IL-6 in COPD was investigated in 2009 by Qu *et al*. by evaluating the transition from emphysema to lung cancer. The increasing levels of pro-inflammatory cytokine IL-6 seem to induce inflammation in the lungs, which have shown to lead to bronchioalveolar adenocarcinoma. The overexpression of MMP12 has shown to result in a continuous up-regulation of IL-6 and (phosphorylation of) STAT3 genes. It was established that increased expression of MMP12 has been linked to the progression of COPD (emphysema) in humans and associated with adenocarcinoma (Figure 4). The gradual increase of IL-6 was found to cause emphysema, in previous studies. In this study, the same pattern was visualised in the bitransgenic mice bronchioalveolar lavage in the presence of doxycycline (Figure 5, left). After nine months, the bitransgenic mice, after treatment, indicated high levels of IL-6 and STAT3 activated genes in the lung (ATII) epithelial cells (Figure 5, right). As mentioned, the persistence of STAT3 and IL-6 contributed to the induction of lung cancer. It is comprehensible that these genes are exacerbating the inflammation in the lungs (developing emphysema) to point where tumours begin to generate (Qu et al., 2009).

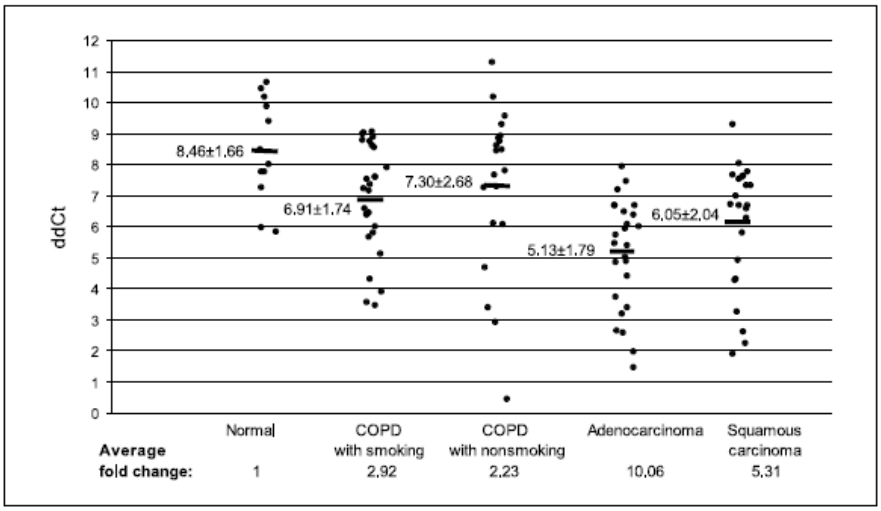

Figure 4. MMP12 upregulation in COPD (smoking and nonsmoking) along with the carcinoma compared to the normal human samples analysed with real-time PCR (Qu et al., 2009).

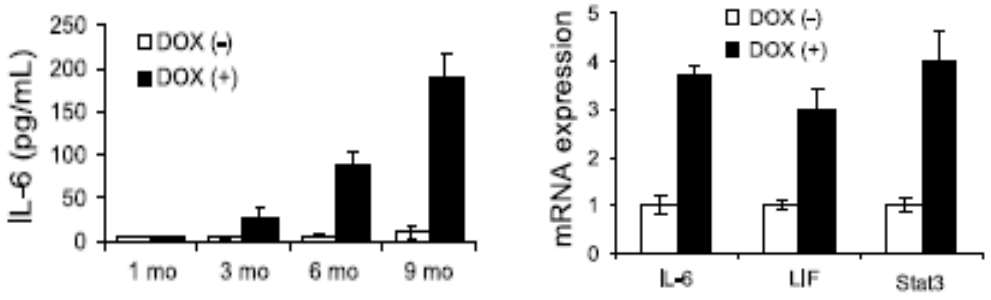

Figure 5. The evaluation of IL6/STAT3 pathway in the lung epithelial cells of bitransgenic mouse model. The left graph depicts the concentration of IL-6 measured by ELISA and, on the right, the mRNA levels obtained after real-time PCR analysis of purified RNA from the epithelial cells (Qu et al., 2009).

It is clear that information linking COPD and LCK is quite limited. The results provided by Grundy *et al*., it appeared to be evident that inhibiting a protein that is found in lower levels than in healthy individuals would not be a logical path to take. Their research tends to depict that LCK is an essential protein which may prevent future exacerbations. However, it is known that LCK associates with both CD8+ and CD4+ T-cells. Concerning the reports by O'Hara *et al*., it may still be plausible to evaluate the role of LCK in COPD pathogenesis in more depth. Their research does not involve COPD but it implicated LCK in a signalling pathway that induced specific inflammatory cytokines that caused injury to lungs by the effect of being in contact with an irritant like chromium. The case may be the same with cigarette smoke, even though studies have not entirely shown the mechanism by which the destruction of the airways in COPD. The induction of IL-6/STAT3 pathway in COPD that was found by Qu *et al*. seems to correlate with the investigation conducted by O'Hara *et al*. Therefore, it is plausible to suggest LCK as a key factor in COPD.

6. Concluding remarks

LCK is an important protein in the TCR-signalling pathway, and its role in cell proliferation and survival. Since LCK is found to be expressed exclusively throughout all stages of thymocyte development and particularly in T-lymphocytes, there is a potential target in terms of therapeutic aspects with regards to COPD. This disease has been researched significantly and yet an actual functioning drug has not been achieved. The reason for that may be for the lack of clarity on the pathological aspect. The role of LCK in relation to COPD has not been targeted previously; however, the availability of a drug that can inhibit LCK function has opened the doors to a future in a prospective therapy/treatment against COPD.

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